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Quality by Design for Biopharmaceutical Drug Product Development

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This work is dedicated to the memory of our friend and colleague Ronald Taticek as a tribute to the imagination that led him to undertake a project of this magnitude and made him a pioneer in this field. It is also a tribute to the dedication that inspired Ron to carry this effort so far to completion before he passed away on April 23, 2014. Ron will be greatly missed.

Preface

Occasionally in one's professional career you become aware that the hand of history is resting on your shoulder. So it was in July 2003, in Brussels, when the members of the International Conference on Harmonisation (ICH) Expert Working Groups (EWG) for quality agreed on a new vision and strategy for ICH. Summarized in the statement, "A harmonized pharmaceutical quality system applicable across the life cycle of the product emphasizing an integrated approach to quality risk management and science," ICH agreed to progress three paradigm-changing guidelines. These were Q8 (pharmaceutical development), Q9 (quality risk management), and Q10 (pharmaceutical quality system). When I called to order the first Q8 EWG, we all thought that we might be able to take the existing European Note for Guidance on Development Pharmaceutics and convert it into an appropriate ICH format and that would be it: a simple task. It took us a little while to appreciate the futility of this approach, especially given the growing interest in the application of process analytical technology (PAT) and the growing appreciation that the goal of pharmaceutical development is to design a quality product and its manufacturing process to deliver consistently the intended performance of the product. The only way to achieve that consistency would be by designing a product from the outset that would meet patients' needs, acquiring comprehensive product and process understanding, and establishing a properly controlled manufacturing process. We needed to tell the world that quality cannot be tested into a product; it has to be designed into a product. But, of course, everyone already knew this, so there was nothing new here, but how could we help move the industry from its traditional 3-sigma processes toward 6-sigma? We needed to talk about Deming, Juran, kaizen, risk assessments, experimental designs, even the value of "failed" experiments. We needed to give the industry permission to share the fullness of their scientific knowledge without the fear of creating an ever-increasing list of regulatory questions that added little value but much time to the review and approval processes.

With these things in mind, the EWG drafted the ICH Q8 guideline. Recognizing that traditional development processes would still be needed, we referred to the new thinking as an "enhanced approach," deliberately avoiding the moniker of "quality by design." Even as Q8 went through its final revisions and adoption, it became clear that outside the confines of the EWG, neither the industry nor regulators had a clear understanding of the new paradigm. We were asked to use the addendum to

Q8 to define and exemplify "quality by design," and we did our best, comparing traditional approaches with an enhanced quality-by-design approach. But even with this effort, and with subsequent Implementation Working Group efforts (which have included question and answer documents, points to consider), there is still mystery and confusion about what QbD really means for the pharmaceutical industry.

Fortunately, our journey has been helped by the foresight and commitment of a number of early adopters. Before the ink was dry on the first part of Q8, a team within the European Federation of Pharmaceutical Industries and Associations developed a mock section P2 (Examplain), which demonstrated some of the key elements of QbD including a quality target product profile, risk assessments, design of experiments, and design space. Two more comprehensive case studies, intended for discussion and teaching purposes, quickly followed. The first, ACE tablets, was aspirational in many respects and explored a number of innovative concepts that industry was contemplating. The second, A-Mab, discussed the application of QbD principles to a biotechnology product, stimulating much discussion between industry and regulators at the same time as the FDA was introducing its pilot programs. Other case studies such as the Sakura mock P2 from Japan and A-Vax (QbD for vaccines) and the several mock ANDA submissions have strengthened our understanding and appreciation of both business and regulatory opportunities.

Many would regard QbD for chemical substances as straightforward: our understanding of kinetics and thermodynamics enables rapid building on prior knowledge to provide scalable syntheses. On the other hand, drug product development still remains a complex blend of art and science which may be behind the often experienced challenges of establishing well characterized, robust manufacturing processes that can be described by reliable models. For biologics, it could be argued that the opposite situation pertains. The drug substance is the process: the processes are often exquisitely designed and engineered with feed-forward and feedback control strategies. While the quality is designed from the outset, the many degrees of freedom and the characterization challenges mean that full application of QbD principles is not easy. The list of critical quality attributes is generally extensive, our ability to directly connect them through analytical techniques back to the critical process parameters and forward to the patient is often not straightforward, and the realization of design spaces becomes challenging, especially when you consider the risks associated with movement with a design space. However, application of QbD principles to the final steps, the drug product, is much more straightforward.

Into one insightful volume is collected a wide range of discussions and practical examples of the application of QbD to biological drug products. For those still uncertain about the business benefit, this is the area to start. Biological drug product manufacturing processes lend themselves to the enhanced approach. The risks, science and engineering are all much better understood than those in many other areas of our industry. The degrees of freedom are manageable. QbD principles facilitate developing an effective control strategy, arguably the most critical deliverable of a well planned and executed development program, including real-time release-testing opportunities.

Most of the leading pharma companies now consider QbD to be "business as usual" for the current development portfolio. An increasing number of publications attest to the business benefits that have accrued from QbD programs and filings. Experience is growing with successful regulatory submissions and approvals. For sure, both industry and agencies have been on a steep learning curve with the new paradigm, but in the USA, the small molecule pilot program followed by the biologics pilot program have provided valuable insight and learning. Similar initiatives have occurred elsewhere. The international agencies have mounted joint assessment and inspection programs—our new paradigm is here to stay, and the publication of this book could not be better timed. Now is the time to wholeheartedly grasp the opportunities, to do the great science that surely motivates us all and comprehensively tell the story to the regulators. What are you afraid of? The patient is waiting.

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Chapter 1
Challenges and Opportunities for Biotech Quality by Design

Cyrus Agarabi, Mansoor A. Khan and Rakhi B. Shah

1.1 Introduction

The goal of biotechnological product development is to design and establish a formulation composition and robust manufacturing process to consistently and reliably meet all the quality standards intended for its therapeutic purpose. Traditionally, products are released onto the market only after successful 'end product testing', however, with the introduction of 'Quality by Design' (QbD) for pharmaceuticals (ICH Q8 2009), quality standards need to be built into the product by design and cannot be met merely at the end-product-testing stage. A scientific knowledge base along with appropriate quality risk management principles (ICH Q9 2005, ICH Q10 2008) and enhanced process and product understanding through process analytical technology (PAT) principles (PAT guidance 2004) can offer advantages for biotech product manufacturing over a traditional approach (Table 1.1).

Bioprocessing is generally divided into two stages; the upstream operations for the generation of the active biological ingredient referred to as the drug substance, and the fill-finish activities that are required to generate a finished drug product. For the scope of this chapter, the terms, biotech molecules and proteins, are limited to monoclonal antibodies or therapeutic proteins.

These active biological ingredients are more complex than small molecules, as their biological activity requires a unique 3-D structural conformation. Additionally, proteins are prone to degradation throughout bioprocessing; examples include

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The findings and conclusions in this chapter have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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Table 1.1 Salient features of pharmaceutical development under traditional and QbD paradigm (ICH Q8 2009)

Aspects	Traditional	QbD
Pharmaceutical development	Empirical; univariate experiments	Systematic; multivariate experiments
Manufacturing process	Fixed; validation on three initial full-scale batches; focus on reproducibility	Adjustable within design space; continuous verification; focus on control strategy and robustness
Process control	In-process testing for go/no-go; offline analysis with slow response	PAT utilized for feedback and feed forward, real time
Product specification	Primary means of quality control; based on batch data	Part of the overall quality control strategy; based on desired product performance
Control strategy	Mainly by intermediate and end product testing	Risk-based; controls shifted upstream; real-time release
Lifecycle management	Reactive to problems and OOS; post-approval changes needed	Continuous improvement enabled within design space

deamidation, oxidation, hydrolysis, aggregation, and denaturation, which can result in activity loss and/or immunogenicity. Often, proteins undergo a post-translational modification in the upstream drug substance processing during biosynthesis. The site of the post-translational modification can vary and potentially produce a protein with more than one form, for example, various glycosylated forms of a monoclonal antibody. Such structural heterogeneity is sometimes inevitable and is challenging to address throughout drug substance and drug product manufacturing.

Due to the complex physicochemical and stability issues, the majority of biotech products are administered via parenteral routes with intravenous and subcutaneous being the most common routes of administration. Biotech-finished drug products can be broadly classified as liquids and lyophilized powders for reconstitution prior to injection. Relative to small molecules, the fill-finish manufacturing steps for biotech drug products do not involve complex multi-step processes, with lyophilization a notable exception. Due to the complex nature of the molecules, there are significant challenges in consistently manufacturing high-quality biotech drug products. Yielding consistent product quality with minimum or no failed batches is a goal for any biopharmaceutical scientists. Rejected or failed batches not only results in loss of revenue but can also bring about negative criticism from the stakeholders and users. Therefore, QbD principles are based upon the idea that quality cannot be tested in the products but should be built-in by design. QbD can offer advantages for complex protein products even as the science and technology to support several elements of QbD are still evolving. Application of QbD to biotech products is not trivial and some of the challenges presented include (i) structural complexity

of the biotech drug substance, (ii) a lack of understanding of interactions between drug substance with formulation excipients, (iii) assigning clinically relevant specifications to a biotech product, and (iv) constructing a multidimensional design space for a biotech product at various scales.

Despite so many challenges, it is possible for biotech industries and regulatory agencies to mutually benefit by adopting QbD principles (Rathore and Winkle 2009; Rathore 2009; Shah et al 2010). In 2008, the FDA announced a notice of a pilot program in Federal Register regarding voluntary submissions of applications under the QbD paradigm for biotech drugs following the successful voluntary program for small molecules QbD (FDA notice, 2008).

1.2 QbD Implementation in Biomanufacturing

QbD implementation is a multi-step approach and is well defined in ICH guidance documents (ICH Q8 2009, ICH Q9 2005, ICH Q10 2008). It is schematically represented in Fig. 1.1, as an iterative risk assessment process in which the quality target product profile is initially predetermined. QbD principles can be very helpful to understand critical quality attributes (CQA), process parameters, and impact of variations in formulation or process on CQAs. Through risk management and statistical approaches a design space can be constructed and followed for a bioprocess manufacturing. The overall approach detailed in the section below for the biotech drug substance and products which are mainly categorized as liquid or lyophilized formulations.

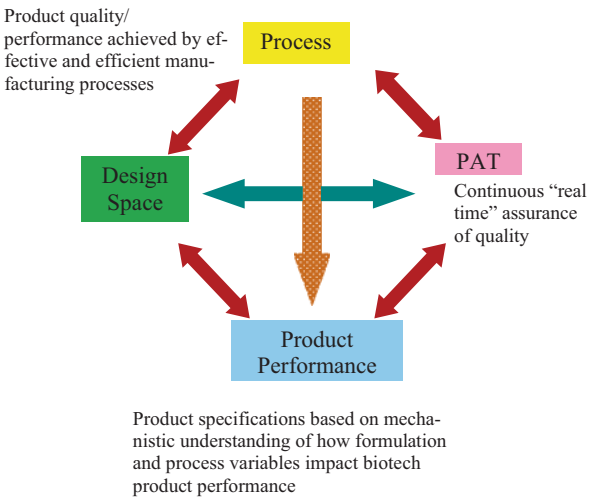

Fig. 1.1 Quality roadmap for a bioprocess

1.2.1 Drug Substance Manufacturing

Figure 1.2 is an example of the unit operations, which comprise drug substance manufacturing. The process begins with the thawing of cells from a working cell bank, the growth and expansion of the cells through different scales into a commercial scale bioreactor. Once the cell culture process is complete, the material is removed from the reactor and concentrated via centrifugation. The concentrate is clarified and purified, usually via chromatographic methods, of unwanted host cell proteins and other impurities to yield a pure protein. The analytics listed in Fig. 1.2 are examples of techniques which may be used in various unit operations throughout the drug substance manufacturing process. The media composition, pH, dissolved oxygen (DO), amino acid (AA) analysis, optical density (OD), viable cell density (VCD), and off gas analysis may be commonly utilized in-line, on-line, or off-line to monitor parameters in the bioreactor during cell culture. The chromatographic methods, such as size exclusion (SEC), cation exchange (CEX), anion exchange (AEX) with pulsed amperometric detector (PAD), are generally studied off-line. While all of the unit operations offer opportunities to explore QbD principles, the cell culture of materials in stirred tank bioreactors is an area of particular interest. Due to the high costs and complexities of utilizing living systems to generate active biologic materials and the potential for irreversible damage which may travel downstream to the final drug product, there is a great demand for the enhanced process understanding which a QbD approach can establish.

1.2.2 Liquid Formulations

Many biologics are formulated as liquid formulations at the end of the downstream purification process. Liquid manufacturing involves mixing the drug substance with other excipients including pH modifiers, tonicity agents, stabilizers, surfactants, chelators, etc. followed by filtration, fill/finish operations. Inspection at the end of line has been done in automated mode by using automated machines for the clarity of the solution (Knapp and Abramson 1990). However, understanding the stability during shelf life in various buffer systems, pH, ionic strength, stabilizers, and preservatives is an important quality attribute. Additionally, there is a growing trend towards more complex delivery systems for liquid formulations, which include prefilled syringes. A prefilled syringe is a single-dose unit of a biologic to which a needle is fixed. Disposable syringes are used for this purpose in which the liquid drug product is filled so that exact dose of the drug is available for the patient without the need of a pre-injection step, i.e. withdrawal from the vial. This eliminates waste due to vial overfilling, it is easier to handle and more convenient for the patients. However, interaction of the drug product with the syringe material poses a technological challenge for such delivery systems (Soikes 2011). Thus in a systematic QbD development approach, the compatibility of the syringe material, stability, and safety should become an integral part of the novel delivery systems for biotech products.

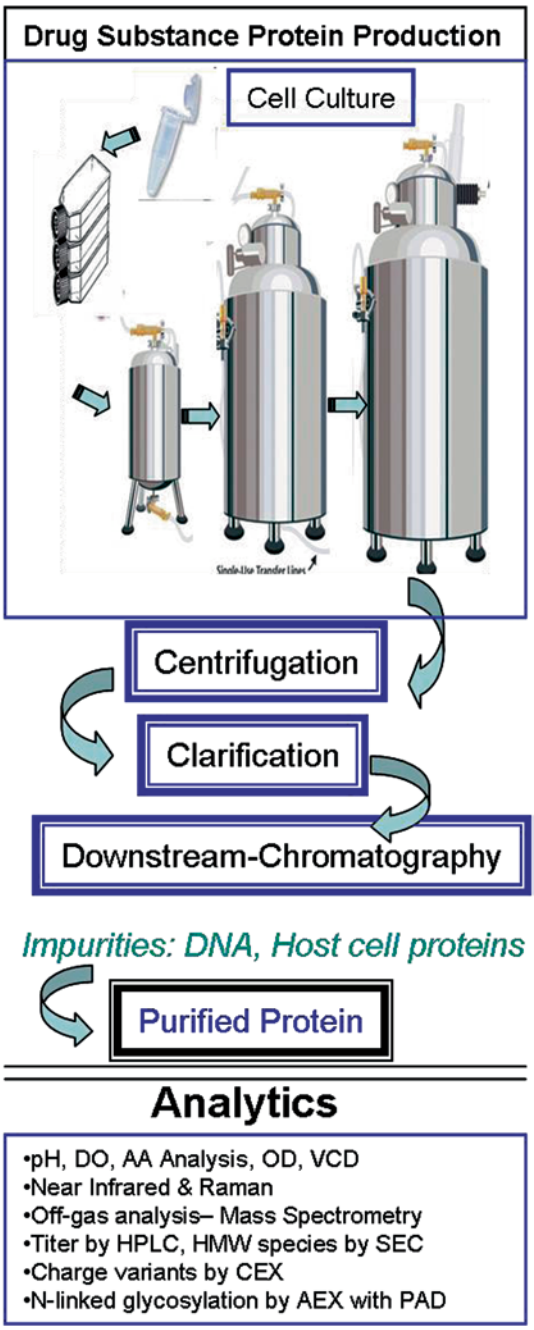

Fig. 1.2 An overview of the unit operations and potential analytical techniques for drug substance manufacturing

1.2.3 Lyophilized Formulations

Lyophilization or freeze drying is a low temperature drying to convert solutions of heat-labile materials into solids. For a biotech drug substance, this process is employed if the molecule is unstable in liquid form to increase the shelf-life of the product. Lyophilization is a multi-step process which includes a (i) freezing, (ii) primary drying, and (iii) secondary drying. In this case, phase transformations occur in this process and therefore present unique challenges during manufacturing to maintain stability of the molecule. The following section describes how a QbD approach can be useful for a lyophilization process and some of the challenges encountered.

1.3 Challenges and/or Opportunities of QbD for Biotech Products

1.3.1 Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs) for Biotech Formulations

A quality target product profile (QTPP) is defined as 'a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product' (ICH Q8 2009). QTPP should consider route of administration, dosage form and strength, delivery system, container closure system, attributes affecting pharmacokinetic characteristics, and drug product quality criteria. Commonly identified QTPP for a biotech liquid product includes clarity, subvisible particulates, pH, concentration, bioactivity, sterility, stability, color, odor, etc. For a lyophilized product, QTPP include pharmaceutical elegance in terms of cake appearance, reconstitution time, moisture content in addition to applicable requirements of liquid dosage forms.

Once the QTPP has been identified, the next step is identifying the relevant CQAs. A CQA is defined as 'a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality' (ICH Q8 2009). CQAs for drug substance, excipients, intermediates (in-process materials), and drug products can be determined based on either prior experience or from experimental designs. The key consideration is an assessment of the extent to which individual CQA variation can have an impact on the overall quality of the drug product. Examples of potential drug substance CQAs include host cell protein (HCP) content, glycan formation/heterogeneity, aggregates, potency, titer, etc. For a liquid drug product, this might include clarity, stability, sterility, etc. Additionally, in the case of pre-filled syringes, compatibility between container closure (i.e. syringe material) and drug substance need to be studied thoroughly. CQAs usually are thought to be a subset

of QTPP. For a lyophilized product, it may include reconstitution time in addition to other applicable attributes listed for a liquid product. Thus, there may be multiple CQAs for a product, and it can become very complex while applying risk assessment principles or other QbD tools.

1.3.2 Setting Specification for a Biotech Drug Product

Traditionally specifications for a biotech drug product were set on the basis of end product testing rather than by design. Under the science and risk-based approaches, QbD may help set the specifications based on user requirements rather than those obtained in the current state of manufacturing. A 'specification' is defined as a list of tests, reference to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges or other criteria (ICH Q6A 1999). For a biotech drug product, conformance to specifications would mean that when tested according to the listed analytical procedure, a liquid or lyophilized product will meet the listed acceptance criteria.

For a liquid or lyophilized biotech product, aggregation of drug substance may be performed as an in-process test, or may be performed as a release test, depending on its relevance to product performance. Justification of specification should include either linkage to performance, stability, safety, efficacy, etc. Thus QbD can help set clinically relevant or meaningful specifications for a biotech drug product.

1.3.3 Quality Risk Management (QRM) for Biotech Formulations

Quality risk management (QRM) helps to identify and control potential quality issues from early stages of development to marketing and large-scale manufacturing for biopharmaceutical drug products. It gives a higher level of product quality assurance. It increases cost saving and efficiency for industry as well as regulators. For sponsors, it facilitates innovation, increases manufacturing efficiency, reduces cost and product rejections, minimizes or eliminates potential compliance actions, enhances opportunities for first cycle approval, and streamlines post-approval changes and regulatory processes. It also offers opportunities for continuous improvement. For regulators, it helps in more focused inspections and reduces burden of post-approval supplements.

The most daunting risk factors are those that occur sparsely and have a catastrophic impact on the product and can only be detected after it is too late to correct the problem. Microbial or viral contamination of a bioreactor can bring a manufacturer to a grinding halt if not handled correctly. During the risk assessment, it is important to understand the causes of contamination, examples include: improper cleaning/sterilization of equipment, improper aseptic techniques by operators, and

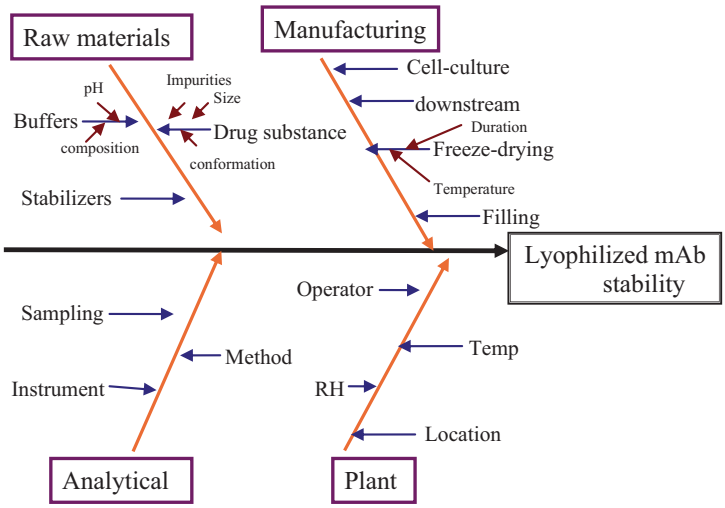

Fig. 1.3 Fishbone (Ishikawa) diagram for CQA of stability for a biotech-lyophilized drug product

contamination during sampling. By identifying potential failure modes during development, proper safeguards and risk mitigation techniques can be established.

For a liquid drug product, challenges to develop a stable solution formulation include multiple degradation pathways and chemical modifications to the molecule. Therefore, assessing the stability risk is of great importance in the overall QRM process for biotech liquid products. Moreover, formulation excipients, processing factors, as well as container-closure systems play an important role in the stability of the molecule. Initial risk assessment can be done by various methods, one of which is by using Ishikawa or Fishbone diagram.

For a lyophilized drug product, risk assessment could be performed for all the identified CQAs, which include but are not limited to cake appearance, reconstitution time, moisture content, stability, potency, etc. An example for a risk assessment is provided in Fig. 1.3. Once the initial risk assessment is performed, all the factors can be identified that could impact the CQAs. However, in order to manage and/control those that pose a significant risk, risk analysis at times is often performed. This process assigns a score depending upon the severity (impact) and occurrence (frequency) as well as ease of detectability in case of an excursion. Risk priority number (RPN) scores are calculated as shown below.

$$\text{Risk priority number} = \text{Severity} \times \text{Occurrence} \times \text{Detectability}.$$

It is often challenging to assign a rating for severity, occurrence, and detectability, especially if there is little institutional knowledge regarding the product or process. A consistent scale should be used each time to prevent bias or variable results (Table 1.2). Often times, low scoring can be justified based on experience, literature, or expert knowledge. However, since this exercise is done without any prior experiments, it is often times semi-quantitative. Once the experimental values are

Table 1.2 Risk priority number[a] score based on ratings of severity, occurrence and detectability (ICH Q9 2005)

Ratings	Severity	Occurrence	Detectability
1	Minimal or no impact	Not likely	High
2	Minor impact	Low likelihood	Moderate
3	Moderate impact; workarounds possible	Likely	Medium
4	Unacceptable impact; workarounds possible	Highly likely	Low
5	Unacceptable impact; no alternatives exist	Near certainly	None

[a] RPN score (severity × occurrence × detectability) will vary from 1 to 125

available, it can be treated as more quantitative and is a very useful tool to focus on high-risk factors. Risk review and reassignment of risk numbers is an iterative process and the model is continuously improved and updated with additional knowledge gained about the impact of the risk factors on the CQAs.

1.3.4 Biotech Formulation Design Space

After QRM, once the risk factors are identified and prioritized, a statistical approach may be useful to perform experiments in a multidimensional manner. This is especially important for the design space determination. Design Space is defined as 'the multidimensional combination and interaction of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide assurance of 'quality' (ICH Q8 2009). Design space is proposed by an applicant and is subject to approval by the regulatory agency. The development of a bioprocess design space requires a careful analysis of process data obtained during development and routine manufacturing along with data from the statistical design of experiments to develop appropriate process models and predict process performance.

Design of experiments (DoE) has been found to be useful for understanding the impact of input process and/or formulation factors on the CQAs. This has been done for small molecules in a two-tiered way (Zidan et al. 2007; Shah and Khan 2005; Shah et al. 2004; Nazzal et al. 2002). In this approach, a screening DoE is first used to identify the main effects of input factors on the dependent variable and those with the highest impact can be studied further using optimization or higher resolution DoEs. In the response surface DoEs, three or four factors can be studied in a multidimensional way that can provide interaction of the input factors in addition to the main effects. It is critical to decipher the impacts of main and interaction effects when creating a design space.

Due to the staggering costs of experimentation at the manufacturing scale, it is essential to have lab scale systems, which accurately reflect the commercial process. The implications for robust low-volume parallel apparatuses with high-throughput

capabilities, including cost-effective disposable systems for supporting DOE and QbD principles have been proposed (Rao et al. 2009). Micro-reactors, stirred tank assemblies below 100 mL have been studied for rapid screening and have shown promise with regard to scalability. Although, significant hurdles remain due to small volumes with regard to emulating equivalent control of gassing resulting in excess foaming, and uneven gas distribution (Chen et al. 2009).

At the bench top scale, commercially available cell culture reactors are available with working volumes ranging from approximately 100 mL to 14 L. Through mathematical modelling of the oxygen mass transfer rates via the calculation of the volumetric mass transfer coefficient ($K_L a$) values, bench top-scale bioreactors have been modelled against commercial scale reactors to guide process scale up (Garcia-Ochoa and Gomez 2003). A case study of 2 L bioreactors was experimentally evaluated against 15 and 110 L reactors and modelled for 2000 L vessel operation (Zhang and Mostafa 2009). The authors successfully used a fractional factorial experimental design for clone selection and process optimization for temperature, pH, and pH shift from parallel 2 L reactors, which was ultimately transferred to the commercial scale.

There are various other approaches to determine design space which includes a first-principle approach where combination of experimental data and mechanistic knowledge of chemistry, physics, and engineering is used to model and predict the performance. Scale-up correlations are also important when the experiments are conducted at a laboratory scale. Verification experiments at a larger scale may be performed for a stronger correlation of the design space at various scales.

1.3.5 Bioprocess Parameters and Control

Process analytical technology (PAT) is gaining momentum after the guidance document was published by the FDA in September 2004 (PAT guidance 2004). A fundamental element of PAT is to increase process understanding and control, and is useful to verify product quality prior to release. A control strategy is defined as ‘A planned set of controls, derived from current product and process understanding that assures process performance and product quality’ (PAT guidance 2004). For a biotech drug product, PAT offers the possibility to increase the product quality, consistency, and reduce product risk through increased process knowledge and understanding with the optimized process control through the use of various tools. PAT has a potential to offer ‘real-time release’ (RTR) in lieu of end product testing. In RTR, material attributes are measured and controlled along with process parameters. Material attributes can be assessed using direct and/or indirect process analytical methods. However, for bioprocesses, it is challenging to obtain RTR due to the complexity of the products. Additional research is required for bioprocessing to help monitor and control the manufacturing process.

During the cell culture process, each cell line requires a unique blend of nutrients, buffers, and other additives to produce a highly active protein with desirable critical quality attributes with a cost effective yield. There are a wide variety of

suppliers, which manufacture chemically defined media and offer an opportunity for evaluation during the development phase to study the effects of initial and in process feeding. At the commercial scale, where tens of thousands of litres are required, it is often more cost-effective to formulate the media in-house with the required nutrients and supplements and to devise a tailored feeding strategy for the individual cell line application. Stand alone or autosampler interfaced nutrient analyzers utilize electrochemical sensors and commonly measure glucose, glutamine, glutamate, lactate, ammonium, sodium, potassium, ionized calcium, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), pH, and use freezing point depression to measure osmolality.

Examples of on-line HPLC of amino acids for cell culture feeding strategies have been seen for over 15 years (Kurokawa et al. 1994) and can play a pivotal role in the quantitation of nutrient consumption during processing. Advances in HPLC capabilities for automated derivitization of amino acids through autosampler programming alleviates the need for tedious off-line manual sample preparation (Frank and Powers 2007) and supports automated feedback loop strategies. Quantitation of nutrient consumption patterns are essential components to develop additional capabilities, which require model building and chemometrics and do not directly measure concentration. A prominent example is near-infrared (NIR) spectroscopy, which has been studied by a number of authors as an in-line tool to monitor the cell culture process within the bioreactor. Quantitation of prevalent components, such as glucose, glutamine, and lactate, is commonly studied, and evaluated against commercially available nutrient analyzers. More advanced models have evaluated biomass (Tosi et al. 2003), protein titer (Mattes et al. 2007), and the application of multiplexing NIR probes for DoE studies (Roychoudhury et al. 2007). A challenge associated with NIR is its sensitivity to water, resulting in a large absorbance of water peaks at 1400 and 1900–2100 nm, which may limit its utility for cell culture medium, which are aqueous in nature. Peak overlap of nutrients has also been identified as a complication of NIR, and may additionally limit the applications of the technique (Arnold et al. 2002).

Raman spectroscopy, which is not affected by water, has emerged as a potential spectroscopic alternative to NIR for cell culture monitoring, and has been demonstrated to be a capable in-line tool for real-time monitoring of nutrient consumption. Multiplexed raman systems to quantitate common media components, such as glucose, lactate, glutamate, and ammonium, as well as osmolarity and viable cell density (VCD) have been studied (Moretto et al. 2011). The applications of this technique are limited and require further investigation.

Process parameters in the case of liquid manufacturing include time, speed, and temperature of mixing, hold time, excipient quality. The process parameters for filtration unit operation include filtration pressure, flow rate, and the attributes that needs to be controlled include aggregate level or filtration type (Sharma et al. 2008). Pressure gauge and/or flow meters have been used to control the filtration unit operation (Sharma et al. 2008). For a filling unit operation, process parameters include fill weight/volume, fill temperature, vial size, filling speed, etc. A balance or even Nuclear magnetic resonance (NMR) for more accurate monitoring has been cited

in the literature (Aldridge 2007). Some of these process control techniques are easily adapted to various scales, for example, automated inspection, pressure gauge, weight measurements, etc. However, sophisticated techniques such as NMR have limitations for use at a commercial scale due to the time and specialized expertise required for the measurements.

For a lyophilized product, optimum freeze-drying process relies on the knowledge of critical properties of the formulation and process parameters and application of this information to process design. The critical formulation properties include the collapse temperature of the formulation, the stability of the drug, and the properties of the excipients used (Tang and Pikal 2004). Above the glass temperature, the freeze-dried cake will be physically unstable, which may result in crystallization of one or more of the compounds in the formulation, or shrinkage of the cake. The stability issue is particularly important for biotech drugs, as some of the biotech drugs may be sensitive to the stresses imposed by freeze drying and may be degraded or decomposed during the process. Also, interplay between the choice of proper excipients and the process have an important impact on quality and stability of the product. The glass transition temperature can be influenced by the moisture content and the choice of excipients.

There is a need for online and in-line measurement sensors which can act as an 'eye' of the process to monitor the real-time phenomena occurring during process (Shah et al. 2011). These techniques are crucial during the liquid or lyophilized formulations manufacturing process. Various sensors which are being considered to monitor the process of freeze-drying are described in the literature (Tang et al. 2006a, b, c; Roy and Pikal 1989; Schwegman et al. 2007; Read et al. 2010). Manometric Temperature Measurments (MTM) is a procedure to measure the product temperature at the sublimation interface during primary drying by quickly isolating the freeze-drying chamber from the condenser for a short time and by subsequent analysis of the pressure rise during this period (Tang et al. 2006a, b, c). The method is relatively easy to use and yields product dry-layer resistance in real time. Figure 1.4 shows a freeze drying process profile for a study of model monoclonal antibody formulations. The MTM was useful in detecting the end point of primary drying with three thermocouples placed in vials and using pirani gauge as well as capacitance manometer readings. Although, MTM is generally limited to lab-scale freeze-drying measurements, it has a potential to be applied for larger scales as well.

While PAT is still an evolving area, it is believed that over time it may be seen as an initiative that helped foster a period of innovation, efficiency, and expansion for the biopharmaceutical industry.

1.4 Summary

QbD methodologies with the identification and justification of target product profiles, and product and process understanding to obtain preset specifications, with appropriate control strategies can ensure a state of control. This provides a potential

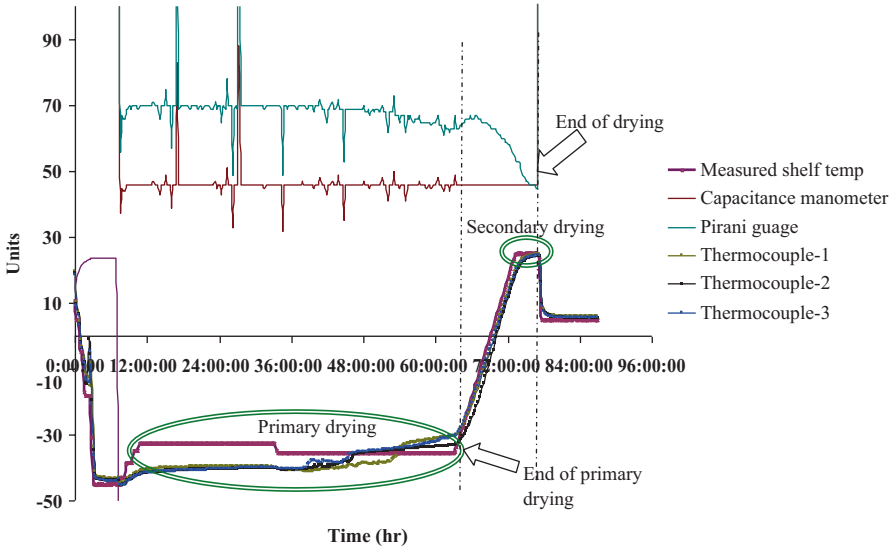

Fig. 1.4 Lyophilization chart for a biotech mAb drug product-process control with online monitoring using capacitance manometer, pirani gauge, and thermocouples

pathway for continuous improvement with real time release and reduced supplement submission burden. Regulatory agencies, industry, and academia need to work together in a multi-disciplinary approach to make QbD a success for biotech products as it requires a significant culture change.

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Chapter 2
Lessons Learned from Monoclonal Antibody Applications to the Office of Biotechnology Products Quality by Design Pilot Program

Barbara L. Rellahan, Steven Kozlowski and Patrick Swann

2.1 Introduction

Food and Drug Administration (FDA) announced the Pharmaceutical CGMPs for the twenty-first century—A risk-based approach in 2002 with the intent of ensuring that the product review and inspection programs operate in a coordinated and synergistic manner. The program was intended to encourage the adoption of modern and innovative manufacturing technologies and, as Janet Woodcock stated at the FDA-ISPE Workshop in October 2005 (AAPS 2005), to encourage the pharmaceutical industry to be "maximally efficient, agile, and flexible" and to produce high-quality drug products "without extensive regulatory oversight." The goal was to create systems where quality would be built into the product, and testing alone would not be relied upon to ensure product quality. Dr. Woodcock further indicated that in the desired state, manufacturers would have extensive knowledge on the critical

Views in this article, other than current Food and Drug Administration (FDA) and International Committee for Harmonization (ICH) guidance, are presented by the authors for consideration. They do not necessarily reflect current or future FDA policy.

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elements of their products and processes and they would strive for continuous improvement. An important objective of the initiative was to facilitate industry application of modern quality management techniques, including implementation of quality systems approaches, to all aspects of pharmaceutical production and quality assurance and to encourage implementation of risk-based approaches that focus on both industry and agency attention on critical areas. To achieve these objectives, the concepts of Quality by Design (QbD), risk management and the quality system approach could be utilized (Food and Drug Administration 2009a, b, c, 2012).

2.2 Quality by Design (QbD) for Biotechnology Products

ICH Q8 (R2) defines QbD as "A systematic approach to product development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" (Food and Drug Administration 2009c). QbD requires an investment of resources to develop an in-depth understanding of product quality and how the manufacturing process impacts product quality. This knowledge is then used to build product quality into the process rather than simply confirming it through testing (Rathore and Winkle 2009).

Due to the high degree of physicochemical complexity and inherent heterogeneity of biotechnology products, implementation of QbD for biotechnology products is a more difficult undertaking compared to most small molecule drugs. The molecular complexity of biotechnology products presents challenges for both the identification of product attributes that impact efficacy and safety, and the manufacturing parameters that impact product quality. Monoclonal antibody (mAb) products represent a somewhat unique biotechnology class in that most share a common structure, which has enabled the development of robust platform manufacturing processes. Platform manufacturing is defined in ICH Q11 as "the approach of developing a production strategy for a new drug starting from manufacturing processes similar to those used by the same applicant to manufacture other drugs of the same type (e.g., as in the production of monoclonal antibodies using predefined host cell, cell culture, and purification processes, for which there already exists considerable experience)." The first mAb product gained FDA marketing approval over 25 years ago and since that time there has been a significant investment in understanding the structure/functional relationships of mAb attributes. This knowledge base and the ability to leverage platform manufacturing knowledge from one mAb product to another is encouraging investment in QbD approaches for mAb products.

While the FDA is in fact seeing QbD principles applied in product characterization and process development sections of Investigation New Drug (IND) submissions and Biological License Applications (BLA), most of these submissions do not utilize all QbD concepts described in guidance (e.g., seek approval of a design space). To help facilitate more complete implementation of QbD for biotechnology

products, in July 2008 OBP initiated a QbD pilot program (Food and Drug Administration 2008). The pilot program was designed to define clinically relevant attributes for protein products regulated by OBP and link them to manufacturing processes. The program considered QbD approaches to unit operations in supplements as well as original BLAs. It was also intended to explore the use of expanded change protocols submitted under 21 CFR 314.70(e) and 601.12(e). The pilot program accepted six original BLA applications and four postapproval supplements. The program has been instrumental in helping the FDA develop an understanding of, and expectations for, how QbD will be applied to biotechnology products. In addition, CASSS and ISPE published the A-Mab case study in October 2009 (CASSS and ISPE 2009). The A-Mab case study exemplified development of drug substance and drug product design spaces for a fictitious mAb product and has been a very useful tool for discussing ways QbD concepts can be applied to biotechnology products.

While the use of advanced QbD concepts such as implementation of design space and expanded change protocols are optional approaches, the use of some QbD elements such as development of a quality target product profile (QTPP), identification and/or ranking of critical quality attributes (CQAs) and a control strategy are regulatory expectations (http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ManualofPoliciesProcedures/UCM242665.pdf). Risk assessments are important tools in identifying CQAs as well as designing a control strategy. As outlined in ICH Q8 (R2), implementation of QbD should be initiated with the development of a QTPP, which identifies the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, followed by identification of CQAs. These standard QbD concepts should be incorporated into product development regardless of whether there is intent to utilize more advanced QbD concepts.

A key lesson that came out of the A-Mab case study was that the drug product (DP) manufacturing process can be viewed as an opportunity for the development and use of advanced QbD concepts. DP processes are generally far less complex than a typical biotechnology drug substance process and consist of several operations (e.g., mixing, sterile filtration and filling) that are often highly similar or identical between products. This similarity allows knowledge of the process to be leveraged from one product to another, thus facilitating development of an enhanced control strategy that includes QbD elements. In addition, while poorly controlled DP manufacturing processes can negatively impact product quality, the DP process does not in general involve steps (other than sterility assurance steps perhaps) that increase the purity/quality of the product. Therefore, risk assessments and process characterization and design can focus almost exclusively on maintenance of product quality.

The fact that the drug product process generally does not include steps that are needed to increase product quality (e.g., steps designed to reduce impurities) does not mean that less information is required to support identification of the product's CQAs or that less rigor is applied to the identification of critical process parameters

(CPPs) and development of an appropriate control strategy. Below are some additional lessons learned in regard to application of the enhanced QbD elements to the drug product process from the A-Mab case study and, in particular, the OBP QbD pilot program.

2.3 Identification of Critical Quality Attributes (CQAs)

Development of a QTPP and CQAs assessment should be initiated early in development. A CQA is defined in ICH Q8R2 as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. While some biotechnology products, such as monoclonal antibodies, have a wealth of publically available information that can be used to assess the likelihood a product attribute will impact safety and/or efficacy, the assessment of attribute criticality still presents a significant challenge. The simplest approach for most products may be to initially use available information to rank a product's attributes by their likelihood to impact safety and/or efficacy rather than formally identifying an attribute as critical or not. Early risk assessments (as described in ICH Q9) may draw heavily on published information when little product-specific information is available, as long as the limitations of this information source and its relevance to the molecule under consideration are kept in mind. The process of attribute ranking can help to consolidate and organize available information/data and provide a venue with which to plan and prioritize future studies. In addition to identifying attributes that are likely to be CQAs, the ranking process may illuminate areas of greater uncertainty which can help focus future investigations into attribute criticality. The initial attribute ranking profile can subsequently be updated and refined as additional product and clinical information is acquired. As described in ICH Q6B, the heterogeneity of biotechnology products defines their quality, thus the degree and profile of the heterogeneity should be characterized (Food and Drug Administration 1999). A systematic evaluation of attribute criticality will not only help identify those attributes that need to be controlled within the tightest limits, but can also be used to support other areas of product development such as demonstration of comparability after manufacturing changes (Swann et al. 2008), and support for product stability.

A common deficiency observed in the OBP QbD pilot program was the inclusion of process capability in the assessment of attribute criticality. It is the FDA's expectation that the risk ranking of product CQAs will focus on their likelihood of impacting the product's clinical activity and safety profile (e.g., severity). The CQA identification process should not include process capability or attribute detectability. It is also common that a score for uncertainty be included in the risk assessment so as to factor in the relevance of the information used to assign the impact score or rank. Experience from the OBP, QbD pilot program highlighted the importance of considering the possibility that attributes can interact and/or impact product stability in the severity assessment. For example, the level of free thiols present during

production or at release has been shown to impact both product potency and the formation of aggregates. Free thiols may therefore have a high severity due to a direct impact on product activity but also due to safety since an increase in aggregates could impact product immunogenicity.

Principles outlined in ICH Q5E may be helpful when assessing quality attribute criticality particularly for the severity assessment (Food and Drug Administration 2005). ICH Q5E states that in cases where differences are seen between products in a comparability study the "…existing knowledge [needs to be] sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety and/or efficacy." It is further stated that determination of whether the product variant affects safety and/or efficacy may require additional evidence from nonclinical or clinical studies, and that the extent and nature of those studies will be determined on a case-by-case basis. Application of these concepts to identification of CQAs suggests that assignment of a criticality designation to a quality attribute may need to be supported by nonclinical and/or clinical data in addition to a thorough in vitro assessment. There is more likely to be a need for nonclinical and/or clinical information when a significant product attribute (e.g., glycosylation, charge, etc.) is designated as noncritical and thus may not be tightly monitored and/or controlled during manufacture and in the postapproval lifecycle management process. While it is obviously not possible to clinically test purified product variants, it is sometimes possible to analyze variants from patient samples (Chen et al. 2009) or to test them in a relevant animal or in vitro model. Another approach is to use information from clinical lot extremes to support the quality attribute's criticality designation if the variant is present at high enough levels for an impact to potentially be observed in the exposed population. Such studies can also be used to justify CQA acceptance criteria both for design space development, and lot release and stability testing.

The direct assessment of some attributes (e.g., oxidation at a specific site, glycation of the antigen binding domains) may not be possible due to their low abundance. In some instances, the level of low-abundance attributes can be increased through the use of accelerated or stressed conditions, but often attempts to increase their abundance results in an increase in the level of other attributes and analysis of the impact from a specific variant is confounded. In these cases, even if the severity assessment indicates a low probability of quality impact, the uncertainty score will be high since a direct assessment of product impact was not possible. The end result may be the classification of the product attribute as critical due to high uncertainty even though at the very low levels present in the process, it is unlikely the attribute would impact product quality. Classifying the attribute as critical is important however so that the uncertainty associated with its potential to impact product quality when present at higher levels is not minimized or forgotten during the product's postlicensure lifecycle management process. For some attributes present at very low, well-controlled levels, a control strategy that only includes assessment of the attribute for comparability after relevant manufacturing changes may be justifiable.

2.4 Identification of Critical Process Parameters (CPPs)

ICH Q8 (R2) defines CPPs as those "whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality". A common deficiency noted in the pilot program was to interpret this to mean that CPPs are only those that impact CQAs if they are varied outside their acceptable range. Just as with attribute assessments, quality risk management principles can be useful in assessing the criticality of parameters. In the risk assessment and subsequent studies, it's important that parameter ranges that are explored are wider than those anticipated during routine manufacturing. Ranges may be chosen based on a combination of equipment design capability studies and previous manufacturing history. When assessing possible sources of variability, considerations should include the functionality and limitations of commercial manufacturing equipment as well as the contributions of variability by different component lots, production operators, environmental conditions, and measurement systems in the production setting. Parameters whose variation has a meaningful impact on a CQA should be classified as critical regardless of how well the parameter can be controlled and whether there is CQA impact within the proposed manufacturing range.

The ability to accurately assess the impact a parameter has on a CQA depends on the extent of process/product understanding and the size of the characterized process space (knowledge space). Narrow investigational ranges may not provide sufficient variability for a CQA impact to be detected and may result in incorrectly labeling a CPP a non-CPP. At the same time, there may be a practical and/or experimental limit to the range(s) investigated and the expectation is not to test each parameter to its limit of failure. A balance must be set between these two considerations so that the range studied is broad enough to detect CQA impact by CPPs, but is not so broad as to have no relation to the range intended to be used for manufacture. This issue was identified most often with parameters such as pH, which are generally well controlled in a narrow range, and was not generally an issue for parameters that are operated (and explored) under a wide range. QbD submissions should therefore contain robust justification for the ranges selected for the process characterization studies based on both the intended manufacturing range and the degree of variability represented by the range studied. The greater the uncertainty that the range explored was sufficient to identify whether variation of the parameter impacts a CQA, the greater the residual risk associated with that parameter. This higher residual risk may need to be taken into account in the overall control strategy.

Another common concern raised in the pilot program was whether there were sufficient data to demonstrate that the small scale models used for characterization studies were representative of the full-scale process. There are well-known cases where small scale studies have not predicted events which happened at full scale (antibody reduction case (Trexler-Schmidt et al. 2010). It is essential that applications that propose a design space address how representative small-scale models are and provide data, when appropriate, to support their linkage. If firm-specific

platform knowledge is used to support a design space, comparative information on product characteristics and process parameters should be provided so regulators can assess the appropriateness of the platform information. While verifying small-scale models of DP manufacturing unit operations may not always represent the same challenges as some drug substance unit operations (such as the production bioreactor), the importance of demonstrating representativeness of small scale models should not be minimized and should be supported by a robust statistical evaluation that supports equivalence. If sufficient full-scale data are not available at the time of submission, information should be provided on how the link between the small-scale and commercial-scale processes will be verified after approval of the design space.

Only a subset of the parameters that is monitored for each unit operation is generally included in the characterization studies performed for CPP identification. Formal risk assessments are used to prioritize parameters for inclusion or exclusion from further study and in some cases to determine which type of study (e.g., univariate or multivariate) will be used to assess the potential criticality of a parameter. QbD submissions therefore should include a list of the parameters in each unit operation, a summary of the information/data that was used in the risk assessment to determine which parameters to include in the characterization studies, and a brief summary of and justification for the final risk-assessment decision.

Formal risk assessments can also be used to determine which CQAs to monitor in the process characterization studies for a given unit operation. As stated earlier, the DP process is geared toward maintenance of product quality, and sterility assurance, and the potential to impact some CQAs is minimal. Quality attributes that are commonly impacted by the DP process are aggregates and particulates including product-related visible particles and subvisible particles. The importance of considering the possibility that attributes can interact and/or impact product stability cannot be stressed enough. As the formation of aggregates and particulates may be linked to other quality attributes such as free thiols, deamidation, oxidation, and/or leachates, the CPP identification risk assessment should consider each CQA individually for impact by the process and for linkage to other CQAs and product stability.

The potential for time and temperature dependent operations to impact CQAs should be considered in the overall framework of DP stability, including product stability over the course of the product's shelf life and possibly during shipping. For example, characterization studies to assess the acceptability of hold and processing times may need to be combined with longer term stability studies to adequately assess the impact to product stability. A common issue observed in biological license applications is the absence of data that adequately supports product quality during shipment. These studies need to include a comprehensive set of assays that are capable of sensitively detecting product degradation in addition to data demonstrating validation of the shipping container(s) and shipping conditions.

It is essential that the DP process be thoroughly assessed for its potential to impact product quality negatively, and sources of variability identified and controlled. For example, there are now multiple instances where raw materials have had an

adverse impact on drug product quality. CQAs based on variability of input materials need to be identified, with formulation of excipients of particular concern (e.g., polysorbates (Kerwin 2008)). Leachates from the container/closure system is another area that requires particular attention as evidenced by recent issues associated with glass lamellae in glass vials (Food and Drug Administration 2011), tungsten with prefilled syringes (Liu et al. 2010), and silicon from vial stoppers or prefilled syringe barrels (Thirumangalathu et al. 2009). Filters have also presented leachate issues; in one case due to incomplete washing of sterile filters by their manufacturer. An additional example is the manufacture of depth filters with the incorrect filter components which resulted in the introduction of unacceptable by-products into the finished drug product. A well-documented example of the drug product process negatively impacting product quality is the shedding of nanoparticles from a filling pump's solution-contact surfaces which may nucleate protein aggregation and/or particulate formation (Carpenter et al. 2009; Tyagi et al. 2009).

2.5 Design Space

The implementation of QbD may allow for the establishment of a manufacturing design space(s) which is defined by ICH Q8 (R2) as "…the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality" (Food and Drug Administration 2009). If approved, changes within a design space can be managed by the firm's quality management system and may provide a measure of regulatory flexibility. It should be noted that the definition of design space in ICH Q8 (R2) does not include the word "critical." A reoccurring issue in the pilot program was the definition of design space to only include parameters that had been defined as being critical. Even with robust CQA and CPP identification programs there is some level of residual risk and uncertainty associated with risk assessments and process characterization studies. This is particularly true in the absence of a large dataset that verifies the design space at full scale. The establishment of a design space should therefore focus on selecting relevant variables and ranges within which consistent quality is assured and may include parameters that have not been defined as critical using the sponsor's prespecified criteria.

The A-Mab case study (CASSS and ISPE 2009) had some interesting examples of potential drug product design spaces which focused on distinct areas of the drug product process such as formulation robustness, compounding, sterile filtration and filling. Other areas proposed for inclusion in a design space have been definition of excipient critical material attributes, and development of a product-specific control system lifecycle management protocol for periodic control system re-evaluation to allow continuous improvement in the process and testing of licensed products.

Experience from the pilot program also revealed that a firm's long-term strategy for implementing enhanced regulatory concepts could impact developmental studies (e.g., quality attribute characterization, identification of relevant process

parameters). For example, if the final control strategy will not include lot release testing for a particular CQA, it may be more important to include that CQA in process characterization studies to define the link between input material attributes, process parameters and product quality. In addition, the risk assessment of process parameters depends upon a firm's current and future manufacturing capability and plans for postmarketing management of process parameters. Therefore, expectations of the ultimate control strategy and postmarketing management plan can impact the design of QbD development/characterization studies and should be considered early in development to ensure sufficient information is available when the design space and control strategy are proposed.

2.6 Change Management

Change management is defined in ICH Q10 as a systematic approach to proposing, evaluating, approving, implementing, and reviewing changes. Successful change management should facilitate continual improvement and enabling change is a critical component of QbD. Postapproval change management is of great interest and concern for regulators since ICH Q8-11 allows for the possibility of more flexible regulatory approaches. An issue that concerns regulators is that the granting of regulatory flexibility can result in a different role for the regulator in the postapproval change management process. The Pharmaceutical Quality System (PQS) as described in ICH Q10 is an important component to an enhanced development and manufacturing approach. Therefore, if a firm proposes to use advanced QbD concepts to gain regulatory flexibility, it may be beneficial to include information in the application on the process that will be followed when the proposed changes are implemented and the criteria that will be used in the change management decision-making process.

For firms that use advanced QbD approaches and gain approval of one or more design spaces, movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process (Food and Drug Administration 2009). To facilitate the approval of a design space for a complex biotechnology product, an applicant may want to provide information on how movement within the design space will be managed post approval, particularly in the case of large or complex design spaces (Food and Drug Administration 2012). In these cases, the level of certainty in regard to design space performance may be less robust in some areas of the design space. Additional product and process monitoring may therefore be warranted if process parameters are moved to fall within areas of greater uncertainty. Movement within the design space would still be managed primarily by the firm's PQS but a commitment to perform more extended studies with specified acceptance criteria could provide regulatory agencies with a detailed understanding of how changes will be managed and a higher level of assurance that product quality will not be impacted.

Another issue that has arisen is how changes to parameters that are not included in the design space will be managed. A concept that has been proposed is to establish classifications for parameters that will dictate how changes will be managed postapproval. A quality risk assessment utilizing prior knowledge, development studies and manufacturing information can be used to categorize process parameters (e.g., high, moderate, or low-risk) based on their relative potential to impact product quality. The categorization of parameters from the quality risk assessment can be used to communicate with regulators regarding a lifecycle management approach to assure continual improvement throughout the product lifecycle. For example, high-risk parameters might be defined as those CPPs and non-CPPs included in the design space. By definition, changes to design space parameters that are within the established design space would not need prior approval from the regulator. However, changes that would extend the range beyond that established by the design space would require submission of a postapproval supplement per 21 CFR 601.12.

Definitions for moderate and low-risk parameters and their postapproval management strategy could also be proposed. For example, changes to moderate-risk parameters not included in the design space that do not exceed the knowledge space provided in the application could be managed by the firm's PQS. Changes that exceed the knowledge space would be supported by studies, tests and acceptance criteria which are outlined in the submission. If no impact to a relevant CQA is observed, the change could be reported in the annual report. If a meaningful CQA impact is observed however, the change would need to be supported by an appropriately categorized postapproval supplement. Changes to low-risk parameters would be managed by the firm's PQS. For any manufacturing change, if the evaluation of the risk is increased by new knowledge, an appropriately categorized postapproval supplement should be submitted. Where regulatory flexibility is being requested, providing information that clearly outlines how changes will be managed, including how they will be reported and additional studies that will be performed to support specific types of changes, makes the postapproval change process more transparent to regulators and will help regulators assess the acceptability of the proposed post-management lifecycle plan.

2.7 Communicating Complex QbD Concepts and Control Strategies

An issue that came up repeatedly in the pilot program was the magnitude of information that is generated in the QbD development process and the challenges associated with communicating QbD knowledge and the proposed control strategy to the FDA. While to date the Agency's experience with review of QbD BLAs is limited, several factors identified in the pilot program that may aid in our review of enhanced QbD concepts have been identified and are outlined as follows.

- The BLA should include a detailed explanation of any risk-assessment tool used including a justification for any cut-off ranges used.
- The CQA assessment section should include information on all quality attributes assessed and consist of a narrative that explains and summarizes the assessment process for each attribute. The narrative should include summary data from characterization studies that were performed to assess attribute impact and links to literature references cited.
- To support the CPP identification process, information on the parameters and attributes that were assessed for each unit operation and a justification for inclusion or exclusion of the parameter or attribute from the characterization studies for each unit operation should be provided. It may be acceptable to provide summary narratives of the studies and study results for each unit operation if full study reports are provided for a sub-set of select unit operations and all full study reports are available on inspection. Detailed information on the statistical analysis performed for the characterization studies should be provided. This would include such information as the analysis performed to demonstrate the representativeness of small-scale models used, and an explanation of and justification for the chosen statistical approach used for the characterization/process validation studies. In addition to the inclusion of full reports and summary narratives, it will be helpful to include tables or figures that summarize findings and the proposed control strategy. For example, a figure that breaks the control strategy down into specific control elements (e.g., raw material control, in process testing, in process parameter monitoring, release and stability specifications etc.) and summaries the upstream and downstream control strategy for each unit operation for each quality attribute (Fig. 2.1) may be helpful. At a higher level, summaries of the control elements used for each quality attribute in the drug substance or drug product processes could be provided. ICH Q11 includes an example of a possible control strategy summary table (example 5a) for biotechnology products.

2.8 Conclusion

Preliminary findings from the OBP QbD pilot program indicated that application of the enhanced QbD concepts to both the drug substance and drug product could result in a significant increase in product and process understanding. Many of the participating firms proposed to use their increased knowledge to optimize their manufacturing process, enhance quality, create a more focused control strategy and incorporate more flexibility/adaptability into the process. It is hoped that biotechnology manufactures will continue to pursue these goals for monoclonal antibodies and monoclonal antibody related proteins and eventually expand their knowledge and experience to other therapeutic protein classes.

Control Element	Description	Step 1	Step 2	Step 3	Step 4	Etc.
1	Direct in process monitoring of QA	None				
2	Control of process parameter related to QA		■	■	■	
3	Control of raw material related to QA	■			■	
4	Drug product release testing	QA Release acceptance criteria				
5	Drug product stability testing	QA Stability acceptance criteria				
6	Etc.					

QA: Quality Attribute

■ **Element is monitored or controlled**

□ **Element is not monitored or controlled**

Fig. 2.1 Abbreviated summary of final control elements for the drug product process for critical quality attribute #1. Information in the figure is only intended to be used as an example of how a control strategy could be summarized and presented in a submission and is not intended to indicate acceptability of the indicated control elements or strategy

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Chapter 3
Definitions and Scope of Key Elements of QbD

Ron Taticek and Jun Liu

3.1 Introduction

The concept of Quality by Design (QbD) has provided both opportunities and challenges for the biopharmaceutical industry. A successful QbD approach can lead to a better understanding of products and more robust manufacturing processes, and offers the potential for timely and flexible regulatory approval (Stevenson and Cochrane 2011a, b). QbD uses a science and risk-based approach that emphasizes the importance of developing scientific knowledge and thorough understanding of both the product and the process. This concept has been successfully applied in many industries and is only recently being introduced in the pharmaceutical industry, first to small molecules and now to biologics (Elliott et al. 2013). The US Food and Drug Administration (FDA) and other health authorities are also engaged in applying QbD to pharmaceutical development and manufacturing. In the past several years, significant progress has been made to establish and implement the concepts of QbD to pharmaceutical development and manufacturing. A number of initiatives within the FDA have described their expectations and objectives and are encouraging pharmaceutical industry to utilize QbD concepts in their product development and manufacturing. Several International Conferences on Harmonization (ICH) guidance documents (ICH Q8, ICH Q9, ICH Q10, and ICH Q11), and the FDA Process Analytical Technology guidance have been published and laid the foundation for pharmaceutical companies to implement QbD in their operations and product

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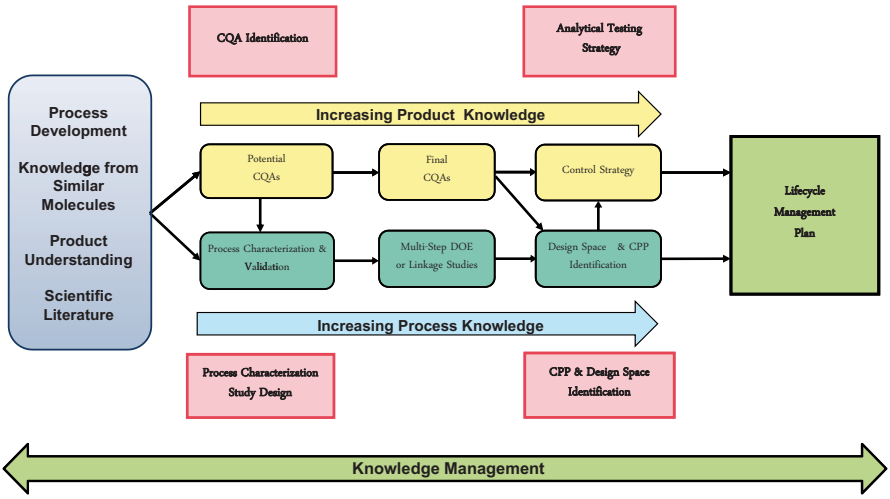

Fig. 3.1 Quality by design (QbD) roadmap

development (FDA 2004; ICH 2009, 2005, 2008, 2012). In addition, a recently published mock case study on an antibody API and Drug Product by the CMC Biotech Working group, with FDA and other health authority feedback, has provided further useful and practical information about how to apply the key elements of QbD to process development (CMC Working Group 2009).

QbD requires a thorough understanding of the product and its manufacturing process. Additional time and resources are required to establish a company's approach and framework to applying QbD for its first few products. But once established, such a program should streamline development by applying consistent approaches and tools, and leveraging data across the same class of products more easily. A successful QbD approach should provide a higher level of assurance of product quality and improved efficiency for industry and regulatory approval.

The key elements of the QbD approach include the quality target product profile (QTPP), critical quality attributes (CQAs), risk assessments, design space, critical material attributes (CMAs), critical process parameters (CPPs), control strategy and product life cycle management which include continuous improvement. Figure 3.1 illustrates the QbD roadmap and how all of the elements of QbD are linked to each other.

Despite much progress having been made in past several years, interpretation of QbD concepts and the scope of its key elements are still an ongoing process and will require further clarification and alignment within industry, particularly for more complex biopharmaceutical products and with regulators. In this chapter, we focus on the basic definition and scope of the key elements of QbD for biopharmaceutical drug development.

3.2 Quality Target Product Profile

3.2.1 Definition

The QbD approach begins with the establishment of quality target product profile (QTPP). The QTPP is a prospective summary of the quality characteristics of a Drug Product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the product (ICH 2009). The establishment of a good understanding of the target product profile (TPP) is an important step in determining QTPP. The TPP provides a statement of the overall intent of the drug development program and gives information about the drug at a particular time in its development lifecycle. Usually, it includes the specific studies (both planned and completed) that will supply the evidence for each conclusion that becomes part of the label (Lionberger et al. 2008). The QTPP is derived from an understanding of the mode of action of the product, patient profile, clinical indication, desired safety profile, and where appropriate, includes quality characteristics related to:

- Route of administration and intended use (in a clinical setting or at home)
- Dosage form, delivery system
- Dosage strength
- Container closure system
- Therapeutic moiety release or delivery and attributes affecting pharmacokinetic characteristics (e.g., dissolution, aerodynamic performance) appropriate to the Drug Product dosage form being developed
- Drug Product quality criteria (e.g., sterility, purity, stability, and drug release) appropriate for the intended marketed product

3.2.2 Interpretation/Consideration for Biopharmaceuticals

The QTPP defines a target for product quality requirements. It forms the basis for the development of other key QbD elements, such as CQAs and control strategy, and drives formulation and process development decisions. The QTPP describes the product design criteria that will ensure the quality, safety, and efficacy of a specific product for patients. A series of critical considerations should be made for the QTPP of a biopharmaceutical product. This includes important information from the TPP or equivalent source that describes the use, safety and efficacy of the product. In addition, it also includes the understanding of scientific knowledge, health authority requirements and in case of the Drug Product intrinsic Active Product Ingredient (API) properties. The flow of inputs to and output of QTPP is shown in Fig. 3.2:

Establishment of the QTPP is a critical step for a QbD approach. The QTPP includes not only the relevant information from the product specification but also patient relevant product performance. For example, if the viscosity of a high concentration product is critical to the reconstitution or delivery of the Drug Product,

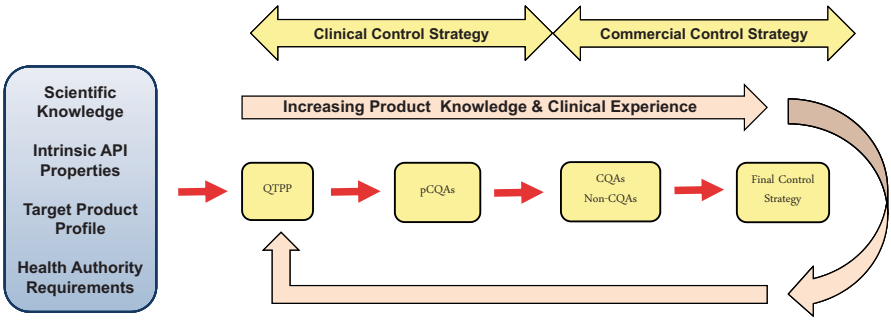

Fig. 3.2 Inputs to and outputs from the quality target product profile (QTPP)

then the QTPP should include viscosity information. The QTPP is a living document that can change as more information become available. When changes are made to the TPP or other key elements, a reevaluation must be performed to assess impact to the QTPP. The QTPP may be updated to reflect new knowledge about the product and changes in the clinical development program.

An example of a QTPP for a biopharmaceutical product is provided below. This example is taken from the A–MAb published mock case study on an antibody API and Drug Product (CMC Working Group 2009). Detailed information in QTPP will vary from product to product based on the differences between indications, intended use, and the characteristics of the product itself. For example, in the A–MAb mock case study, Drug Product quality criteria, such as aggregate, fucose content, galactosylation, and host cell protein were listed in detail (Table 3.1).

3.3 Critical Quality Attributes

3.3.1 Definition

ICH Q8 defines a CQA as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality (ICH 2009). Product quality is typically interpreted as product safety and efficacy. A CQA is a product attribute and not an analytical test and is generally associated with the Drug Substance, excipients, intermediate and Drug Product. It should be noted that the intended safety, efficacy, stability, and performance of the product are generally not considered as CQAs. Safety and efficacy clearly fall under the domain of the TPP. CQAs can be further categorized as an obligatory CQA. An obligatory CQA is an attribute required by a health authority to be either monitored or controlled as part of the product's control strategy.

CQAs are managed throughout the product lifecycle (see Fig. 3.2). During product development, potential CQAs (pCQAs) are identified based on an itera-

Table 3.1 Quality target product profile for A-MAb1 (CMC working group, 2009)

Product attribute	Target
Dosage form	Liquid, single use
Protein content per vial	500 mg
Dose	10 mg/kg
Concentration	25 mg/mL
Mode of administration	IV, diluted with isotonic saline or dextrose
Viscosity	Acceptable for manufacturing, storage, and delivery without the use of special devices (for example, less than 10 cP at room temperature)
Container	20R type 1 borosilicate glass vials, fluro-resin laminated stopper
Shelf life	≥2 years at 2–8°C
Compatibility with manufacturing processes	Minimum 14 days at 25 °C and subsequent 2 years at 2–8 °C, soluble at higher concentrations during UF/DF
Biocompatibility	Acceptable toleration on infusion
Degradants and impurities	Below safety threshold, or qualified
Pharmacopoeial compliance	Meets pharmacopoeial requirements for parenteral dosage forms, colorless to slightly yellow, practically free of visible particles and meets USP criteria for sub-visible particles
Aggregate	0–5 %
Fucose content	2–13 %
Galactosylation (%G1 + %G2)	10–40 %
Host cell protein	0–100 ng/mg

tive application of risk-based tools. The list of pCQAs and their risk scores will be further modified as product knowledge increases through the various stages of product development. When changes are made to the QTPP, an evaluation must be performed to assess the impact on the pCQAs. At the time of filing for approval, the potential CQAs become CQAs and should reflect the current knowledge and understanding of the impact on patient safety and product efficacy. The CQAs must be described, justified, and documented. As more knowledge is gained about quality attributes post-licensure, the criticality of those attributes may change (increase or decrease) and the CQAs should be updated. When there is a change in a CQA, the impact on the design space and control strategy should be assessed and updated if necessary.

3.3.2 Interpretation/Considerations for Biopharmaceuticals

The approach to identifying CQAs should be dependent on the category of the quality attribute (QA) being assessed. Quality attributes for a biopharmaceutical product may be divided into the different assessment categories (see Table 3.2). Dividing

Table 3.2 Categories of antibody product quality attributes

Category of Attribute	Assessment	Rationale for approach
Product variants Charge, size, thiol/disulfide, glycans, oxidation, sequence	Risk assessment	Impacts on patient safety and product efficacy are specific to variant in question, the product's mechanism of action, route of administration, clinical experience, etc.
Process-related impurities Host cell protein, DNA, leached protein A	Risk assessment	With appropriate justification, data from similar products can be used to assess safety in the absence of product-specific clinical experience
Composition and strength pH, buffers, protein concentration, appearance	not required, obligate CQA	Potentially high impact on patient safety and product efficacy
Adventitious agents Potential viruses, bioburden, mycoplasma, endotoxin, microbial contamination	not required, obligate CQA	Potentially high impact on patient safety
Raw materials and leachables Cell culture and recovery components (nutrients, trace elements, salts, buffers, etc.) and leachables	Safety and toxicity/process clearance	Extensive data are often available from safety and toxicity assessments

quality attributes into categories enables distinction of QAs that are product or process specific and require a risk assessment, from those that are common across products and processes and can therefore be assessed generically, or those that must be controlled based on regulatory agency requirements or expectations.

In general, the risk assessment tools developed for evaluation of quality attributes are science and risk based, and designed to allow consistent CQA identification that is independent of process capability and applicable throughout the product lifecycle. This approach enables early identification of high-risk product variants and impurities that may be need to be studied further to lower the uncertainty and modify the impact based on the new information obtained. The risk assessment tool should be applied at defined stages of product development to incorporate new information and help guide development studies to better understand product quality attributes. Consistency among products and users determining QA criticality is assured through training, subject matter expert facilitation of assessments, standardized documentation, team and expert review, and management approval of CQA identification.

3.3.2.1 Product Variants and Process-related Impurities

Product variants and certain process-related impurities should be evaluated carefully using a risk assessment approach that assesses the impact of each QA on safety and

efficacy. Product variants are assessed on a product-specific basis to account for the unique modifications, mechanism of action, indication, route of administration, preclinical and clinical experience, in vitro studies, and other factors that influence risk assessment. General product and platform knowledge from similar molecules can also help this process. Process-related impurities are often common among similar products and process. Therefore, prior knowledge is often applied to assess risk for these attributes in products manufactured using similar processes. Criticality of each QA is assessed independent of actual levels present or the ability of the process to control the QA. Process capability should be considered later, during development of the control strategy and any post-approval lifecycle management plan. An exception to this is the assessment done with raw materials and leachables (see 4.3.2.3).

3.3.2.2 Risk Assessment Approach

Many biopharmaceutical companies, with input from the regulators, have adopted the use of a risk ranking and filtering (RRF) approach to assess criticality of QAs (Martin-Moe et al. 2011). The risk-ranking approach typically incorporates two factors: impact and the uncertainty of that impact. Impact is the potential affect a variant or impurity may have on patient safety and product efficacy (together these constitute "harm"). Uncertainty is related to the degree of confidence that the impact is correctly assigned for the QA of interest. Also, the impact and uncertainty rankings may have different scales to reflect the relative importance of the two factors, with impact outweighing uncertainty. Numerical values are assigned to impact and uncertainty and multiplied to generate a relative risk score, which is used in ranking. Filters, in the form of cut-offs for risk scores, are then used to identify attributes that are high risk (classified as CQAs) and low risk (classified as non-CQAs).

Application of risk assessments to identify the criticality of QAs should not take the place of the need for review by subject matter experts and technical management before final CQA classifications are endorsed. Business practices should ensure review of QA classification by technical experts and management. In the event that a QA is categorized incorrectly with a risk assessment tool, this practice would enable reclassification, with appropriate justification. Moreover, the justification for the assigned classifications will also be presented to health authority reviewers as a part of the summary of the outcomes of the risk assessment. As a result of this internal and external oversight, there is assurance that no high-risk QAs are inadvertently classified as low risk due to strict application of a risk assessment.

The criticalities of composition/strength and adventitious agents are assessed in a different approach. Regulatory requirements specify that certain attributes in the composition/strength and adventitious agent categories must always be controlled due to their potentially significant impact on safety and efficacy of products. Therefore, these attributes have been classified as obligatory CQAs and do not require using a risk-ranking tool for further evaluation of criticality. For these attributes, appropriate process and analytical controls should be implemented. Examples of such attributes are summarized in Table 3.3.

Table 3.3 Examples of obligatory critical quality attributes

Category of attribute	Examples
Composition and strength	Protein concentration, pH, excipient and buffer concentrations, osmolality, extractable fill volume
Adventitious agents	Virus, bioburden, mycoplasma, bacterial endotoxin, sterility

3.3.2.3 Raw Materials and Leachables

The assessment of the criticality of raw materials is a challenging task. One approach is to consider the toxicity of the raw material assuming no clearance in the manufacturing process as a worse case. This approach evaluates and expresses theoretical risk to patients related to the direct impact of the presence of these materials on the Drug Product. In practice, many of the raw materials used in the manufacturing process have been studied extensively in animal or clinical studies. For example, extensive data are available for culture additives such as insulin (Smith et al. 1980), and process chemicals such as phosphate and acetate (Haut et al. 1980).

Raw materials are evaluated for criticality by assessing the potential toxicity of the compound itself. This approach evaluates the theoretical risk to patients related to the direct impact of the presence of these materials on the Drug Product. Those raw materials that pose a potential toxicity risk are considered potential CQAs (pCQAs). Those pCQAs are then assessed for clearance and the potential toxicity is reassessed based on the levels determined in those studies. Not all compounds identified as pCQAs can be measured directly. In those cases, clearance was supported by removal of detectable compounds with similar physicochemical properties. For example, clearance of a subset of ionic salts may be used to demonstrate clearance of all ionic salts (e.g., magnesium, chlorides, and sodium). Raw materials that are still potentially a toxicity risk after considering that clearance through the process becomes CQAs, and a control strategy will need to be developed for them. The impurity of a raw material that affects a CQA is also known as a CMA. A CMA is defined as a physical, chemical, biological, or microbiological property or the characteristic of a raw material whose variability has an impact on CQAs. Therefore, they need to be monitored or controlled to ensure the desired product quality. CMAs may include purity of raw materials, physical properties of excipient, and chemical and/or microbiological purity of API or excipient. Acceptable ranges for CMAs must be specified to ensure that the CQAs of the final product will be within the acceptable ranges.

Identification of specific leachables as CQAs is much dependent on whether a specific compound or its impact can be detected. Leachables are compounds that leach into the drug or biological product from elastomeric or plastic components or coatings of the primary container and closure system. If development and stability data show evidence that leachables are consistently below levels that are demonstrated to be acceptable and safe, no leachables will be classified as CQAs. Typically, no leachables are classified as CQAs. However, if leachables are shown to have

significant impacts on CQAs of the final product (e.g., glue, tungsten, or silicone oil from a prefilled syringe) by a stability study, they can be classified as CQAs.

Examples of identifying critical quality attributes have been presented recently (CMC Working Group 2009). These examples have demonstrated the importance of using prior product knowledge, laboratory data, nonclinical data, and clinical data for the criticality assessment.

3.4 Critical Process Parameters (CPPs) and Design Space

3.4.1 Definition

Once pCQAs are identified, the next important step in the QbD process is to define CPPs and design space. This work is usually done in parallel with the identification and characterization of CQAs. A CPP is defined as a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored and controlled to ensure the process produces the desired quality (ICH, 2006a).

The concept of design space has been defined in ICH Q8 (R2) as "The multidimensional combination and interaction of input variables (e.g., material attributes and process parameters) that have been demonstrated to provide assurance of quality within an acceptable range." A design space can be applied to a single unit operation, multiple unit operations, or the entire process. The establishment of a design space for a manufacturing process is based on a good understanding of how the process impacts the CQAs. The limits of the design space should correspond to the acceptable ranges for the CQAs. In general, a change within the established design space for a manufacturing process is not considered as a significant change, while moving beyond the established design space is considered to be a significant change and would require pre-approval by the health authorities. In addition, more extensive preclinical or clinical data may also be required to support such a change.

3.4.2 Interpretation/Considerations for Biopharmaceuticals

The concepts of CPPs and design space are used in the manufacturing process development studies to define the acceptable ranges for the manufacturing process parameters and formulation conditions for biopharmaceutical industry (Jameel and Khan 2009; Martin-Moe et al. 2011). Key steps for establishing CPPs and design space include performing risk assessments to identify which process parameters should be studied; designing those studies using design of experiments (DOE) and using qualified scale-down models; and executing the studies and analyzing the results to determine the importance of the process parameters, as well as define the design space.

Prior knowledge from early manufacturing development experience with the molecule in question and knowledge from similar molecules is used to assess initial criticality. The initial criticality of the process parameters is usually assessed based on likelihood that a parameter can impact a pCQA on its own or in combination with other parameters. For a product with limited prior knowledge, small-scale pilot studies are often conducted before doing more thorough DOE studies.

Process characterization and validation studies are the final steps for establishing CPPs and design space. Process characterization is a systematic investigation to understand the relationship between key operating parameters and critical quality attributes. Objectives of process characterization include identification of key operational and performance parameters, establishment of acceptable range for key parameters, and demonstrating process robustness (Li et al. 2006). Process validation is establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-defined specifications and quality attributes. Process validation often includes results of full-scale runs under target manufacturing conditions, and the collection of data on an appropriate number of production batches. Cumulative impact of multiple unit operations on pCQAs may be assessed by doing multi-step DOE studies or by assessing the impact on a given pCQA by running the various process steps that impact that pCQA at a worse case (process step linkage study). DOE is used during process characterization studies to establish CPPs and design space for the manufacturing process. DOE is a systematic and rigorous approach to determine the multidimensional relationship among input variables and their influences on outputs of a process. The input variable can be a process parameter (e.g., process time and/or temperature) and formulation attributes (e.g., concentration and excipients), while outputs are the product quality and impurity levels which usually are defined by CQAs.

For a DOE study, it should be emphasized that a combination of acceptable ranges based on univariate experiments can provide supporting data but may not be sufficient to establish a design space. The acceptable ranges may need to be based on multivariate experiments that take into account the main effects, as well as interactions of the process parameters and formulation attributes. For many of biopharmaceutical products, site- and scale-independent characterization studies that support identification of CPPs and define the design space are conducted using scale-down models of the manufacturing-scale unit operations. Site-specific studies include characterization and validation studies conducted at manufacturing scale in the intended commercial facility that demonstrate manufacturing process consistency with regard to meeting pre-specified process parameter ranges, process performance indicators, and CQAs. When considering a scale-down model, additional experimental work is typically required to demonstrate that the data generated using the small-scale model is adequately representative of the commercial manufacturing scale.

Recently, several case study examples for both Drug Substance and Drug Product manufacturing process have been published and provided useful information for define CPP and process design space during QbD process. Harms et al. have

presented a case study, involving *P. pastoris* fermentation process to demonstrate a stepwise approach for defining process design space (Harms et al. 2008). Similar work has also been conducted to define process design space for Drug Product manufacturing process. Martin Moe et al. have recently published a paper that describes the use of QbD concept for Drug Product process development for an antibody.

3.5 Control Strategy and Control System

3.5.1 Definition

The control strategy is a key element of the QbD process. The control strategy refers to a set of planned controls, derived from current product and process understanding that ensures process performance and product quality. One of the important parts of control strategy is to establish a control system. A control system is a set of defined controls and their established acceptance criteria (or limit) based on product understanding that assures product quality. The control strategy comprises several elements including

- Raw material controls
- Process control via procedural and process parameter control
- In-process, lot release, and stability testing
- Testing to demonstrate comparability
- Testing done as part of process monitoring.

Raw material controls are controls relating to raw materials, excipients, buffer components, etc. used in the formulation and manufacturing processes, including supplier quality management, raw material qualification, and raw material specifications. Procedural controls are a comprehensive set of facility, equipment, and quality system controls that result in robust and reproducible operations and product quality. Process parameter controls are linked to CPPs that must be controlled within the limits of the design space to ensure product quality. In-process testing is conducted using analytical test methods or functionality test to ensure that selected manufacturing operations are performing satisfactorily to achieve the intended product quality. Lot release testing is related to the testing at final lot release on a set of quality attributes to confirm quality of the Drug Substance or Drug Product. Some of the attributes will also be tested as part of the stability testing. Characterization and comparability testing are often used to test certain attributes beyond lot release testing for the purpose of intermittent process monitoring or demonstration of comparability when a change is being implemented (e.g., licensing a new production facility or modified manufacturing process). Process monitoring is the testing or evaluation of selected attributes or parameters to trend product quality or process performance within the design space and/or enhance confidence in an attribute's normal distribution. The frequency of monitoring is periodically reviewed and adjusted based on trends. The process monitoring program may include limits for evaluating data trends.

3.5.2 *Interpretation/Considerations for Biopharmaceuticals*

The focus of the control strategy for a biopharmaceutical is typically the testing strategy for each attribute. It should be determined using a risk-based assessment related to the understanding of the potential impact of the quality attribute on the safety and efficacy of the product and the ability to control the level of the attribute through the manufacturing process and during storage (see Fig. 3.3). The testing strategy for each attribute is typically developed using a risk assessment tool and is also often confirmed using a separate risk assessment to determine the robustness of the resulting testing strategy.

One approach for determining the testing strategy for each identified attribute is to use a risk assessment tool that incorporates that quality attribute criticality and the risk that an attribute will exceed the acceptable range for the CQA when the process is operated within its design space or during with Drug Substance and Drug Product storage in the recommended conditions. The assessment would be performed for each quality attribute during Drug Substance manufacturing, Drug Product manufacturing, Drug Substance stability, and Drug Product stability (see Fig. 3.4).

From this evaluation, one of three possible outcomes is identified for each quality attribute

1. Control system testing is required (in-process, lot release, and/or stability testing)
2. Testing is required as part of process monitoring or to support comparability
3. No testing is required.

Once a testing strategy has been defined for each attribute, an overall robustness assessment should be performed using a risk assessment to determine the risk to the overall program that a more critical quality attribute is not controlled adequately by the proposed control strategy. In this evaluation, the type of measurement (i.e., direct versus indirect measurement), as well as its sensitivity and robustness, are considered in the overall risk assessment for each attribute.

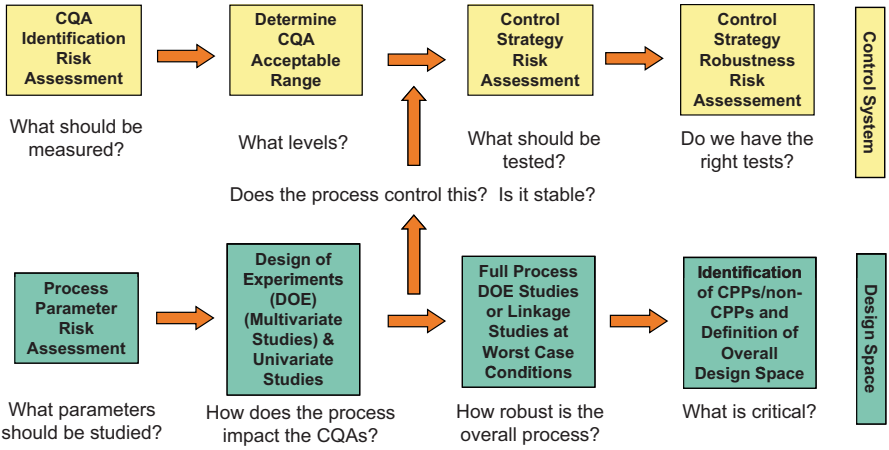

Fig. 3.3 Interrelationship between control strategy and design space

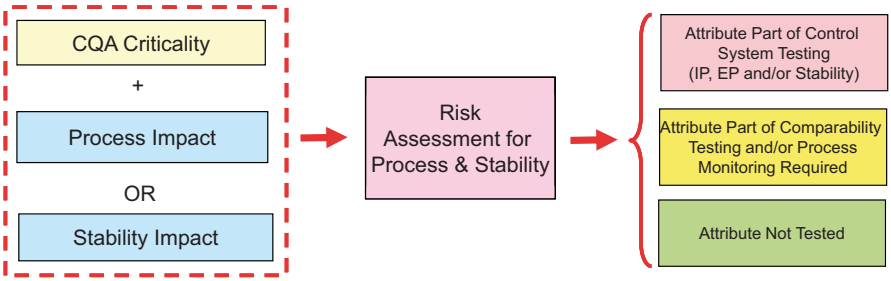

Fig. 3.4 Establishing the control strategy

In some cases, starting with a "minimum" control system and then adding additional tests based on the outcome of the risk assessments may be required by the health authorities as those tests are considered useful in monitoring product consistency and for further mitigating risk to patients due to unanticipated sources of variation.

3.5.2.1 Control System Testing

Control system testing includes in-process testing (e.g., bioburden, endotoxin), product release testing (e.g., product attributes, adventitious agents, impurities) and stability testing (e.g., stability indicating product attributes).

3.5.2.2 Process Monitoring

Process monitoring programs should be designed to provide ongoing assurance and verification that product quality is appropriately controlled during routine commercial manufacturing. The process-monitoring program is designed to meet the following criteria:

- Provide assurance that the process is operating in a validated state
- Provide knowledge to enhance process understanding
- Identify adverse trends and opportunities for process improvements

Continuous process monitoring is key element in a lifecycle approach for process validation. A process monitoring system collects data on CPPs, key performance indicators (KPIs), and CQAs. The attributes monitored have been selected based on knowledge gained during development and execution of the process validation lots.

3.5.2.3 Comparability Assessments

Comparability assessments of both product and, if appropriate, process, are performed to ensure that there is no adverse impact on the quality, safety, or efficacy of the prod-

uct as a result of a change made to the manufacturing process or licensing a new manufacturing site. The comparability assessment considers product quality (physicochemical characterization of the product), stability (degradation), and process performance (key performance indicators and removal of process-related impurities).

3.6 Lifecycle and Knowledge Management

3.6.1 Definitions

A post-approval lifecycle management (PALM) plan is a formal document that explains how a product is managed within the QbD framework post regulatory licensure. Health authorities expect that a product developed using QbD has a formalized lifecycle management plan. The health authorities also expect that there is a formal knowledge management program that archives and updates documents associated with product and process knowledge, as well as the documents summarizing the outputs of the QbD strategy.

3.6.2 Interpretation/Considerations for Biopharmaceuticals

The elements of the PALM for a biologic product and the interrelationships between them are illustrated in Fig. 3.5. The LMP for a biologic should include a description of how process and product attributes will be monitored to ensure that both remain in a state of control post regulatory licensure. The frequency that a product attribute is measured is attribute-specific and dependent on the risk associated with that attribute. Some attributes may be monitored in every production batch (more critical) or intermittently on some subset of batches (e.g., every fifth batch). Additionally, product and process monitoring results, including adverse trends, serve as the scientific basis for continuous verification and improvement of the

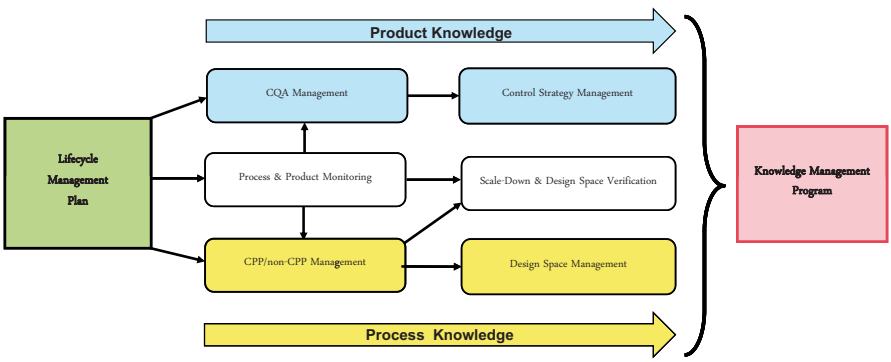

Fig. 3.5 Components of the lifecycle management plan (PALM)

initial control system and manufacturing process. The lifecycle management plan also explains how changes to the critical process parameter (CPP) operating targets are managed within and outside of design space. For a change to a CPP target within the design space, the level of pre- and post-implementation testing is determined by the level of risk and the potential for the change to impact a CQA. The risk is assessed based on considerations such as CPP criticality, the product quality attributes affected, and the classification of those product quality attributes. There is pre-implementation and post-implementation/verification testing. The post-change assessment testing is meant to verify that the change had the desired result and that the design space continues to be valid for the manufacturing process. The PALM also explains how changes to a non-CPP (operating target and/or ranges) are managed as well. Since non-CPPs do not impact CQAs, the assessment of these changes typically focus on key performance indicators. If a non-CPP is associated with a step that has an influence on product quality, changes to the acceptable range for non-CPPs for these steps usually require additional justification, based on scientific literature, historical data, or new studies performed similar to the ones that established the acceptability of the original range.

The strategy for updating CQAs, the overall control strategy, and CPPs as further process or product knowledge gained post approval is also described in the LMP.

It is also an expectation of the health authorities that the elements of the PALM are integrated in the company's pharmaceutical quality system (PQS) and that changes to CQAs, CPPs, etc. are documented and justified appropriately. In the majority of the cases, the same risk assessment tools are used to support those changes.

The lifecycle management plan may also be included in the product registration documentation and, if so, then the PALM becomes a regulatory agreement between the health authority and the company. In that circumstance, the company may be able to get agreement on some level of regulatory flexibility following the plan. Any change that does not meet the predefined requirements specified in the PALM would be reported to the health authorities following the standard regulatory reporting approach.

The PALM is a key facilitator of knowledge management as it requires the outputs of the QbD strategy to be re-evaluated as new process and product knowledge is gained and requires that information and any changes to CQAs, CPPs, design space, and control strategy to be documented and justified.

3.7 Summary

QbD has provided opportunities for biopharmaceutical companies to develop better understanding of their products and their associated manufacturing processes. This approach can provide a higher level of assurance for product quality, and offer the potential of improved efficiency for industry and regulatory approval. Over the past several years, significant progress has been made to establish the industry approach and framework for applying QbD concepts to biopharmaceutical product development. The key tools and strategies are developed and implemented to assess the key elements of QbD, including CQAs, CPPs, and the control strategy. Some of

these key QbD elements have been successfully included in the recent regulatory filling for complex biopharmaceutical products and likely will be a requirement for biopharmaceuticals in future. Close attention will be paid both by industry and regulators on how this novel approach will help to realize these potential benefits.

3.8 Acknowledgments

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Chapter 4
An Overview of Quality by Design for Drug Product

Sheryl Martin-Moe and Carol Nast

4.1 Introduction

Quality by Design (QbD) implementation and regulatory approval of design space has been successful for small molecules but remains a work in progress for biologics. A summary of key QbD guidance's and programs is shown in Fig. 4.1 and includes: (1) major guidelines from regulators that contribute to defining QbD for pharmaceuticals, (2) responses from the industry that interpret these guidelines and propose QbD program definition (case studies, mock submissions), and (3) pilot programs in place between regulators and industry. The QbD pilot program for small molecules began in 2005, followed by a similar pilot program for biologics in 2008. By mid 2011 there were 11 design space approvals for small molecules and none for biologics (Miksinski 2011). It was not until late in 2013 that the first new biologic, Gazyva by Genentech was approved by the FDA with design space (FDA approval letter 2013). There had been approval by the FDA in 2010 for an expanded change protocol (eCP) with CBE-30 for a multiproduct/multisite drug substance transfer for existing products as part of the QbD pilot program (Krummen 2013). Implementation of QbD for small molecules appears to have gone relatively quickly for standard synthetic compounds but requires more time and discussion for biologics.

The complexity of the manufacturing process for a biologic (especially cell culture) and resultant product is reflected in the complexity of defining design space and QbD. This point is made in Fig. 4.2 for cancer treatment options where a chemically synthesized product (vinblastine, 811 Da) is compared structurally to a biologic antibody product produced by living cells (IgG, ~150,000 Da). It has not been simple to interpret QbD for biologics, but progress has been made and implementation has been shown to be possible. Using process validation as an analogy, challenges of cell culture validation historically were daunting as compared to chemical synthesis, but were eventually overcome and are now status quo.

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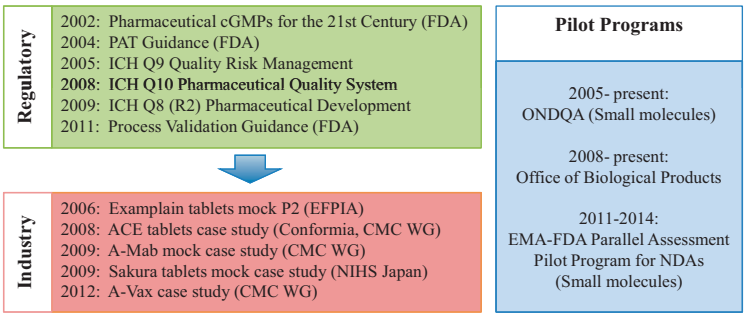

Fig. 4.1 Summary of QbD key guidances and programs

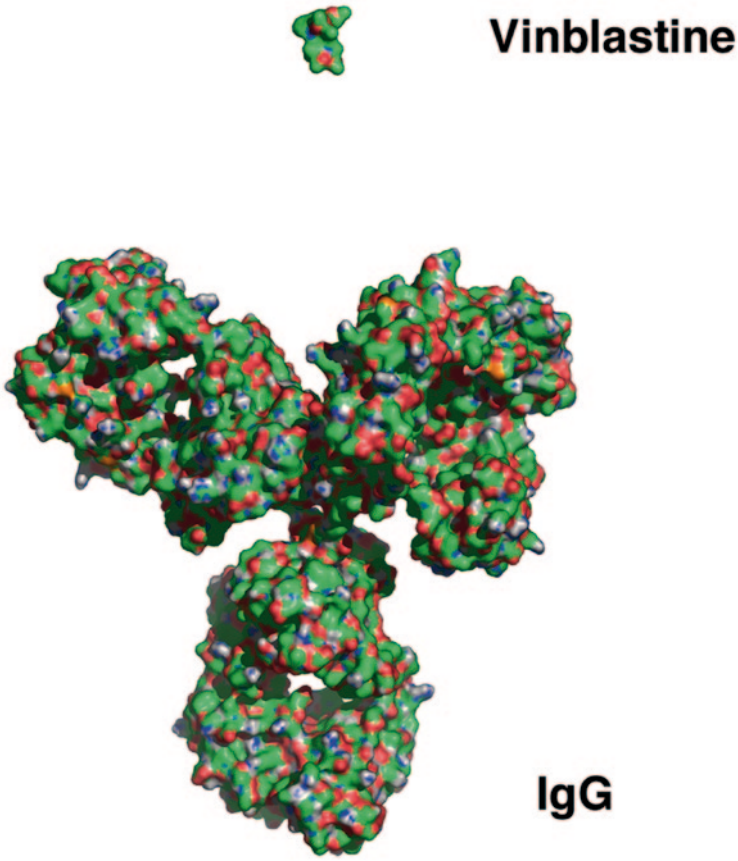

Fig. 4.2 The complexity of a biologic compared to a small molecule, example of two molecules used in cancer treatment, monoclonal antibody (IgG) and Vinblastine. Figures were produced using the PyMOL molecular graphics system, version 1.5.0.4 Schrödinger, LLC and are scaled for size comparison

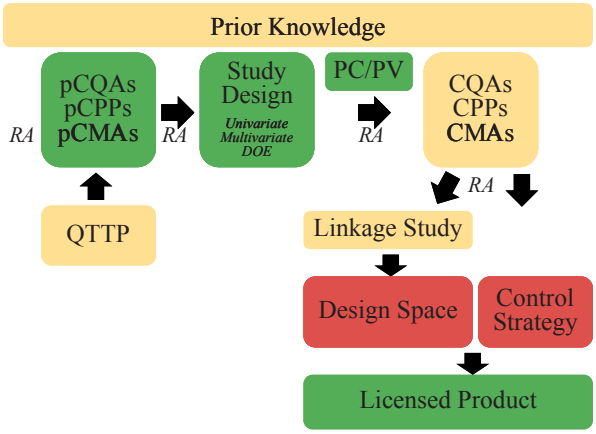

Fig. 4.3 QbD elements and current implementation status. *Green* = Elements regularly defined by risk assessment, *Yellow* = Elements still not routinely defined by risk assessment, *Red* = Elements occasionally defined by risk assessment

The industry response to adopting QbD for biologics has been mixed, with concerns expressed about cost and benefit (Cook 2013; Kouri 2012). Many companies have taken a wait and see attitude to QbD, others have incorporated QbD elements within their development programs and a few have actively pursued QbD submissions for design space. An estimate of the current status of the industry with respect to QbD implementation for biologics is shown in Fig. 4.3 based on the authors' experience and mostly consistent with a survey conducted for both small molecules and biologics (Cook 2013). Beginning development by defining a Quality Target Product Profile (QTTP) formally has increased, especially for combination products (CP), but is still not routine. Prior knowledge has always been used by developers but not often formally in risk assessments or documentation, and has now become an important part of determining criticality. CQAs (critical quality attributes), CPPs (critical process parameters), and CMAs (critical material attributes) are now often regularly defined by formal risk assessment (RA) by companies, as are process characterization (PC) and process validation (PV) study designs. Defining design space by linkage studies and risk assessment mapped back to clinical impact and defining control strategy by risk assessment is occasionally done and needs more examples of approval from health authorities to supply concrete feedback to the industry for what is acceptable.

Despite the limited design space approval for biologics, the foundation for success of QbD for biologics is in place. QbD practices have already added significant value to developing biologic processes and to a deeper understanding of manufacturing processes. The beneficial impacts seen to date of using QbD elements in a drug substance or drug product development process are listed in Table 4.1 and are quite remarkable. These include: improved communication of standardized elements used to describe the process across departments, sites, companies, and the

Table 4.1 High level development benefits of QbD seen to date for drug substance and drug product

QbD Element	Change	Impact
All elements	Common Language: Clear and consistent terms used (CQA, CPP, etc.) for processes by companies and regulators	Improved communication, streamlined end-to-end process standardization and consistent approach to generation and review of documentation. Clear and consistent risk-based process descriptions
Prior knowledge	Leverage prior knowledge; especially in risk assessments and decision making	Enhanced ability to differentiate criticality: experience with platform molecules/processes, to streamline life cycle management, technology transfer
Risk assessment	Formally used for most decision making for process, product, and patient	Enhanced ability to differentiate criticality: focus on risk reduction and testing rationale
Testing and specifications	Testing method mapped to CQAs; Control Strategy developed to cover process as well as product	Testing, control, and process validation logic well defined, rationale present to reduce testing or widen acceptance criteria

industry, an enhanced ability to differentiate criticality, a process to define testing logic, and mechanisms to increase process knowledge.

In the case of drug product, processes are similar for many products and prior knowledge can be extensively leveraged. Interactive effects, linkage of unit operations, and design space are more easily studied than for active pharmaceutical ingredient (API) because conditions are intentionally not variable nor have wide ranges, and no further purification occurs (other than sterile filtration). For these reasons, the authors believe that biologics drug product is particularly well suited for the application of QbD principles and approaches, both in terms of the relative ease of application and the potential benefits to the industry. In the following chapters, authors will discuss the examples of defining design space and interpretations of the associated QbD elements for biologic drug product operations, materials and products. We hope these examples will help to catalyze further development and broader application of the QbD methodology to drug product development, with gains in terms of greater process knowledge, more rapid development, avoidance of pitfalls, and greater ease of implementing changes within a well-defined design space.

4.2 Drug Product Design Space

What does design space mean for drug product when the main goals are: (1) to maintain the quality attributes of the API, (2) to demonstrate the product is sterile, (3) to deliver label claims for composition, and (4) to ensure the patient is dosed properly and as comfortably as possible? Formulation excipients, process impu-

rities, and sterility are mapped directly to label and packaging claims, subject to adulteration law. There is no desire to have ranges for these values other than those associated with assay variability. However, there is room for some movement of operational parameters during the drug product processing operations, and for changes in product contact materials. The drug product process typically starts with thawing the API, additional compounding if needed, sterile filtering, filling, lyophilization if that is the final format, stoppering and capping the primary containers, and packaging. Delivery from the final package into the patient is also studied. Subsequent chapters cover each of these operations from the standpoint of QbD development approaches and outcomes, including definition of design space.

4.3 Formulation Development

Historically, formulation development often started with the use of a service formulation such as phosphate buffered saline (PBS) for early phase clinical use. For commercial phase formulation development, the service formulation was usually further refined using a series of univariate or multivariate excipient studies and/or an excipient-based DOE. This approach can result in a loss of insight into comprehensive interactive effects. Another approach was to start formulation development from "square one" and survey all excipients (albeit, preferably those that were generally recognized as safe or GRAS). This was very time and resource intensive and did not make much use of prior knowledge, as there was no formal way to incorporate that. Despite the effort expended, the robustness of the end formulation was therefore highly variable. With a QbD approach, prior knowledge is used to inform development. Platform experience (molecule, materials, testing, and processing) is used to define and streamline testing and processes using risk assessments to determine what and how to test. Design space definition is expanded by including CMA's and assessing impact on quality, performance, and on the patient. The impact of processing interactive effects is also included in determining the formulation. Formulation development simultaneously optimizes the combination of excipient composition with container contact materials, filling/storage/handling unit operations, and delivery systems. Linkage studies are performed to study the impact of formulation CQA ranges on the final product quality. An example of an approach to drug product formulation QbD is found in Martin-Moe et al. 2011 as well as in the formulation development chapters by Perez-Ramirez et al., Sreedhara et al., and Jameel et al.

QbD impact: Prior physiochemical knowledge of "platform molecules" such as antibodies now contributes formally to molecule design, screening strategies and rationales to reduce the risk of poor physical, chemical, or biological stability (see also chapter by Seidler and Fraunhofer). This can improve the probability of technical success and expedite development of a molecule. The comprehensive evaluation of CMA's and process impact is an integral element of drug product design space

definition. This is a good illustration in which QbD does not merely equally perform an extensive DOE, but rather is much more comprehensive and leads to a much more robust result.

4.4 Drug Delivery and Device/Combination Product Development

The delivery requirements for patient administration of drugs include both commercial/market considerations and technical drivers. Ensuring patient compliance and sustaining or improving market share often can be achieved with life cycle improvements in approaches to drug delivery. Technical drivers can be prompted by changing from a lyophilized product to a liquid product, moving from intravenous to subcutaneous delivery, replacing a vial and syringe configuration with a prefilled syringe system or adding an auto injector to a product currently provided in a prefilled syringe—as some common examples.

The interdependence of the device, drug, and patient and/or end user highlights the need for an integrated systems approach to development that is often underestimated by companies. As a result of shortfalls in the initial development of requirements, a common industry challenge is unanticipated additions and changes in target specifications during the design and development process that can compromise product design and/ or result in significant delays in timelines. In a QbD approach, a QTTP is a critical input to design inputs and rigorously captures many of the essential targets (Table 4.2) including the patient interface. Required delivery volume, viscosity, dosing regimen, user preferences, and limitations of the patient population, and product contact materials are critical elements that must be considered while designing or selecting a delivery device. The fundamental difference between an engineered electromechanical device and the development of drug with biological origins can complicate the integration process. Sophisticated analytical techniques with high levels of reliability can be utilized to develop and characterize a device. Robust characterization of the device design and comprehensive risk management processes are common in the medical device industry. The principles of QbD are much easier to apply where these types of product characteristics and analytical techniques are available. On the other hand, the ability to analyze and understand a biologic relies heavily on experimental results and prior experience. Uncertainty levels are higher and more unpredictable than for devices.

Insulin delivery is the most prominent example of the value of advances in injection delivery systems that span many decades and technologies. The range of currently approved delivery systems for insulin (FDA 2014) include manual administration by syringe, a variety of pen injectors, a needleless injector, and numerous automated pumps. Significant patient benefits have occurred as a result of the evolution of easier to use and/or more automated injection devices for the delivery of insulin. These advances have been dramatic and the rapid adoption of increasingly

Table 4.2 QTTP for a prefilled syringe/antibody combination product for arthritis

Product attribute	Target
Dosage form	Liquid, single dose
Dose/PFS	100 mg
Concentration	100 mg/mL
Administration mode	Subcutaneous, thigh or abdomen
Viscosity	< 10 cP at room temperature
Container	20R Type 1 borosilicate glass syringe, fluoro- resin laminated plunger, staked needle 27G
Ergonomics	Easy to use by arthritic patients
Biocompatibility	Acceptable tolerance on injection
Compatibility with manufacturing process	Soluble at high concentration at UF/DF
Degradants and impurities	Below safety threshold or qualified
Pharmacopoeia compliance	Meets pharmacopoeia requirements for parenterals, colorless to slightly yellow, practically free of visible particles and meets USP criteria for subvisible particles
Aggregates	0–5 %
Fucose content	2–13 %
Galactosylation (%G1 + G2)	10–40 %
Host cell protein	0–100 ng/mg
Shelf life	NLT 2 years at 2–8C

featured rich delivery systems highlights the value that accrues from integrated, patient-centric designs of combination products.

Now and increasingly in the future, self-injection will be highly desirable for the treatment of chronic diseases such as rheumatoid arthritis and multiple sclerosis. The use by patients of injection delivery devices has placed an increasing emphasis on human factors in device design and development. The FDA has followed with draft guidance "Applying Human Factors and Usability Engineering to Optimize Medical Device Design" (FDA 2011). Design Inputs are required by design control and specifically require that the needs of the "user and patient" be included. User studies include formative usability testing during development and summative usability testing under realistic conditions during final product testing. While features of the device design heavily influence the user experience, the drug formulation also plays a key role. Formulations that sting or cause other injection site issues have a direct impact on the patient experience.

In the near term, further enhancements to delivery systems will include addition of electronics and software that will promote compliance and add in patient status monitoring. The challenges associated with the emergence of more sophisticated drug delivery systems can be more successfully met if rigorous use of the principles and tools associated with QbD are utilized.

In January 2013, the FDA published the final rule regarding current good manufacturing practice requirements for combination products. The final rule became

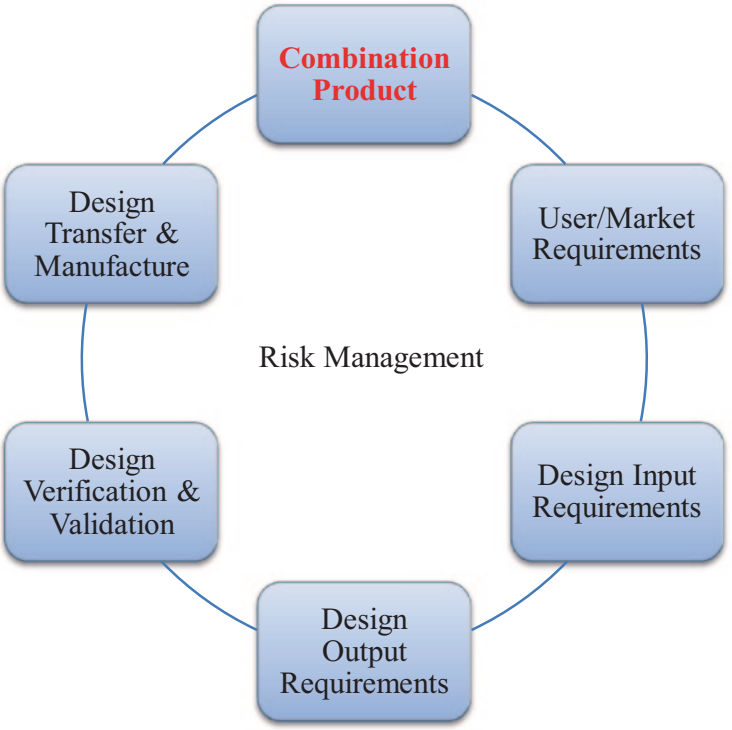

Fig. 4.4 Quality system design control

effective in July 2013 (Federal Register 2013). This rule clarifies the regulatory requirements for quality systems used to design, develop, and manufacture combination products. Fundamentally the rule states that the biologic must comply with applicable regulations (21 CFR 600 through 680) and the device must comply with applicable regulations (Quality System Regulation (QSR), 21 CFR Part 820). The combination product can benefit from adoption of specific provisions of the QSR's. The specific provision having the most impact during development is the need for design control. All elements of design control apply to the device and certain elements logically apply to the combination product. The design control process (Fig. 4.4) is well understood by device development organizations but the approach to utilizing applicable elements of design control of the combination product varies widely across the industry. Some companies currently employ a robust, highly integrated process for the development of combination products. Over time an industry standard will evolve that fosters an integrated approach to development. The device and combination product chapter by Givand et al. contains a number of examples of device development and the value of a comprehensive approach to integrated design and development of combination products.

QbD Impact: There is a focus, emanating from the QSR's, during development of combination products to provide comprehensive product requirements. Leverag-

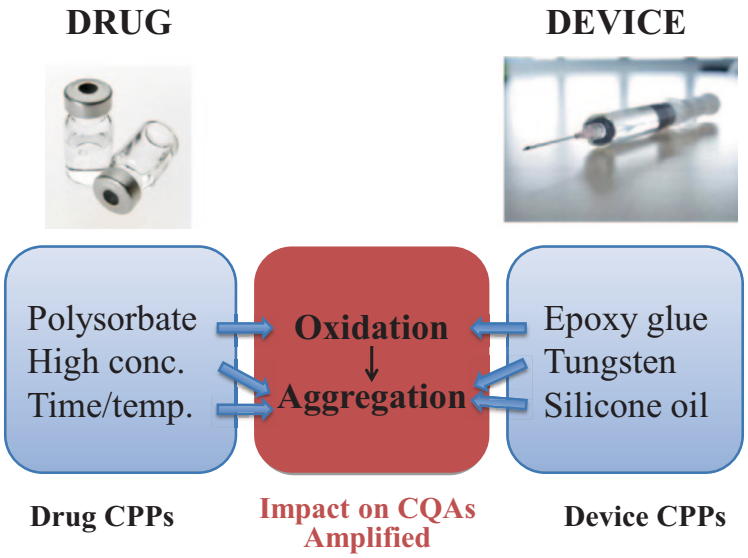

Fig. 4.5 Combination product potential interactive effects on CQAs

ing QbD principles for drugs/combination products aligns with design control for devices. The QTPP is the guiding, multifunctional requirement map for integrated development that assures that the product or combination product is safe, efficacious, and usable. As noted above, compromised requirements definition can lead to uncertain or changing design inputs for either the delivery device or the combination product late in the development process. Increased attention to comprehensive product requirements early in the process is the essential element of QbD that fosters robust combination product development.

Currently industry standards for QbD for a combination product have not been defined or consistently utilized. However, it is clear that studying interactive effects and defining design space for the combination product systems are critical to develop and characterize a new combination product. An illustration of how interactive effects can occur in a combination product is shown in Fig. 4.5. A CQA such as product oxidation can be caused by the drug product excipient polysorbate (Ha et al. 2002) as well as by epoxy glue used for staked needle in some prefilled syringes (Markovic 2006). Similarly, a CQA such as product aggregation may be amplified by contributions by the drug at high concentration or high temperature (Cleland et al. 1993), and/or by tungsten remaining from staked needle hole formation in the device (Bee et al. 2009) and/or by silicone oil used in the syringe barrel (Thirumangalathu et al. 2009). In subsequent chapters (see Perez-Ramirez et al. for combining proteins with sustained delivery matrices, Verdine and Degrazio for primary container QbD and Givand et al. for combination products), provide additional insight into the benefits of the QbD approach in ensuring the well-functioning integration of all the elements of a combination product (e.g., drug, primary container, and injection device).

The CQAs, CPPs, risk assessments, design space, control strategy, and continuous improvement process must be conducted on the biologic, the delivery device, and the combination product. Structuring an organization that fosters this approach significantly enhances product quality, efficacy, and safety. Organizations that successfully bridge this divide have established a significant competitive advantage that can be leveraged as self-administration and enhancements in delivery technology evolve.

4.5 Manufacturing of Drug Product

Drug product manufacturing process parameter ranges are typically defined by facility and equipment capabilities and tend to be narrow. Exceptions include lyophilization unit operations, hold times, and temperatures. Edge of failure and range finding studies are very limited due to material availability and cost. Using a QbD approach, small scale, worst case, and surrogate models allow for studying the impact of the process on product quality and interactive effects (Martin-Moe et al. 2011). In the following chapters on unit operations, small scale and worst case models are heavily leveraged in order to define design space, for example in chapters by Lam et al. for freeze and thaw operations, Shultz et al. for UF/DF, and Patel et al. for lyophilization.

QbD impact summary—Validated models are used to study ranges for all unit operations and deepen process knowledge. This information and capability is also useful for defining broader operation ranges, in some cases finding the edge of failure, and for investigation of discrepancies.

4.6 Control Strategy and the Validated State

Key elements of the control strategy include the control system (testing plan such as in-process, release, stability and stress, and extended characterization) and life cycle and knowledge management of the validated state of the product and process. Throughout this book there are numerous examples of determining CQAs by risk assessment and in particular by risk ranking and filtering (RRF). RRF allows pCQAs to be assessed against a spectrum of criticality (impact on safety and efficacy and uncertainty of impact) and thereby allows for change in criticality. As more medical knowledge is accumulated to support the safety of a CQA for example, a CQA may decrease in criticality even to a point of no longer being critical, and in theory a test may be dropped from the control system. Or the converse may occur and a QA becomes critical. The same situation could occur for CPPs. This may explain why there has been feedback from the FDA to include non-CPPs in a submission, as they will also need post approval surveillance as knowledge accumulates.

Once a risk-based control strategy is established, acceptance criteria/specifications are established by risk assessment. Often there will be a discussion that can be controversial with respect to the balance of running tight clinical processes vs. wider processes in order to test the presence of low levels of certain degradants in human subjects and justify specifications for impurities (see discussion in Kozlowski et al. 2012). The ability to use prior knowledge as part of risk assessments to determine specifications can reduce or eliminate the need for this type of decision.

Traditional processes are defined in more narrow ranges whereas QbD allows for wider processing ranges. There is some discussion that validation/conformance runs ($n=3$) could be replaced by continuous process verification though monitoring to detect variability as part of an integrated lifecycle practice during routine production rather than being a specific point in time. Real time online detection with feedback could permit controlled changes of in process parameters within design space. As discussed earlier, most of the drug product unit operations actually require tight ranges, but lyophilization is one good example of where PAT could be implemented to control a design space; this is presented in a later chapter by Jameel and Kessler. Additional analytical (PAT) development may be required to enable other applications of real-time QbD.

QbD impact: Product specifications are set based on rigid process performance and tend to narrow over time. In a QbD Design space, specifications are set based on product and process knowledge and may be wider and stay wider as a QbD process is more flexible, the controls are more dynamic and adaptation to variability can occur such as to raw materials or a new facility. Process analytical technology (PAT) may become even more essential for real time process control.

4.7 Future Directions and Opportunities for Regulatory Impact for Drug Product

Defining drug product design space allows more opportunity for flexibility and postapproval optimization. Some key opportunities include:

- Technology transfer—There is an opportunity to use design space and eCPs for technology transfer, as exemplified by the first approval for an API technology transfer for a biologic mentioned earlier. For a drug product, it would be less complex. Even without a formal QbD design space, simply defining the transfer elements by QbD enables an enhanced control of transfer. In a technology transfer to another manufacturing site, the elements of transfer can be QbD elements as shown in Fig. 4.6. Process and product characterization, clinical lots and commercial lots all contribute to prior knowledge. Equivalent design at the second site drives comparability success. Assurance of transfer is governed by the quality risk management program including a transfer FMEA, a technology transfer plan and a comparability testing exercise where process and product (including stability lots) from both sites are compared. A full chapter by Lim et al. is dedicated to this.

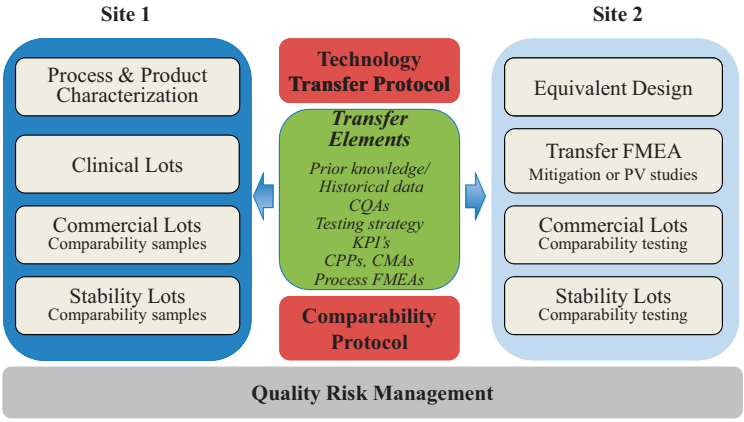

Fig. 4.6 Technology transfer for a commercial product to another site and comparability program overview. *KPI* key performance indicator, *FMEA* failure mode and effects analysis

- Reduced risk of manufacturing discrepancies—Using validated models, discrepancies can be minimized by proactively understanding and even defining wider operating ranges.
- Continuous improvement within a design space—A design space that includes CMAs would be very useful for primary packaging changes, devices, or kit elements. It may also be useful for software changes in the case of mobile apps or web portals.
- Biosimilars or biobetters—A design space for an API and drug product could allow for transition to a biosimilar or biobetter. Originator companies could leverage their known design space to move into this arena themselves or outside companies could leverage aspects of QbD to streamline bringing products to market.

In general, submissions may be stronger and more standardized across the submission package, company, and industry. The downside of longer submissions (as more information is included for drug product operations) would be counterbalanced by wider operating margins and simplification of process improvements, as well as ease and presumably increased speed of review. As continuous improvement is further enabled, we can expect to see great improvements to safety, efficacy, and in particular for drug product—the user experience.

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Chapter 5
Development of Drug Product Formulations: Molecular Design and Early Candidates Screening

Michael Siedler, Vineet Kumar, Ravi Chari, Sonal Saluja and Wolfgang Fraunhofer

5.1 Introduction—Implementation of Quality by Design (QbD) in Late-Stage Discovery and Transition

During the development of biotherapeutics such as monoclonal antibodies (mAbs) and other proteins, CMC issues are likely to arise if the molecule possesses less-than-ideal drug-like properties, caused by poor physical, chemical, or biological stability (Daugherty and Mrsny 2010; Shire 2009). These stability issues may arise if a stability assessment is not included in the candidate selection process. Figure 5.1 depicts the optimal sequence for candidate selection, in which a stability assessment is conducted prior to final candidate selection.

The overall goal in formulation development is to accommodate any molecule identified within discovery, even those with less favorite drug-like properties. In some cases, a decision may be made to accept stability issue and manage it through appropriate formulation and drug product process development, due to inherent difficulties in editing the degradation site; or an issue may arise during late stage development or even postlaunch that was not detected earlier.

However, it should be recognized that the attempt to compensate the lack of intrinsic stability of a given molecule within drug product formulation development is

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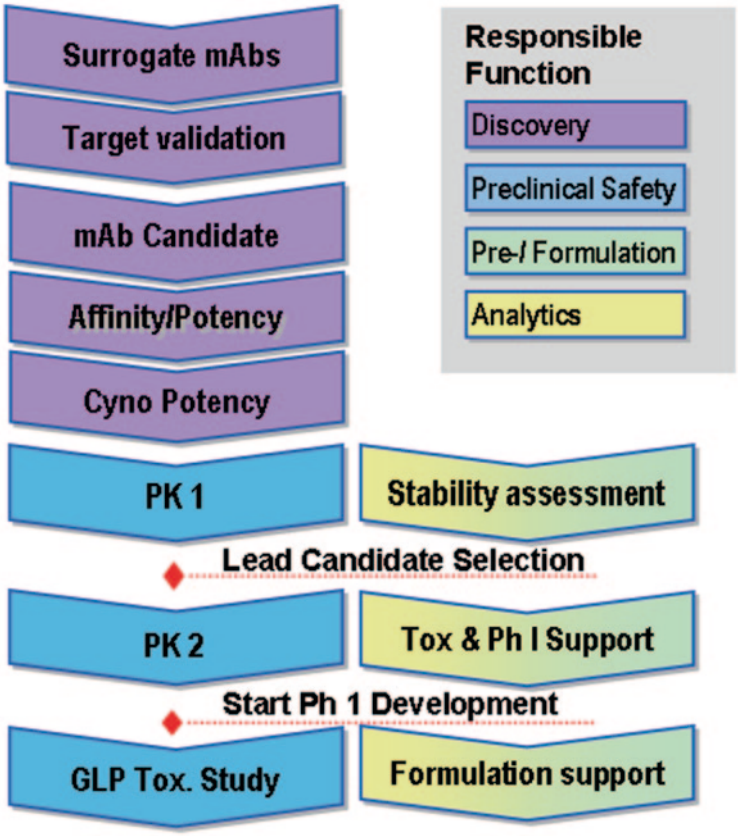

Fig. 5.1 Overview of the development activities during the conventional development of monoclonal antibodies (mAbs)

often less efficient and may result in a dosage form that is less than optimal for the respective indication when it reaches the market.

Implementation of a QbD approach, combining the capabilities of all involved functions, enhances product and process understanding leading to a more efficient development process as well as more robust manufacturing processes, resulting in a better quality of the final drug product.

A further important aspect of this holistic effort is that all those challenges caused by the molecule (e.g., liable sequence motifs or aggregation propensity) that may decelerate drug product development will be circled back into discovery to guide the generation/design of new molecules at a stage where such issues can be engineered out. This feedback loop will allow implementation of continuous improvement into the pharmaceutical development leading again to higher quality drug products. However, such an approach will significantly affect the role and the scope of every function involved. In fact discovery, analytics, preformulation/early formulation development, and drug metabolism and pharmacokinetics (PK) need to

become partners in order to define an aligned development strategy to identify the most viable molecule candidate, hence increasing the overall probability of success.

In the following section, we will focus mainly on the interaction between discovery and preformulation/early formulation development by outlining the concept of a systematic and standardized screening process to enable the use of development platforms. Platform technologies for drug product development are the key element to enable both the business needs to increase efficiency during the development (e.g., optimize costs and timelines) and the expectations by regulatory authorities toward an increased product quality by in-depth product and process understanding. The examples below focus on mAbs and closely related structures but the principles can be applied across protein therapeutic classes.

5.2 Molecular Engineering and Screening

Significant advancements in technologies to generate antibodies have been made to identify suitable candidates on a routine base. However, the focus was mainly toward screening for affinity to a given target and optimizing potency but not necessarily toward optimizing drug-like properties.

Nevertheless, the ability to generate a number of different candidates with adequate binding characteristics is the prerequisite to allow additional screening processes to evaluate the drug-like-properties of the molecular candidates and select the lead candidate based on all available data. Therefore, modern development approaches leverages the capabilities within preformulation/early formulation development as an essential tool within the discovery process to screen and select candidates.

Historically, the main function of preformulation/early formulation development was to analyze a given protein to such an extent that allows defining the composition of the drug substance buffer for subsequent formulation development and in some cases to define an early formulation for IND-enabling preclinical studies and early clinical development. With the addition of a stability assessment to the candidate selection process, the aim shifts toward contributing to the systematic and standardized screening process implemented within the discovery phase to leverage the capabilities of each function at a development stage where quality can be engineered into the sequence of the molecule. The joint goal is to identify molecules that meet a predefined subset of characteristics indicative for favorable drug-like-properties based on prior knowledge. These characteristics are summarized in the target molecule profile (TMP) shown in Box 1 and is used to guide the generation of molecules in all therapeutic areas. The TMP is the result of a joint effort from all functions involved during late discovery/early development and defines all properties needed to assure a safe, efficacious, and stable molecule, based on prior knowledge. It specifies the ranges for all drug-like properties needed to assure comparability to previous mAbs. Hence, verifying the leverage of prior knowledge, for example the use of existing platform technologies established in drug substance

and drug product development. Therefore, the TMP is of paramount importance to define and design quality into the molecule.

Once a candidate matches the TMP, it is considered a "next-in-class" molecule. This means that this particular molecule is comparable to previously developed molecules and the knowledge gained with the previous molecules (prior knowledge) can be fully applied for the development of this new molecule. Consequently, the overall CMC development risk assigned to this class of molecules is low. Following the concept of QbD the advantage of this approach is to focus activities only on those candidates showing most promise for successful drug product development.

Example of a Target Molecule Profile (TMP)1,2

The TMP defines the key characteristics of a molecule needed to assure favorable drug-like properties. Assays performed during candidate selection assess to what extent the respective molecules match predefined criteria justified by prior knowledge to select the most suitable molecules as lead and backup candidates. In addition, the outcome of this assessment can be used to predict CMC development risk and justify the use of established technology platforms in DS/DP development. Some of these may be overlapping.

- High affinity and potency
- Avoids known unstable sequence motifs (hot-spots)
- Favorable secondary, tertiary structure
- High solubility and low viscosity (high pI) to enable high-concentration formulations, if needed
- High physical and chemical stability (manufacturability, long shelf life)
- Acceptable stability in serum (biological stability)
- Acceptable human *PK* (SC bioavailability and T1/2—depends on indication and target)
- Compatibility with implemented DS and DP platform processes
- Low-immunogenic potential

To assess whether the drug-like properties of the initial pool of candidates matches with the TMP, a systematic and standardized screening process must be implemented between clone generation and lead and backup candidate selection. The aim of such a screening is to identify and select from an initial pool of molecule candidates only those that match the predefined criteria of the TMP for "next-in-class" molecules and/or to edit early lead molecules to fit the TMP criteria. An example of such a standardized screening process is shown in Fig. 5.2.

The screening process may be divided into different tiers, taking into consideration the different needs and restrictions at the respective development stage. The screening process starts with the generation of suitable molecules that will undergo an initial in-silico screening for known sequence liabilities (hot-spots). The aim is to identify labile sequence motifs prone to chemical modifications upon manufacturing and/or long-term storage. Examples of labile motifs are shown in Table 5.1. Based on the respective findings, a risk will be assigned to each molecule.

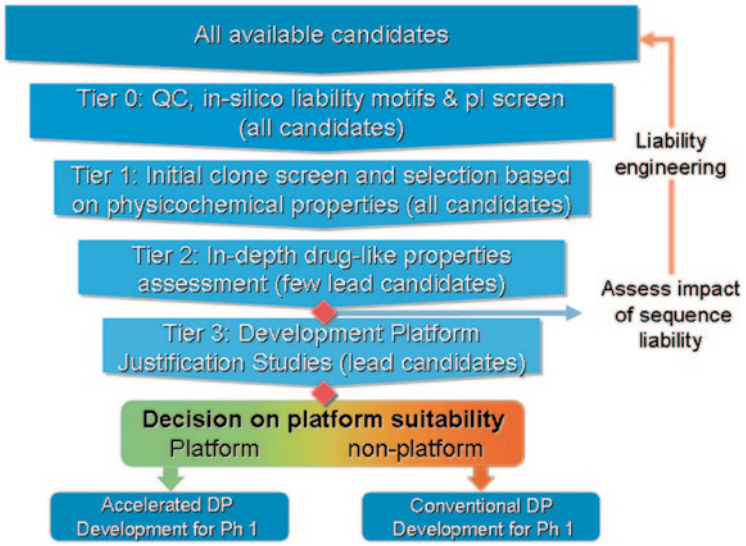

Fig. 5.2 Flow-chart of a standardized screening process to assess the drug-like properties of monoclonal antibodies (mAbs)

Table 5.1 Example of weight scale categories of screening assays to predict drug-like properties

Impact of assays on drug-like properties based on prior knowledge	Weight scale
Strong impact on DS/DP stability based on data	10
Strong impact on DS/DP stability is expected	7
Moderate or unknown impact on DS/DP stability	5
Minor impact on DS/DP stability based on data	1

However, not all labile sequence motifs will finally result in chemical degradation. Therefore, stability studies may be implemented to verify the associated risk, especially for high-risk candidates during the later tiers of the screening process.

The sequence of assays to be performed is typically structured in a way to initially accommodate a larger number of molecules in tier 1, while minimizing the material needs and turn-around times by using methods enabling a high throughput. Transferring assays to standard plates and automated liquid handling systems can help to minimize operator error, allowing statistical approaches to analyze the results.

Ideally only an identified subset of molecules showing most promising drug-like properties will further proceed into tier 2. At that stage focusing on fewer molecules allows more in-depth characterization. Forced degradation/accelerated stability studies are typically an integral part of the stability assessment at this point, in which the molecules are subjected to a variety of stress conditions, such as storage at elevated temperatures, multiple freeze-thaws, shaking, or stirring.

In the course of these studies basic formulation parameters such as pH, buffer system, and detergent concentration are often screened systematically. The results finally allow a decision as to whether the identified lead and backup molecules

match the TMP, hence enabling the use of established development platform technologies based on prior knowledge.

5.3 Definition of Prior Knowledge, Design, and Control Space for Platform Technologies

The most efficient way to leverage prior knowledge is by establishing standardized platform technologies to be applied in formulation development, manufacturing (e.g., standardized manufacturing equipment and processes), and analytics.

Following the concept of QbD, a systematic and standardized screening process to identify and select only molecules that fit the TMP criteria for further development will not only increase the quality of drug products in the future but also enables a "routine development for monoclonal antibodies" that will allow a reduction in the resources needed per project and shorten project timelines to initiate clinical studies. As previously described the stability data gained during the various screening assays to determine the drug-like-properties of the molecule links the molecule to the existing prior knowledge. If the data confirm that the molecule fit the TMP, this information verifies that the established prior knowledge, e.g., DS and DP platform technologies for this class of molecules, can be applied as shown in by the flow chart in Fig. 5.3. If the screening data reveal unfavorable drug-like properties for the lead candidate, such as aggregation propensity or high viscosity, the molecule may not be covered by the existing platforms. The decision whether to proceed with such a nonplatform molecule into drug product development or reengineer such a molecule is a case-by-case decision and dependent on the quality attributes to be affected.

The screening data not only contributes to defining the drug substance composition and may guide the subsequent formulation development but also becomes

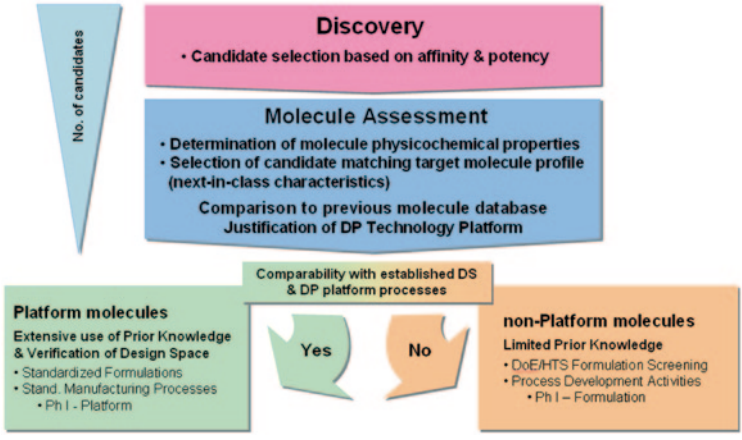

Fig. 5.3 Flow chart of screening process to determine platform compatibility

of crucial importance to establish that a given molecule candidate meets with the predefined criteria of the TMP thus, justifying the application of platform processes later in DS and DP development.

As described in the published QbD guidelines, assurance of quality is achieved by exploring the knowledge space that allows definition of a design space, which is defined as:

A multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality [ICH Q8(R2)].

An example of how this general concept can be transferred to biologics drug product development is described in the published A-Mab Case Study (CMCBiotech Working Group). In addition to the already defined knowledge and design space, this document introduced another area, defined as control space. The control space defines the operational limits for process parameters and input variables used in routine manufacturing. The control space can be a multidimensional space or a combination of univariant process ranges.

The definition of a technology platform follows the same principles. The key idea is that molecules of the same class, for example mAbs, can have comparable characteristics. If this is the case and sufficiently justified, it is possible to define a design- and control space that is valid not only for one particular molecule but also can be applied to all molecules of this class sharing the same characteristics. Following the concept of QbD a technology platform can be defined as:

A systematic approach to ***leverage prior knowledge*** *for* ***standardized process****, that can be applied for a class of molecules with comparable characteristics* ("next-in-class" molecules)*, wherein the multiple combinations of input variables and the interaction have been demonstrated to* provide assurance of quality.

However, there are some prerequisites that need to be fulfilled in order to establish and verify the use of a technology platform:

- A systematic approach for clone section to assess and select molecule candidates with comparable characteristics ("*next-in-class" molecules*)
- Standardized formulations and manufacturing process (e.g., unit operations) to be applied for all molecules
- Adequate justification studies to establish the technology platform for the intended class of molecules (*Platform* design space) and to provide assurance of quality

The concept of a platform design space is depicted in Fig. 5.4. If the data for at least three different molecules of the same class (x-, y-, and z-mAb) demonstrate that the respective design and control spaces will significantly overlap, a platform design and control space can be defined.

Once established, a platform design and control space can be applied for all molecules that meet the TMP since the screening data justify that the target molecule falls into the design space of the established platform(s).

As described in the previous paragraph the whole platform technology concept is build on the comparability of a new molecule with previous molecules used to define the platform design space.

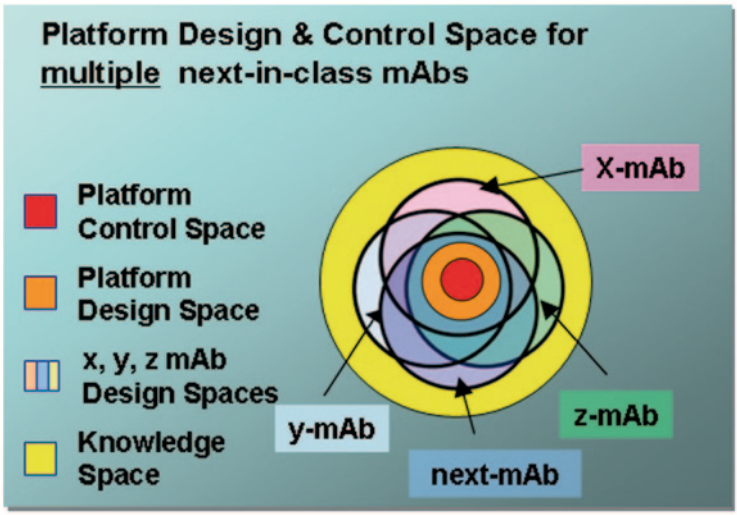

Fig. 5.4 Illustration of the concept on how a design and control space can be applied for a technology platform

5.4 Outline of a Risk-Based Screening Approach to Screen Drug-Like Properties of Molecule Candidates

The previous paragraphs described the importance of a systematic screening process to identify molecules with favorable drug-like-properties based on a TMP. The following sections describe the various stages of such a screening process in more detail.

A common element for all assays to be performed is the use of a risk assessment tool to predict and assign the development risk based on the individual result. As an example of how to implement this in a systematic and transparent manner, a table can be developed to summarize for tier 1, 2, and 3 every assay that will be performed regarding:

- The quality attribute to be evaluated
- The weight-scale of the respective assay based on the anticipated impact to predict the development risk
- The analytical method to be used
- The amount of drug substance required to perform the assay
- The benchmark criteria to assign a low-, medium-, or high-development risk

In the example shown in Table 5.1 the weight scale is sorted into four different categories ranging from assays known to have a strong impact (weight-scale of 10) to assays that have virtually no impact (weight scale of 1) to predict the development risk. The most important information to assign a development risk is the benchmark data for prior representatives of this class of molecules. This information is linked

to the TMP and represents the available product and process understanding (prior knowledge) for the respective class of molecules. Depending on the amount of relevant data (e.g., commercial products) and the respective development strategy the benchmark criteria may vary. However, since the benchmark criteria are predefined, the result of the performed assay allows the assignment of a low-, medium-, or high-development risk. It is important to note that a finding of high risk would not necessary result in abandoning this particular molecule, especially if there are no further candidates available. There may be options to compensate weaknesses by changing the formulation and/or dosage form. However, it is quite obvious that such a molecule, depending on the severity of the findings, will likely require longer development timelines and/or resources and may not fit the development platform.

5.4.1 Tier 0 Assays—In-Silico Assessment and Molecular Engineering

Tier 0 assays are performed during the later discovery stage when discovery teams have developed a series of affinity matured candidates; this can include up to 50 or more in number, depending on the approach used to generate the candidates. Therefore, selecting one or more for further development involves considering other characteristics, including drug-like properties. However, the high number and limited material at this stage means that extended characterization may not be possible.

To address this, the tier 0 assays may be applied as a virtual candidate screen requiring no material. The assays are meant to flag candidates that may have potential issues regarding drug-like properties. Those candidates may then be eliminated from further consideration. However, if selection is based on other properties such as affinity or potency, then development teams know early on that more robust characterization outside of standard approaches may be required.

The tier 0 assays may include:

1. Basic characterization:
    a. Isoelectric point calculation via primary amino acid sequence
    b. Molar extinction coefficient calculation via primary amino acid sequence (may be performed for reference only; no effect on protein stability)
2. Sequence screen for amino acid motifs that are prone to chemical degradation
3. Candidate reengineering based on computer modeling to improve drug-like properties (e.g., solubility, viscosity)

The isoelectric point is calculated based upon the number and type of ionizable residues in the protein (Chari et al. 2009). Although it may not match the experimental isoelectric point in all cases, usually a high correlation between experimental and calculated values can be found. The isoelectric point provides an indication of whether stability issues will arise during liquid formulation. The formulation pH of most commercial liquid formulation biologic drugs range from 5.0 to 6.5

Table 5.2 List of known sequence liabilities (hot-spots) that potentially lead to chemical modifications. (Daugherty and Mrsny 2010; Elizabeth et al. 2010)

Development Risk	Chemical Degradation	Sequence Motif
High	Deamidation	NG, NS, QG
	Isomerization	DG, DS
	Cleavage	DP
	Oxidation	M, C
	Glycosylation	N{P}S{P}, N{P}T{P}
	N-terminal pyroglutamate form.	N-terminal Q
Medium	Deamidation	NH
	Hydrolysis	NP
	Cleavage	TS
Low	Deamidation	SN, TN, KN

(Daugherty and Mrsny 2010). This range is most conducive to minimizing chemical degradation, which occurs through a variety of pathways (Table 5.2; Daugherty and Mrsny 2010; Topp et al. 2010). However, if the isoelectric point of the candidate is close to this range, physical stability may be a concern. When the pH is near the isoelectric point, charge–charge repulsion among proteins in solution is reduced and attractive forces such as dipole–dipole interactions or hydrophobic attractions become more prominent. This can result in lower solubility, higher viscosity, and/or a greater tendency for aggregation or particle formation. Therefore, candidates with isoelectric points further away from this pH range are desired.

A primary amino acid sequence screen may be performed to flag candidates with motifs that may be prone to chemical degradation as listed in Table 5.2. Chemical degradation during expression, purification, manufacturing, or storage may result in CMC issues and can affect drug safety and efficacy (Liu et al. 2008). This is especially a concern if the drug is an antibody and the motifs are present in the Complementarity determining regions (CDR) regions. Ideally, such candidates would be eliminated from further consideration. However, if they are selected, they should be further characterized to establish the extent of their propensity to degrade, as it is possible that the motifs are in a location in the antibody that renders them less labile.

Finally, a more recent development is the use of computer modeling to guide candidate reengineering to improve drug-like properties. This involves creating three-dimensional models of antibody candidates and mapping the surface-charge distribution and surface-hydrophobicity distribution (Voynov et al. 2009). The surface-charge distribution can determine if there is a prominent separation of positive charge and negative charge. Such a profile can indicate the possibility of dipole–dipole interactions which may manifest in low solubility and/or high viscosity (see

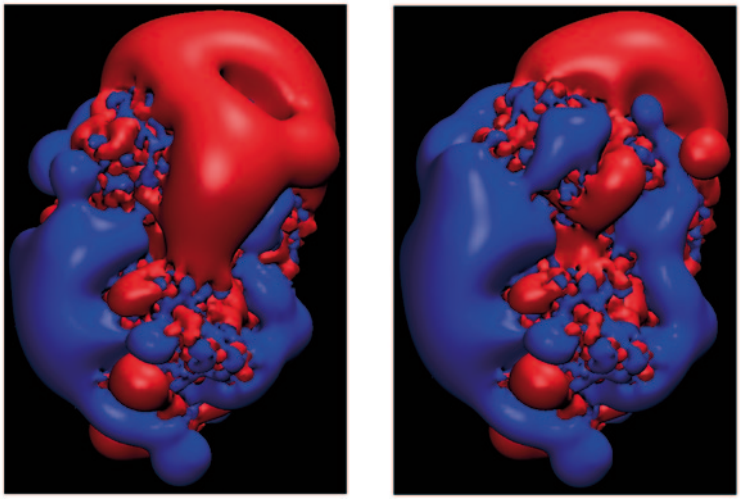

Fig. 5.5 The surface charge distribution of the Fab of a poorly soluble antibody shows that it contains a fairly even separation of positive and negative charge resulting in an apparent dipole (*right*). Specific point mutations were performed to lower the dipole by increasing the positive charge (*right*). As a result, the solubility was increased. *Red* negative surface, *Blue* positive surface

Fig. 5.5; Chari et al. 2009; Long and Labute 2010; Chari et al.). Mutations can then be proposed to reduce such interactions (Long and Labute 2010). Similarly, the hydrophobicity distribution can indicate whether hydrophobic patches are present which can serve as nucleation sites for aggregation (Voynov et al. 2009). Mutations can also be proposed to reduce the size of these patches.

5.4.2 Tier 1 Assays—High-Throughput Molecule Screening (Thermodynamic Solubility and Stability)

Candidates who pass the screen in tier 0 feed into tier 1, where they are assayed for drug like properties for the first time. Not all candidates at this stage may have the physiochemical properties to meet the TMP. Hence, the purpose of early screening is to rank the candidates in order of the risk involved in moving forward to the next stage. It also gives an indication at an early stage about the drugability of candidates, i.e., their susceptibility to degradation during the unit operations involved in converting a biologic from a drug substance to a drug product. Tier I is an initial assessment, hence material constraints are most often present. Another important consideration is to adapt the process to a high-throughput mode because there may be a relatively large number of candidates from early screening. High throughput and automation can not only address the issue of quantitative analysis but may also improve the quality of development in a timely manner to meet patient's needs.

Table 5.3 Example of tier 1 assays to enable a high-throughput initial assessment of drug-like properties

Quality attribute	Weight-scale	Analysis	Prior knowledge/benchmark criteria
Solubility	10	Real solubility using µ-con. centrifuge filters	High solubility
			Medium–low solubility
Thermodynamic stability	7	Dynamic scanning fluorimetry	High-unfolding temperature
			Medium-unfolding temperature
			Low-unfolding temperature
Intrinsic stability	5	Enzymatic degradation	High stability against enzymatic degradation
			Low stability against enzymatic degradation

An example of a scheme of assays to characterize initial physiochemical properties with the goal of candidate selection in a high-throughput manner is shown in Table 5.3. Each assay is weighted based on its impact on candidate selection, a knowledge base gained from prior experience, to enable a continuous trending and improvement of new molecules. The outcome of the tier 1 screening allows an overall assessment and ranking of the predicted development risk to allow a focus on the most promising molecular candidates for further development.

5.5 Solubility

For products intended for self-administration continuous improvements are targeted at improving patient acceptability by reducing the administration frequency and at the same time mitigating pain associated with the frequent use of needles. A step toward this goal is formulation of high-concentration products, such that subcutaneous self-administration is possible with volumes less than 1 ml. Solubility determinations thus form an integral part in the early candidate screening to identify those that may have a propensity to precipitate during manufacturing, storage, or administration.

Polyethylene glycol (PEG) precipitation is a traditional method for solubility estimations using relatively small amounts of material (Arakawa and Timasheff 1985). The amount of PEG required to induce precipitation of the test protein is benchmarked against a protein with known solubility. However, this method only estimates the protein solubility. The true solubility can be better assessed by methods such as concentrating the protein sample in microconcentrators.

5.6 Thermodynamic Stability

Thermodynamic stability of a protein at temperatures of interest can be correlated to its tendency to unfold at elevated temperatures. When a protein unfolds, it exposes its hydrophobic interior which can then serve as a nucleation site for the

hydrophobic regions of other unfolded proteins, thus leading to protein aggregation. Exposure of hydrophobic patches may also lead to degradation via interaction with the walls of the container during long-term storage, with the air–liquid interface when shaken, or at the ice–water interface during freezing. Dynamic scanning fluorimetry (DSF) can be employed to assess the propensity of a protein to unfold. Proteins that have lower intrinsic stability will be more prone to unfold and will have lower unfolding temperature than proteins with greater intrinsic stability. The technique involves studying the change in the fluorescence intensity with the addition of Sypro Orange to the protein samples and is described in detail elsewhere (He et al. 2009).

DSF is an orthogonal technique to the traditional and well established differential scanning calorimetry (DSC) for predicting thermal stability of biologics. It has the advantage that it can be formatted as a high-throughput technique for analysis of 100's of samples per day with low material needs (approx. 0.1 mg material needed). In general, the data generated by both methods correlate very well as shown in overlay in Fig. 5.6a for an antibody measured by DSF and DSC. The graph in Fig. 5.6b shows a correlation of the onset of unfolding for multiple antibodies measured by using DSF and DSC.

5.7 Intrinsic Stability

Stability to protease degradation can be evaluated by exposure of the protein to proteolytic enzymes (Sanchez-Ruiz 2010; Arlandis et al. 2010). In the authors' experience, a protease which cleaves peptide bonds at hydrophobic residues is the key to the assay. Since, hydrophobic residues in the interior are more likely to be exposed and accessible in unstable proteins, the half life of degradation gives an indication of the protein stability. Figure 5.7 represents a schematic summary of the assay representing the factors that govern the half life, namely length of exposure and intrinsic stability of the protein.

5.8 Case Study

A set of 10 mAb's were tested in the tier 1 screen to identify those candidates showing the most favorable drug-like properties. The subset of low-risk candidates will proceed to tier 2 for further characterization. Table 5.4 summarizes the final results for all candidates tested. Based on the established criteria (prior knowledge), the candidates were benchmarked as low/medium/high risk, indicated by the different color coding. Final rank is calculated as a weighted cumulative of all the assays performed at a particular tier. The tier 1 results combined with the respective potency/bioassay data form the basis for a decision as to which candidates will be moved to tier 2 for extended characterization.

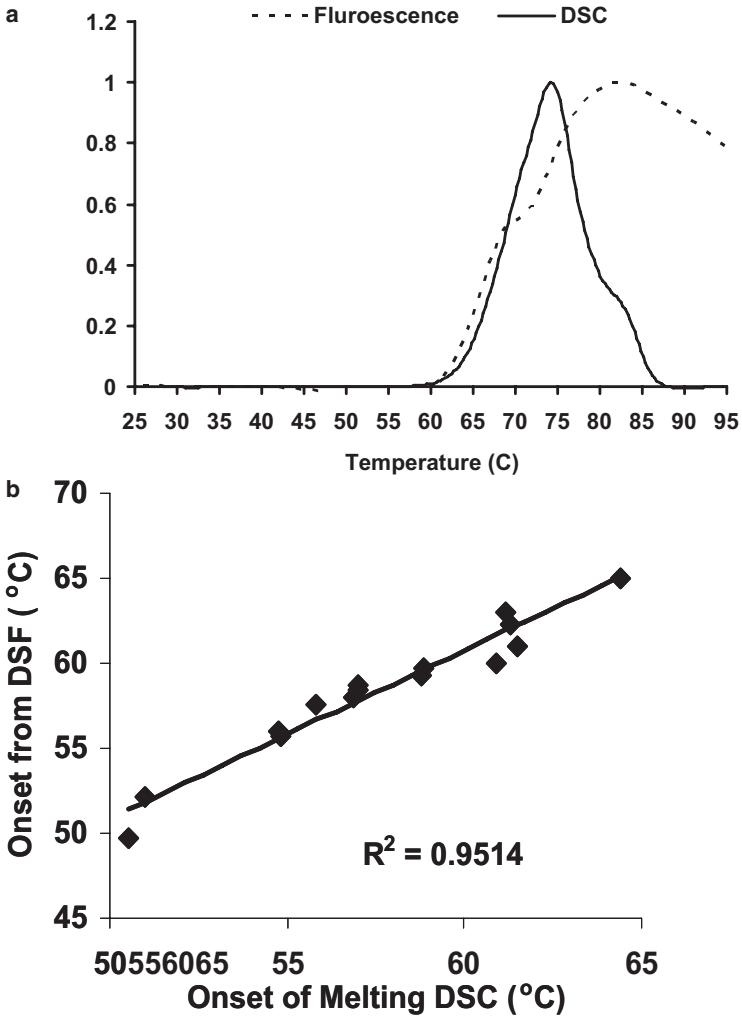

Fig. 5.6 Comparison of DSC and DSF a mAb thermogram for an antibody. b Correlation between the onset of unfolding as obtained using the two techniques for multiple antibodies

5.8.1 Tier 2 Assays—Development Platform Compatibility Screening

Results from the tier 1 assays in conjunction with the efficacy and potency data can be used to narrow down the number of candidates a reasonable number for more extended characterization, typically up to about five. It must be realized that because of the limited availability of the material at tier 1 (usually from 1 to 3 mg), only basic assays can be performed in order to rule out weak candidates and proceed

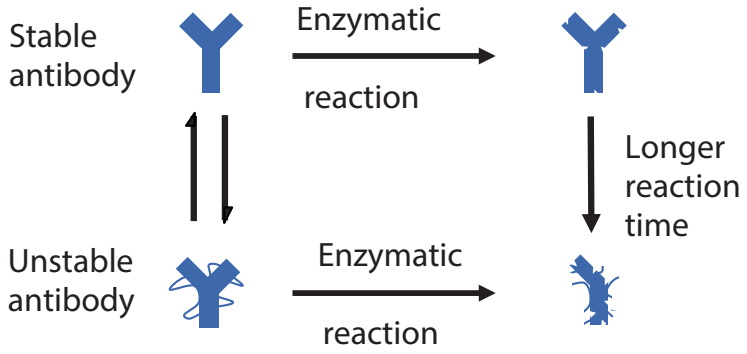

Fig. 5.7 Schematic of enzymatic degradation assay

Table 5.4 Overview of typical tier 1 assay screening results to be used to rank and select molecule candidates according to their predicted development risk

mAb	Solubility (mg/mL)	T (Onset °C)
1	High	Medium
2	Medium	Medium
3	Medium	High
4	High	High
5	Medium	High
6	High	High
7	High	Medium
8	High	Medium
9	High	High
10	High	Low

further with the best ones. Development of a biologic candidate into a successful drug product, however, can be complex and all of the requirements for a target product/molecule profile should ideally be addressed before final candidate selection. For some classes of proteins (e.g., mAbs and related structures) this may be facilitated by defining standardized formulations. The formulation(s) selected should be safe, easy to manufacture, and stable. In addition, the formulation(s) need to be designed for their route of administration and marketability, which takes competition into account. Evaluation of properties that help address all these concerns as early as possible in the drug development process allows resources and effort to be allocated toward only those candidates that show most promise for successful drug development. The purpose of the tier 2 assays is to select the one which satisfies most of the above mentioned desired characteristics and may hence fulfill the basic requirements needed to pass the second stage of candidate selection and hence the requirements to be conferred as a suitable "next-in-class" platform molecule. Even in the event that none of the candidates meet the criteria for a platform molecule, the tier 2 assays none-the-less help to identify a suitable candidate for platform or nonplatform formulation development.

Table 5.5 An example of tier 2 candidate screening assays designed for in-depth characterization of drug-like properties enabling the selection of the lead and backup candidates

Quality attribute	Weight-scale	Analysis	Prior knowledge/benchmark criteria		
Physical stability	10	In vitro serum stability	Biological stability		
			High	Medium	Low
Secondary/ tertiary structure	5	FT-IR and near UV-CD at various pH values	Percentage ß-sheet		
			High	Medium	Low
Intrinsic stability	7	Thermodynamic stability at various pH values	Unfolding temperature		
			High	Medium	Low
Physical stability	10	Freeze-thaw processing at various pH values	Aggregates/particles		
			Low	Medium	High
Shelf (accelerated) stability	10	At low concentration and various pH values SEC and IEX	Loss monomer/main species		
			Low	Medium	High
Shelf (accelerated) stability	10	At high concentration and fixed pH value SEC and IEX	Loss monomer/main species		
			Low	Medium	High
Viscosity	7	At high concentration and fixed pH value and ionic strength	Viscosity		
			Low	Medium	High
Turbidity	5	At high concentration and fixed pH value and ionic strength	Turbidity		
			Low	Medium	High

The aim of the tier 2 studies is to characterize the effect of various formulation variables on different types of degradation reactions that may occur during manufacturing, preclinical studies, and storage of the target drug substance and drug product utilizing fairly low amounts of available protein material (in the authors' experience 30 mg). Table 5.5 shows an example of multiple analytical techniques that can be used toward the identification of an optimum candidate for antibodies and like molecules. A preliminary stability risk assessment based on prior results and literature is used to establish those degradations (and hence assays) that are likely to have the greatest risks of impacting the critical quality attributes. Data in the literature and prior knowledge from internal databases with similar molecules can be used to identify adequate benchmark criteria for the various tier 2 assays (e.g., pH; Kingman and Rajagopalan 2009). In addition, the initial risk assessment is also used to establish the criticality of those modifications identified to have greater risk. A criticality scale for the various assays is provided based on prior knowledge dependent on the importance of the assay to the requisite characteristics of the molecule. A numerical value for the criticality scale of 10 may thus indicate high importance and 1 may suggest that the assay is less critical toward to predict the development risk of a molecule candidate. An overall rank order list for the candidates is finally provided based on the criticality scale of the assay and the results of individual assays for each candidate.

Most of the assays mentioned in the table above are straightforward with regard to their importance and the underlying analytical approach. For mAbs literature shows that most marketed products have been formulated between pH 5.0 and 7.2 (Daugherty and Mrsny 2010; Shire 2009; Saluja and Kalonia 2008). In fact 80% of the liquid mAb formulations are between pH 5.0 and 6.0. This is consistent with the previously mentioned optimum between pH 5.0 and 6.5. In the author's experience the optimal physicochemical stability for most antibodies and comparable compounds that have pI values in the range 7.5–9.0 has been observed to be around pH 6.0. This observation may be used to define the pH values of the formulations that are used in some of the above mentioned assays. Where material availability is limiting a single pH value (e.g., around 6.0) may be selected. Properties requiring high concentrations, such as solubility, viscosity, and high-concentration stability, may then be tested under the selected condition.

For most of the other assays that are conducted it may be feasible to survey a range of pH conditions using prior knowledge and internal databases to help define the conditions. The range would most likely fall within pH 4.0–8.0.

Below is a brief discussion based on the authors' experience of specific analytical methods, the importance of the assays, the design space and the risk assessment.

5.9 In Vitro Serum Stability

Serum is a complex mixture usually composing approximately 85 mg/mL of protein material, including antibodies and a multitude of proteases, (Vitez and Vilma 1958). Since, serum is the final intended environment of the therapeutic, it is reasonable to assume that intra- and intermolecular aggregation and fragmentation that may occur under these high-concentration conditions may alter the efficacy, toxicity, and residence time of a protein in vivo. In addition, although less literature and confirmed information is available, in vivo aggregation, fragmentation, and/or modifications may also affect the immunogenic potential of the candidate molecule. Therefore, it may be valuable to obtain real time information regarding the in vivo stability of new drug candidate by doing in vitro stability studies in serum (Demeule et al. 2009; Correia 2010). Rat or cyno serum may provide an alternative if human serum is unavailable. The chromatographic analysis of the desired protein can be done by utilizing an appropriate labeling method (Leung et al. 1999).

5.10 Integration of the Secondary and Tertiary Structure Elements

It is well recognized in literature that in most reactions that lead to physical instabilities such as aggregation, the rate-limiting step may be the formation of a partially unfolded protein species (Fast et al. 2009; Chi et al. 2003). Maintenance of a proper

secondary structure and/or a compact folded tertiary structure is thus often desirable to mitigate and/or minimize physical instabilities. Rates of deamidation, oxidation, and other chemical degradations have also been found to have significant dependence on the buried nature of the concerned amino acids and hence on the folded nature of the protein molecule (Rivers et al. 2008). The information generated in regard to the structural elements could thus be of immense scientific importance and can help understand the observed/anticipated physical and/or chemical instability issues.

Secondary structure analysis may be performed using techniques such as far UV-CD or FTIR. These techniques need minimal amounts of protein (see Table 5.5) and the wavelength and/or wavenumber scans that are obtained can be deconvoluted to obtained detailed information about the various secondary structural elements (Cai and Dass 2003; Luthra et al. 2007).

Near UV-CD, fluorescence scans, and other techniques can provide information about the environment of the aromatic amino acids of the protein molecule (Kelly and Price 2000). These techniques, although indirect, can still provide valuable information about the compact folded nature of the protein in question. For example, minimal ellipticity in the 260–290 nm region would suggest that the aromatic residues are exposed to the solvent hence possibly indicating absence of a compact tertiary structure.

5.11 Thermodynamic Stability

Although, a clear relation between the thermodynamic stability and the kinetic long-term stability has not been established, it has been recognized that the first step in the process of aggregation is partial unfolding of the protein molecules (Saluja and Kalonia 2008; Vitez and Vilma 1958; Twomey et al. 1995; Kashanian et al. 2008). In the second step, the partially unfolded molecules come together to form aggregates (colloidal stability). Thus, the thermodynamic stability may or may not correlate with the long-term storage stability depending on which of the two steps is rate-limiting. An additional factor that should be considered in such a scenario is the ionic strength of the formulations. Some authors have published data which shows that above certain low-ionic strength conditions (~ 15–20 mM), colloidal stability is more-or-less independent of the pH and ionic strength of the solution (Saluja et al. 2007; Kumar et al.), although there is also considerable literature to the contrary (Chi et al. 2003). Assessing the conformational stability (exposed hydrophobic residues) may be important in estimating/determining the differences in the aggregation rates between formulations. DSC, for example, provides a measure of the overall thermodynamic stability and the results can be correlated and extrapolated to the storage temperatures of interest in some cases. The thermal unfolding studies may therefore form an integral part of candidate screening, and candidates may be differentiated based on the onset of unfolding, midpoints of transitions, and/or any

other thermodynamic parameter obtained using DSC. It is also important to mention the fundamental difference between DSC and the DSF (see tier 1; Goldberg et al. 2011). Although both techniques give similar information, DSC requires much greater amounts of protein and takes much longer (not really a HTS method). In addition, while the DSF data is dependent on the binding of the dye to the hydrophobic patches of the protein molecule, DSC is based on the heat changes that accompany any unfolding process. The fundamental difference of the techniques may result in differences between the two data sets.

5.12 Freeze-Thaw Stability

Since, proteins are susceptible to cold denaturation, unfolding at ice–water interfaces and degradation during freeze-concentration, it is important to study the impact of typical freeze-thaw steps usually employed during processing and handling of the drug substance and or the drug product (Lazar et al. 2009; Bhatnagar et al. 2007). An early screening of freeze-thaw susceptibility can also provide feasibility information concerning a lyophilized formulation. Typical assays that can be done to evaluate the formation of aggregates during freezing and thawing include size exclusion chromatography, $UV_{280,}$ and subvisible particle measurement using light obscuration, coulter counter, microflow imaging, and/or other techniques (Sharma et al. 2010; Huang et al. 2009). Though many antibodies are resistant to freeze-thaw stress, it is more common to observe an increase in the number of subvisible particles with multiple freeze-thaw steps (Barnard et al. 2011). Rising expectations by authorities to provide subvisible particle data in recent years (Carpenter et al. 2009) has greatly enhanced the importance of an early detection of freeze-thaw susceptibility.

Drug substance is typically stored frozen under a variety of conditions including containers such as stainless steel tanks, bottles, and cryo bags and temperatures that can range from −20 to −80 °C. Frozen storage at temperatures in the range of −20 to −30 °C may require additional characterization or prior knowledge concerning excipients that may cause problems at such storage temperatures. Sodium chloride for one is known to form a eutectic mix with water at −21.2 °C (Lashmar et al. 2007). Considering that most antibodies have been observed to have a cold denaturation temperature of less than 0 °C, freeze concentrated protein in a eutectic mix is of concern. Similarly, mannitol containing formulations stored at −20 °C have been observed to have a greater freeze-thaw degradation susceptibility (Izutsu and Kojima 2002), presumably due to crystallization of that reagent during storage, which has also been reported for trehalose at −20 °C (Singh et al. 2011) and sorbitol at −30 °C (Chengbin et al. 2007).

5.13 Accelerated Stability at Various Concentrations

A key performance criterion for any formulation is to achieve sufficient shelf-life stability for a drug product formulation. mAbs, bispecific Ig's and novel Fc fusion proteins that are increasingly being utilized for the treatment of immunological and oncological disorders constitute a major portion (>60 %) of the total protein drugs currently in clinical and preclinical testing (Dierdre et al. 2007; Morhet 2008). Since many diseases that are being targeted by these relatively low-potency proteins (need higher and/or frequent dosing) are chronic, providing an at home administration option to patients by the subcutaneous or intramuscular route is needed to enable the therapy or increase patient compliance. In many cases, high dose requirements combined with the volume limitation (~1.5 mL) for these delivery routes necessitates a high-concentration formulation (defined here as >100 mg/mL; Liu et al. 2005). Development of stable, easy to use, high-concentration liquid protein formulations poses several challenges including those associated with the issues of aggregation, viscosity, and solubility. Nonideality leads to enhanced protein–protein attractive interactions which increases the propensity of the molecules to aggregate. It has been shown that aggregation and concentration usually have a nonlinear relationship, and that aggregation can increase tremendously with an increase in the concentration of the protein (Saluja and Kalonia 2008). For proteins for which at home administration is anticipated, it is highly recommended that aggregation propensity be evaluated at a range of concentrations very early during preclinical development. Typical analytical assays that can be used to assess the instabilities may include size exclusion chromatography, UV_{280} and ion exchange chromatography.

5.14 Viscosity/Solubility

Viscosity/solubility evaluation can be performed during tier 2 if the material availability at tier 1 stage is limited. Around 100 μL of high concentration material (>50 mg/mL) is sufficient to perform a multitude of critical studies. The concentrated material can be used to assess the overnight solubility at 5ºC under appropriate pH and other conditions. The same material can then be used to evaluate the viscosity using any of the low volume, nondestructive techniques such as ultrasonic rheometry or viscometry (Yadav et al. 2009; Saluja and Kalonia 2004). Finally, the sample may be utilized for accelerated stability testing.

5.15 Risk Assessment and Ranking

CMC risks associated with the manufacturing and development of candidates can be appropriately defined based on prior knowledge including literature and available internal databases for a wide variety of candidates and molecules. The

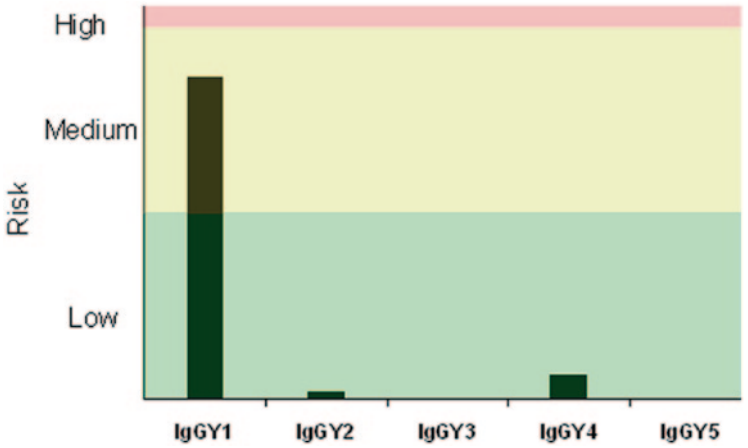

Fig. 5.8 Shelf stability under accelerated conditions for five commercially available mAbs at 60 mg/mL and pH 6. Risk assessment for accelerated studies can thus be based on prior knowledge with such external and internal stable and unstable molecules. Color codes represent risk factor for CMC development

candidates can be assessed as low, medium, or high risk (green, yellow, or red flag) of development based on the evaluation. For example, a certain percentage of aggregates formed in a predetermined time period (e.g., 7 days) at a predetermined elevated temperature (e.g., 40 °C) may be used as a criterion to define the aggregation risk for the candidates. Figure 5.8 shows an example in which several commercially available mAbs were evaluated for stability at 60 mg/ml at pH 6.0. The study was done to define the risk assessment for internal candidates. Figure 5.9

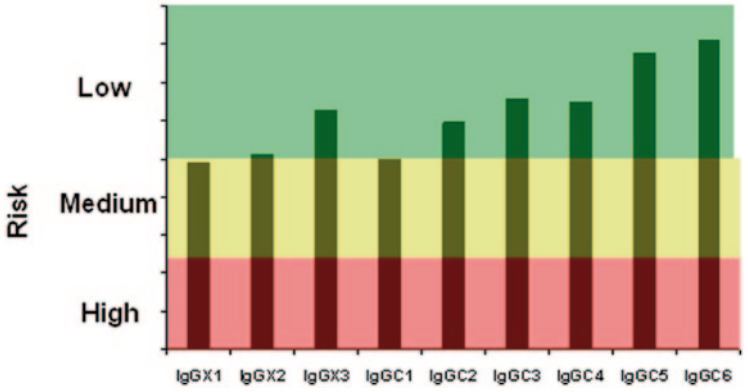

Fig. 5.9 Thermodynamic stability as assessed by DSC for three IgG candidates (IgG-X1, IgG-X2, and IgG-X3) at 1 mg/mL and pH 6. Risk assessment is based on competitive knowledge with 6 marketed IgG molecules (IgGC1-IgGC6). Color codes represent risk factor for CMC development. The average onset of unfolding for the marketed molecules is 59 °C

Table 5.6 Standard tier 3 assay program to determine drug-like properties of the lead and backup molecule candidate in platform formulations to be used for toxicity studies and bulk drug substance

Quality attribute	Weight-scale	Analysis performed/formulation	Evaluation technique	Prior knowledge/ benchmark criteria		
Physical/ chemical stability	10	Freeze/thaw stability (up to 100 mg/ml)	SEC/IEX	Aggregates/particles		
				Low	Medium	High
Physical/ chemical stability	10	Stability in quiescent solution (up to 100 mg/ml)	SEC/IEX	Loss monomer/main species		
				Low	Medium	High
Physical/ chemical stability	10	Stability against shaking stress (up to 100 mg/ml)	SEC/IEX/ OD_{500}	Aggregates/particles		
				Low	Medium	High

shows a similar evaluation for in-house Abbott candidates in a different study that were taken through a thermodynamic stability assessment. The risk factor was again based on a previously generated database with marketed IgG molecules. Two of the candidates evaluated in this study were predicted to have a moderate risk of thermal stability; IgGX3 was predicted to be highly stable in this regards.

Based on the criticality of the assay, an overall risk ranking for the candidates that have been taken through the tier 2 assays can be provided. In this example, a simple evaluation may be based on the multiplication of the rank obtained in individual assays by the criticality factor and summing the overall score.

5.15.1 Tier 3 Assays—Development Platform Justification Studies

1. *Long-term stability assessment*
2. *Justification of prior knowledge for lead candidate*

The aforementioned tier 2 studies are typically performed to aid in the selection of a lead candidate, support subsequent in vivo evaluation, and explore the composition of a pH 1 drug substance formulation. In tier 3, larger quantities of the candidate are manufactured for non-GLP PK studies. In addition, CHO cell line development is initiated for the large-scale GMP manufacture of the lead drug substance for clinical trials. In both cases, the concentration of the drug substance may be set at high concentrations. In the authors' experience, dosing for PK studies may require up to 100 mg/ml, especially for subcutaneous administration. In the latter, the lead drug substance may range from 70 to 100 mg/ml once bottled, although transient concentrations during manufacture can reach as high as 150 mg/ml. Both processes employ specific platform formulations as the drug substance vehicle.

Therefore, the purpose of the tier 3 assays is to assess the stability of the drug substance at low and high concentrations and within the standard platform formulations. Table 5.6 details the experiments performed.

The experiments and evaluation techniques are similar to those performed during tier 2. They include stability assessment of the lead candidate in the platform tox formulation vehicle(s) at the drug substance concentration(s) required for the tox studies. It also includes stability assessment of the drug substance in the drug substance buffer at 100 mg/ml and at the target pH recommended by the tier 2 assays and at pHs ± 0.5 of that pH. This allows us to fine-tune the optimal pH for stability and also provides stability data to justify the target pH range during GMP drug substance manufacturing. Quiescent stability is assessed at storage conditions of + 5 °C. In addition, accelerated quiescent stability studies at + 40 °C, freeze-thaw studies, and shaking studies are also performed to verify the robustness during the various fill and finish unit operations. SEC and IEX are used primarily to evaluate the degradation due to stress. However, orthonogonal methods may also be used. These include light scattering and particle sizing for aggregation and association and SDS-PAGE and CE-SDS for chemical stability such as cross-linking or fragmentation.

The results of these studies provide the information needed to verify that the molecule meets the TMP and can be considered a "next-in-class" molecule, thus enabling the use of already established platform processes. If the drug-like properties assessment reveals an issue, the molecule might be still considered to be processed by the established platform processes, if robust enough. Otherwise an extended formulation and/or process development will be required to ensure good product stability. In either case, further studies must be carried out to test drug-like properties at the new conditions.

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Chapter 6
Approaches for Early Developability Assessment of Proteins to Guide Quality by Design of Liquid Formulations

Bernardo Perez-Ramírez, Nicholas Guziewicz, Robert Simler and Alavattam Sreedhara

6.1 Introduction

The quality of a liquid formulation has a major impact on clinical performance and drug product manufacturing. Therefore, development of liquid formulations for therapeutic proteins should be focused on quality from the beginning—prior to the transition of a molecule into development. The International Conference for Harmonisation (ICH) guideline Q8(R2) Pharmaceutical Development states that quality cannot be tested into a product; that is quality should be built in by design (ICH 2009). This guideline is the basis of Quality by Design (QbD). Several QbD milestones need to be specifically addressed by the formulator. First, the main instabilities of a candidate molecule can be discerned. The identification of these preliminary critical quality attributes (pCQA) enables the design of proper stabilization and control strategies during formulation development. Second, multiple candidate molecules of comparable biological function can be screened by the formulator to evaluate their stability with respect to the pCQAs during storage and other pertinent stresses. This "manufacturability" assessment best ensures that a molecule with

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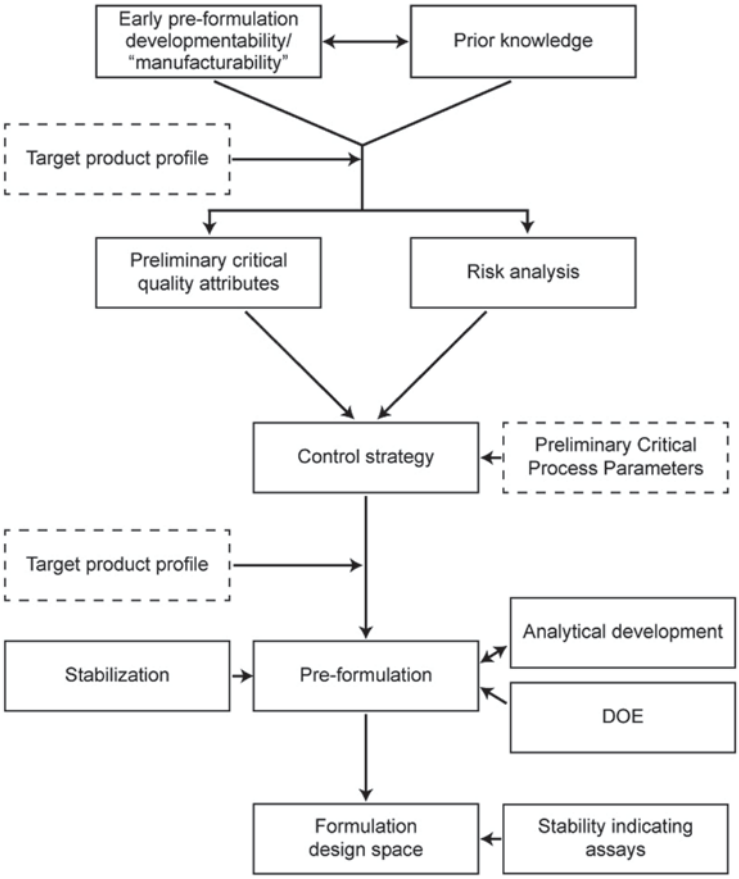

Fig. 6.1 Schematic representation of the essential elements in a QbD strategy for protein liquid formulations (*dashed-line boxes* represent parameters that may not be totally defined)

robust stability is carried forward while candidate molecules with compromised stability profiles that would jeopardize success later in development are eliminated.

Figure 6.1 illustrates schematically the essential components of QbD for liquid formulations for protein therapeutics that will be discussed in this chapter. Early involvement by the formulator is critical to ensure that QbD principles be successfully implemented. Strengthening the interface between the formulation group and the discovery research group is crucial for successful early preformulation and identification of pCQAs. Leveraging this interface allows for a mutual understanding of the needs of each group that can dramatically improve the process of identifying good candidates to move into development. Ideally, the formulator should become involved during the molecular biology research and initial purification efforts of new protein candidates (Perez-Ramirez et al. 2010). Candidates for clinical development should be chosen not only for their biological behavior but also their potential for manufacturability.

Conducting preformulation at this early stage is challenging since the amount of protein available for formulation research is usually limited, often in microgram or milligram quantities. The availability of protein specific analytical methods is also limited at this stage. The use of methods that are based on fundamental physical principles that require minimal or no development is thus preferred. Analytical methods that can monitor changes in physical and chemical stability that can provide a global fingerprint of the candidate proteins are helpful in early development studies and are discussed in this chapter.

Development of liquid formulations for antibodies has become an established practice due to the extensive prior knowledge accumulated. We know, for example, that from a stability standpoint, an IgG1 scaffold is preferred to that of an IgG4, as it has a lower propensity for hinge-region fragmentation (Aalberse and Schuurman 2002). There is also a need to re-engineer particular residues to eliminate chemical instabilities such as deamidation linked to losses in biological activity (Wakankar et al. 2007b). Nevertheless, proteins—even antibodies—are unique in terms of solubility and stability. Although sharing many of the same structural features in the nonconserved regions of the antibody, the complimentarily determining regions (CDRs) are always unique and have important "hot spots" (e.g., deamidation, isomerization, oxidation). Due to this uniqueness, generic approaches to protein liquid formulation development utilizing only prior knowledge are of limited value. Instead, risk assessments that take into account prior knowledge as well as experimental data obtained in the laboratory can be an important tool in the identification of critical quality attributes (CQAs).

The quality target product profile (QTPP), including route of administration and expected shelf life among other parameters, should be defined during the marketing research evaluation to aid in the QbD assessment. If a working QTPP is available at this early stage, it can help guide early preformulation. For example, an important factor to take into consideration is whether there is a need to combine the liquid formulation with a delivery system to improve local retention. Such a scenario presents an additional set of challenges in that the impact of the delivery system on the active protein needs to be considered and the formulation strategy should be reevaluated during the manufacturability assessment. Such considerations for drug delivery systems will be discussed in detail. However, since QTPPs are generally living documents and tend to change as more information is gathered as the project proceeds, QTPPs are not often available during early preformulation research and the formulator must often make several assumptions to proceed with development.

During early preformulation development, the manufacturing process is evolving and not yet fully developed. As a consequence, specific critical process parameters (CPPs) are limited or lacking. The identification of CPPs becomes the main goal during late stage formulation development. While this limitation does not preclude the identification of pCQAs that is the main goal during early formulation development (Fig. 6.1), knowledge about CPPs can be implemented as such knowledge becomes available to guide preformulation research. In this chapter, we will provide examples of some general CPPs that can be implemented into the QbD approach to liquid formulations.

Selection of appropriate excipients for a formulation is also important since many of the excipients employed in formulation development may interact with each other or with the protein of interest. In order to understand potential interactions, a design of experiments (DOE) approach could be employed. The DOE strategy can vary depending on the number of parameters to evaluate. Thus, screening DOE such as fractional and full factorial DOE may aid during preformulation research as well as during the optimization of the dosage forms. This chapter describes our approach to QbD and the advantages of a DOE approach to preformulation development of liquid formulations.

6.2 Early Preformulation Research and Risk Assessment for Liquid Formulations

6.2.1 Using Prior Knowledge to Initiate Preformulation

Preliminary understanding of the solution behavior and stability profile of a candidate molecule leads directly into the identification of preliminary CQAs (pCQAs) for the candidate molecules. These are referred to as "preliminary" because they can be defined based on the accumulated laboratory experience, preclinical, clinical, and manufacturing and process knowledge from developing liquid formulations in the biopharmaceutical industry. This body of information is referred in the ICH guidelines (Q6B, Q8(R2), and Q9) as prior product knowledge (ICH 1999; ICH 2005; ICH 2009). In the absence of a final QTPP at this early stage, pCQAs are identified in a risk assessment using the results emerging from preformulation research, as will be discussed later.

In one example, thermodynamic characterization by differential scanning calorimetry (DSC; Guziewicz et al. 2008), has resulted in a database of information for the melting temperatures of IgG molecules. We can see that IgG molecules with melting temperatures in the Fab region > 70 °C (a pCQA) are preferred for optimal liquid formulation stability. In another case for subcutaneous (SC) dosage forms of a monoclonal antibody (mAb) where high protein concentrations (> 50 mg/mL) and minimal injection volumes (0.5–1.0 mL) are required (not discussed here), the focus was on preventing and managing shear stress, aggregation, and self-association linked to solution viscosity (a pCQA; Hall and Abraham 1984; Liu J et al. 2005). Additional examples of pCQAs include aggregation, fragmentation, and chemical instabilities such as oxidation, deamidation, and isomerization that impact potency.

Identifying modes of degradation is critical to the development of a proper formulation strategy (Perez–Ramirez et al. 2010). Some of the most common parameters evaluated that impact stability are pH, ionic strength, freeze/thaw, shear, and temperature stresses. Prior knowledge is valuable to help narrow what can be tested experimentally. For example, proteins have limited stability as a function of pH, temperature, and other solution variables; therefore, it is not necessary to include

extreme conditions, such as pH 3 or 11, in the evaluations. Most biopharmaceutical proteins, and in particular antibodies, will show better stability between pH 5 and pH 7. Thus, the design space, or process range, for an antibody liquid formulation, will not oscillate far from the pH range indicated above.

6.2.2 The Interface Between Discovery Research and Formulation Development in Successful QbD

To maximize the benefits from a QbD risk assessment approach for liquid formulation development, it must be initiated at the drug discovery stage. The formulator will start to become familiar with the solution behavior, stability profile, pCQAs, and the potential challenges (e.g., high viscosity, chemical hotspots, etc.), that the candidate proteins may present. By incorporating the formulator in the discovery research team, valuable advice can be provided in terms of initial handling of the protein and the selection of storage buffers. This type of intervention will prevent the use of low purity or degraded material in biological screening studies and will maximize the odds of identifying robust candidates. Finally, and perhaps most importantly, this approach will provide an additional set of criteria by which to screen candidate molecules, assuming several molecules are identified with comparable efficacy for a given target.

At the discovery research phase, purified protein is typically at a premium. This presents several challenges to the formulator to institute QbD principles. For a successful iterative development interface, the formulation team must be highly trained for the handling and characterization of sub-milligram amounts of protein (see Sect. 1.2.3). As cell culture and purification processes have yet to be defined, the formulator must also be prepared to deal with the dynamic nature of the final product. It is imperative to realize that the data obtained at this stage will not be absolute, but rather serve as relative indicators of stability trends. Optimized studies will likely need to be executed when larger quantities of more highly purified material are available. The final consideration for the formulation/drug discovery interface is the lack of product specific assays. Again, the formulator needs to rely on experimental approaches that not only minimize the amount of protein consumed but also utilize generic probes of physical, chemical, and functional stability (Perez-Ramirez et al. 2010).

Physical stability typically refers to changes in secondary, tertiary, or quaternary structure without affecting chemical bonds. Ideally the discovery group will provide to the formulator the primary structure and posttranslational modifications of the protein candidates. This information can be rapidly obtained using intact mass spectrometry analysis (Hayter et al. 2003). Primary structure determination is not typically of much concern to the formulation development scientist (except for typical chemical reactions such as deamidation and isomerization where primary sequence plays a big role in addition to tertiary structures). Changes in protein secondary structure are not usually very discriminating when comparing several

candidate proteins for liquid formulation development. Most of the changes relevant to liquid formulations occur at the surface of the protein and are reflected in disruptions in tertiary and quaternary structure (Perez-Ramirez et al. 2010). Nevertheless, far ultraviolet (UV) circular dichroism (CD) can be employed to monitor potential changes in secondary structure as a function of solution variables such as pH, ionic strength, and buffer composition utilizing minute quantities of protein (Simler et al. 2008; Perez-Ramirez et al. 2010). Fourier transform infrared spectroscopy can provide similar information, but the low sensitivity of the technique requires large amount of protein, making the approach impractical for use in support of early discovery research. Analysis of the tertiary structure remains the most predictive approach for long-term stability. Both near UV CD and fluorescence spectroscopy can be used to probe tertiary structure. While not a direct probe of secondary or tertiary structure, DSC is an invaluable tool for characterizing global structural stability (Guziewicz et al. 2008). Finally, the tendency of a protein to oligomerize and aggregate must be evaluated (see Table 6.1). While asymmetrical field flow fractionation (Kowalkowski et al. 2006) and analytical ultracentrifugation (Perez-Ramirez and Steckert 2005) are powerful techniques under appropriate circumstances, size exclusion chromatography coupled to multiangle laser light scattering (SEC-MALLS) remains the workhorse technique for quaternary structure characterization (Li Y et al. 2009) due to its high-throughput capabilities (HTP).

Chemical stability encompasses the mechanisms of degradation in which chemical bonds are made or broken in the presence of water. Chemical instabilities are most concerning when they occur in critical areas of the protein that impact target binding and biological function. While less concerning, chemical modifications in noncritical areas of the protein are still important as they may lead to subtle changes in structure impacting oligomerization propensity or immunogenicity (Kumar et al. 2011). Peptide mapping combined with mass spectrometry (PM/MS) is the definitive approach for analyzing the chemical stability profile of a protein (Hayter et al. 2003). Standardized digestion procedures, advances in software analysis, minimalist material requirements, and near universal application make this the ideal technique. While PM/MS has become the gold standard, alternate techniques such as isoelectric focusing (IEF) polyacrylamide gel electrophoresis, capillary isoelectric focusing, and reverse phase chromatography can also be used to probe various aspects of chemical stability.

Finally, methods need to be employed to link potential chemical and physical instabilities to some measure of biological effect. In vivo studies and cell-based in vitro experiments cannot practically serve this purpose due to their low throughput. Binding analysis based on surface plasmon resonance or enzyme-linked immunosorbent assay techniques provide a viable high-throughput alternative to these biological assays. Nevertheless, cell-based assays are typically needed for late stage formulation development.

Table 6.1 Analytical paradigm for apparent manufacturability potential evaluation

Stability	Analytical method	Attribute
Physical	Near-UV-CD	Tertiary structure
	Far-UV-CD	Secondary structure
	DSC	Thermal stability
	SEC	Aggregation/fragmentation
	SDS-PAGE	Aggregation/fragmentation
	MALLS	Molar mass distribution
	Turbidity	Precipitation-visual appearance
	DLS	Oligomerization
	MFI	Subvisible particles
Chemical	IEF/cIEF	Charge heterogeneity
	PM/MS	Chemical integrity
Apparent function	Surface plasma resonance (Biacore)	Functionality

CD circular dichroism, *DSC* differential scanning calorimetry, *DLS* dynamic light scattering, *IEF/cIEF* isoelectric focusing/capillary isoelectric focusing, *MALLS* multiangle laser light scattering, *MFI* microflow imaging, *SDS-PAGE* sodium dodecyl sulfate polyacrylamide gel electrophoresis, *SEC* size exclusion chromatography, *PM/MS* peptide mapping-mass spectrometry, *UV* ultraviolet

6.2.3 The Role of Stress Studies and Manufacturability Assessments in Identifying Critical Product Attributes

In accordance with ICH Q8(R2), Q9, and Q10 (ICH 2005; ICH 2008; ICH 2009), risk analysis for developing a liquid formulation is performed to identify instabilities and obtain a process understanding with the ultimate goal of generating proper process controls. The typical research and development timeline is not long enough to make formulation development decisions based on real-time storage data. Properly executed stress studies provide a means to identify risks and guide the formulation strategy more rapidly. Temperature is a common variable used to stress proteins and help identify CQAs. For these studies to provide meaningful data, proper elevated temperatures should be chosen to provide assurance that stability trends are consistent across storage temperature. A useful approach is characterization of the thermal stability of the candidate proteins by DSC to identify proper stress temperatures (Guziewicz et al. 2008; Perez-Ramirez et al. 2010). For example, measurements of onset temperatures of calorimetric transitions as opposed to melting temperatures can help guide the selection of proper temperature for accelerated studies (Guziewicz et al. 2008). This minimizes the likelihood of enabling irrelevant degradation pathways resulting from partial unfolding at higher temperatures (Guziewicz et al. 2008; Perez-Ramirez et al. 2010). In general, mAbs (and complex proteins with multi-domains) may have many transitions as observed by DSC, making the interpretation of the data challenging in some instances. In addition to guiding stress temperature studies, thermodynamic analysis can also be predictive of long-term physical stability, as stated earlier. Similar information can be obtained

at very early stages in development by thermal melts employing CD or fluorescence spectroscopy incorporating single value decomposition analysis (Hayter et al. 2003; Guziewicz et al. 2008; Perez-Ramirez et al. 2010).

Although, DSC, CD, and fluorescence spectroscopy are key predictive techniques early in development, they cannot be used alone. The identification of the pCQAs requires a concerted analysis of physical, chemical, and functional responses to stress conditions that mimic processing steps. At this early stage, CPPs have usually not yet been identified. However, some general process parameters, such as freeze/thaw, exposure to extreme pH conditions, and shear stress can be assumed to be process parameters that will be encountered during the projected life of the protein. Prior to transitioning a molecule to formulation development, the top (biologically comparable) candidates emerging from discovery research (see Sect. 1.3.1) should undergo a thorough develop ability/manufacturability analysis to assess their stability with respect to these process relevant stresses.

This preprocess development information will be useful to the formulator developing liquid dosage forms and can constitute the database for large-scale process development and future comparability assessments. Although it is difficult to directly extrapolate from laboratory scale to what will happen during manufacturing of the drug product, some approximations can be obtained making worst case scenario assumptions. It is not uncommon for therapeutic proteins to be exposed to low pH as part of viral inactivation, shear stress during ultrafiltration/diafiltration, compounding, fill finish and shipping, and freeze/thaw during in-process hold steps and shipping. These stresses can be approximated in the laboratory and their impact on the physical, chemical, and biological integrity of the candidate molecules can be evaluated. The goal of this approach is twofold. The first is to identify CQAs that may be detrimental to the development of a given molecule. The second goal is to rank candidates based on the stability response to various stresses and identify the most suitable ones to move into development. A manufacturability case study highlighting this QbD approach to screening several candidate mAbs for one biological target is summarized below.

6.2.4 Case Study: Early Preformulation and Manufacturability of a Humanized Antibody

The antibody humanization process, in which murine framework residues are replaced with human framework residues, can easily produce a large number of biologically comparable molecules. Rapid biophysical screening of these mutants can help to quickly screen out structurally destabilizing mutations. After confirmation of comparable oligomerization states by SEC-MALLS (not shown), DSC analysis was conducted on four mAb-C humanization mutants (Fig. 6.2). Where C-1, C-2, C-3, and C-4 are permutations of two neighboring residues, hydrophilic and aliphatic.

Antibodies mAb-C-1 and C-2 showed typical three-domain unfolding of F_{ab}, F_cC_H3, and F_cC_H2, consistent with mAbs (Fig. 6.2a). No differences in thermal

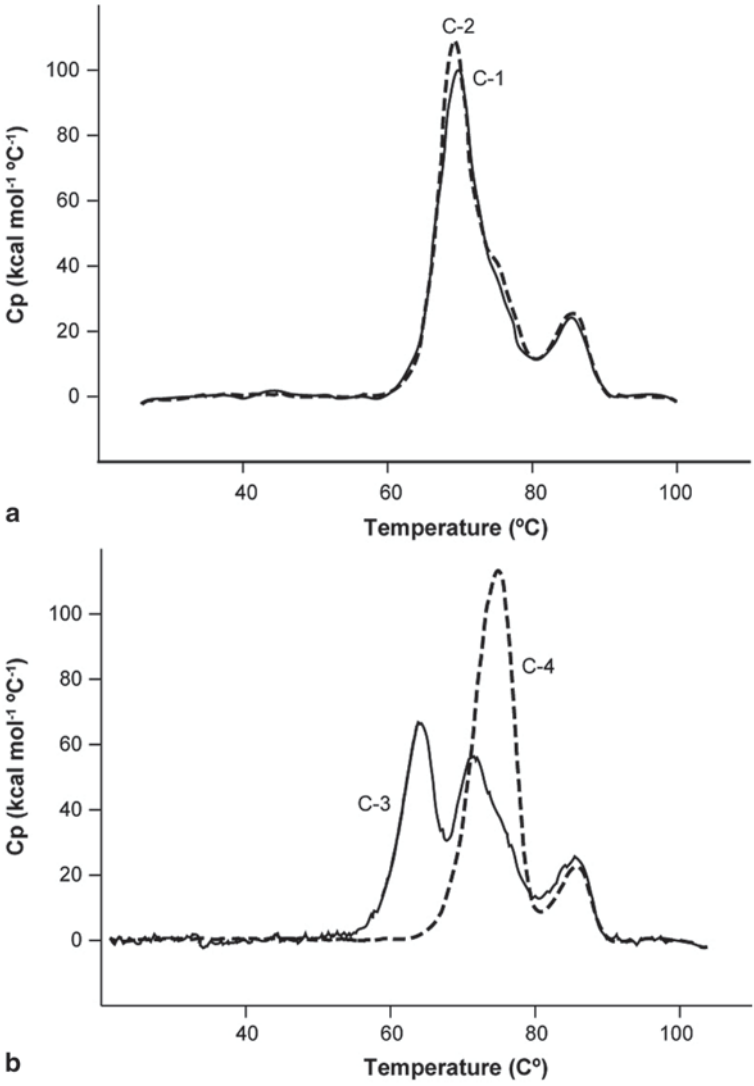

Fig. 6.2 Differential scanning calorimetry thermograms of four human IgG1 monoclonal antibody CDR humanization mutants. The constructs differ in two residues by permutations of aliphatic and hydrophilic residues: **a** hydrophilic/aliphatic (C-1) and aliphatic/hydrophilic (C-2) substitutions, **b** constructs with hydrophilic/hydrophilic (C-3) and aliphatic/aliphatic (C-4) substitutions

stability were observed between these two mutants. Antibody C-4 showed a typical unfolding profile, only with the F_cC_H3 transition buried beneath the large F_{ab} transition due to its increased stability (Fig. 6.2b). In contrast, the unfolding characteristics of mAb-C-3 were dramatically altered. Four unfolding transitions were observed. Utilizing a non-2-state modeling approach, enthalpy analysis (data not

shown) confirmed that the mAb-C-3 F_{ab} domain undergoes a 2-transition unfolding process. This is highly unique, and indicative of significant destabilization of the F_{ab} domain as a result of the mutation. Altered structural stability in mAb-C-3 was confirmed with far UV CD analysis (Fig. 6.3). Altered β-sheet features were observed in mAb-C-3 compared with the other mutants. While functionally comparable, mAb mutant C-3 had a clearly destabilized structure, causing increased aggregation propensity. This characteristic makes mAb-C-3 an undesirable development candidate, and as such, it was eliminated.

When the number of candidate molecules is limited to fewer than ten, a more detailed screening process can be applied. Comparing candidate molecule behavior across a wide range of pH values can identify key instabilities and provide insight into the pCQA. Far UV CD spectra are shown in Fig. 6.4 for three candidate antibodies for same receptor target as a function of pH. Based on the UV spectra, there was no significant impact on secondary structure across most of the pH range for all three antibodies. At the low pH extreme, all of the antibodies demonstrated some level of partial unfolding. The partial unfolding was minimal and comparable for mAb-A and mAb-C. In contrast, mAb-B was found to be most sensitive to low pH demonstrating near complete unfolding.

Similar pH response curves were generated using DSC (Fig. 6.5). Consistent with the CD results, mAb-B F_{ab} thermal stability was lowest of the three antibodies. Additionally, the thermal stability of the mAb-B F_{ab} domain was most sensitive to changes in pH. The structural data obtained from CD and DSC analysis implies that mAb-B has decreased physical stability compared to the other two mAbs.

A wide range of manufacturing stress studies can be designed based on time, material availability, and potential hot spots identified by inspection of the primary structure of the candidate proteins. In this particular example, four stresses were employed: low pH viral inactivation, shear, freeze/thaw, and elevated temperature. Viral inactivation was mimicked in the laboratory by adjusting the pH of the antibody solution to pH 3.8, holding for 90 min, and subsequently adjusting back to pH 6.28 (Guziewicz et al. 2008). Shear stress can be induced simply by repetitive pipetting of the antibody solution (50 cycles × 1 mL pipette). If larger volumes of protein are available (~4 mL), then a small-scale tangential flow filtration recirculation experiment provides a more elegant means of inducing process relevant shear stress. Freeze/thaw stress was induced by exposing the protein to five cycles of freezing at −80 °C and thawing at room temperature at the working pH 6 identified by DSC. Finally, the candidates were also evaluated in terms of stability at 37 °C for up to 4 weeks. This analysis was conducted with only 12 mg of each protein candidate, and it could easily be optimized for higher material availability. For example, more material allows two pHs incubations to be done and identify degradation pathways that could be differentially populated at different pH. Selective assays were employed to provide insight into the physical, chemical, and functional stability response to these stress conditions.

A brief review of the critical results shows that mAb-B possesses decreased physical stability as it was the only antibody to aggregate over the 4-week incubation as shown by SEC—high performance liquid chromatography (HPLC) (Fig. 6.6) and in

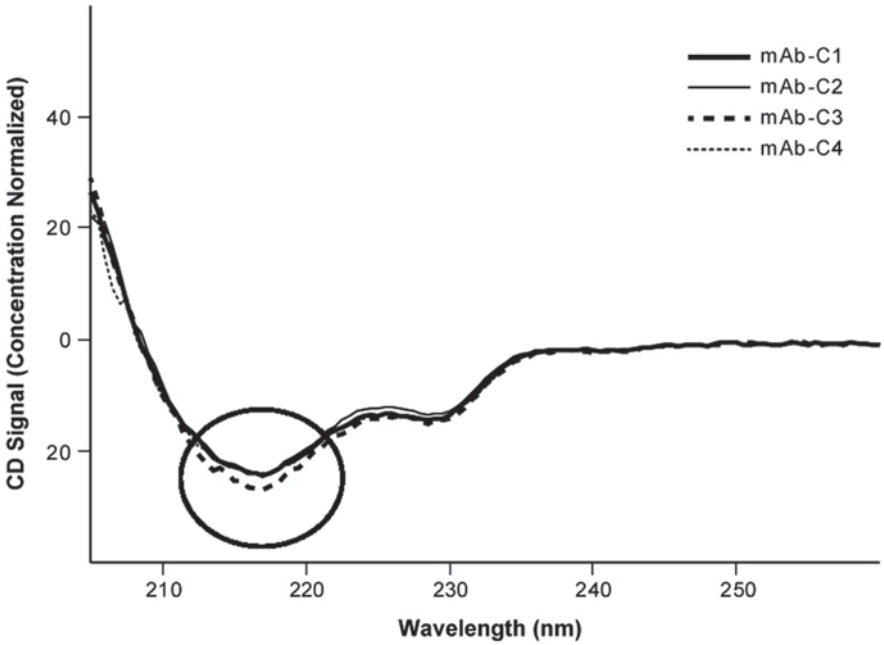

Fig. 6.3 Far UV circular dichroism spectra of four human IgG1 monoclonal antibody CDR humanization mutants (see legend of Fig. 6.2 for details)

response to low-pH viral inactivation (not shown). In contrast, a decrease in target binding affinity was observed in mAb-C after 4 weeks at 37 °C (not shown). An IEF gel showed a significant shift in the banding pattern at 37 °C over the same time period (Fig. 6.7a) consistent with the formation of acidic isoforms. Subsequent PM/MS analysis confirmed the charge heterogeneity as deamidation of an asparagine residue (Fig. 6.7b). Based on the primary structure, this asparagine was located in the CDR, clearly impacting target binding.

After all the stress response information is collected for the different constructs, it is then categorized based on the possible level of impact on manufacturability. An example of the analysis summary is shown in Table 6.2. From this analysis, it can be seen that in terms of physical stability, mAb-C is most desirable whereas mAb-B demonstrates compromised physical stability. The chemical stability of mAb-C, however, has highly undesirable properties in response to elevated temperature—a function—critical residue susceptible to deamidation. Based on the lack of negative response to all stress conditions, mAb-A represents the most desirable candidate to transition into formulation development.

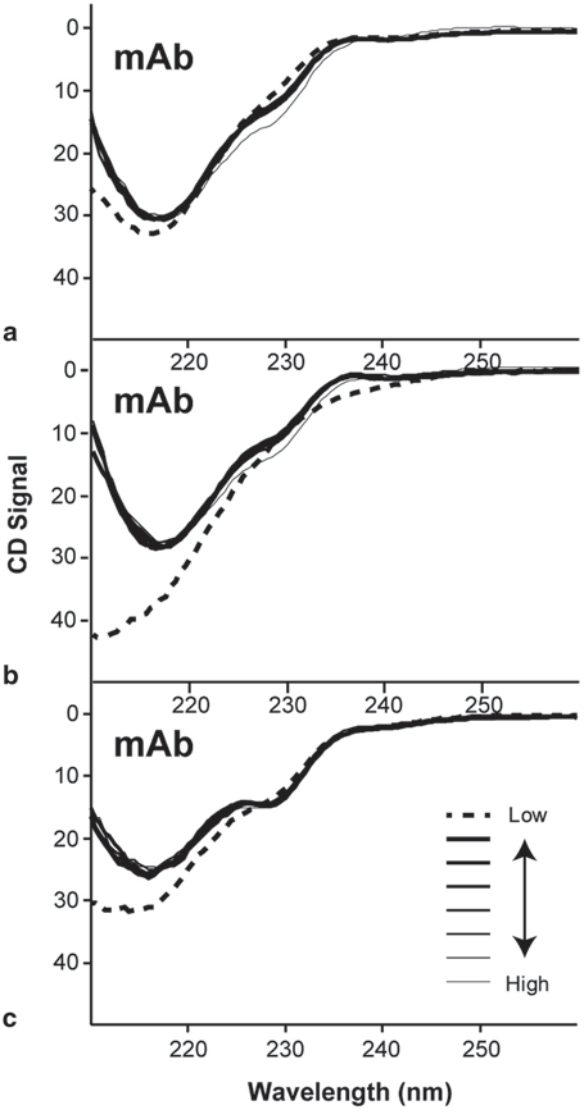

Fig. 6.4 Far UV circular dichroism spectra of human IgG1 monoclonal antibody candidates as a function of pH from low to high; mAbA, mAbB, and mAbC recognize the same target receptor

6.2.4.1 The Role of Risk Assessments in Identifying pCQAs

Often the analysis of experimental data is not simple and other parameters need to be taken in consideration, such as the severity of a particular instability affecting the potency. The severity should also be linked to the probability that the given instability becomes rate limiting in the development process. Under those circumstances, it is also useful to perform a risk analysis to formally quantify the risks and help

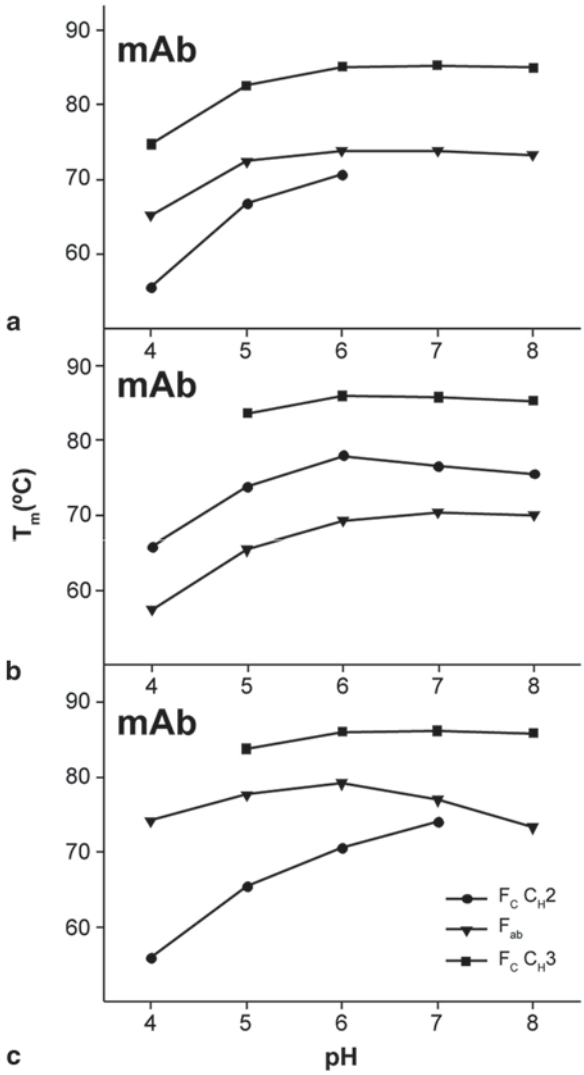

Fig. 6.5 Differential scanning calorimetry thermograms of human IgG1 monoclonal antibodies mAbA, mAbB, and mAbC as a function of pH

the formulation development strategy. A failure modes and effects analysis tool can be used to rank the importance of factors based on probability, severity, and ability to be detected (Stamatis 2003). For example, an acid catalyzed degradation like aspartic acid isomerization to iso-aspartic acid has a high probability to occur if the pH of the formulation is within a particular region of the acidic range (Wakankar and Borchardt 2006; Wakankar et al. 2007a). If this instability is not linked to a loss in potency and/or biological activity then the severity will be low to medium and may be controlled through a specification (after examining several lots, understanding the assay limitations and a full evaluation of the stability profile), allowing for a manageable risk (Fig. 6.8). Conversely, if the isomerization reaction is linked to

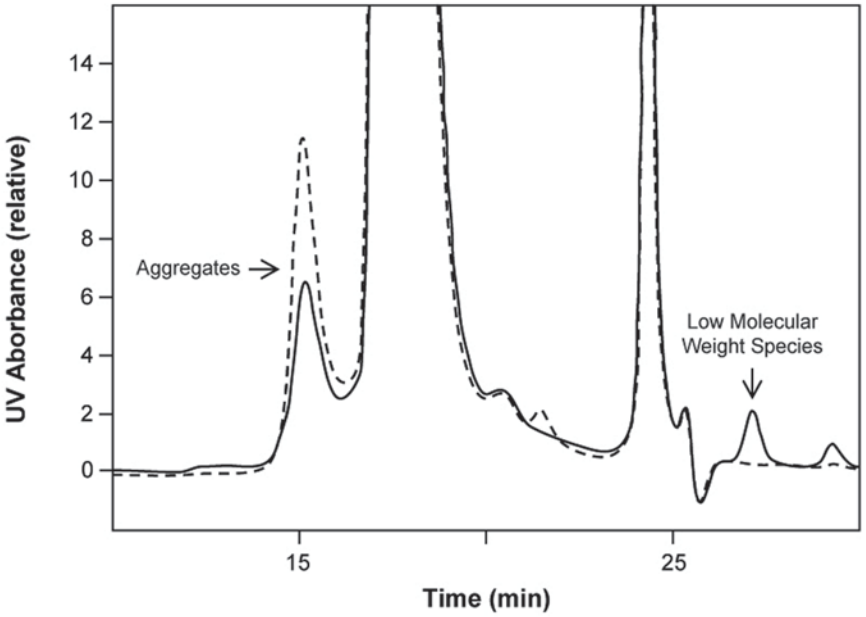

Fig. 6.6 Size exclusion chromatograms of mAb-B after storage at pH 6 for 4 weeks at 4 °C and 37 °C

a loss in potency, then the severity in the risk analysis will be high, in such a way that probability × severity will give a final score of an overall high risk (Fig. 6.8) wherein either a specification is required or a change in construct is deemed necessary. The decision to select a different protein construct that could eliminate the instability linked to loss in potency could potentially have impact to project timelines and needs to be carefully assessed. By performing the comparability stress response analysis before the candidate proteins are in development, the formulator has options to create a better dosage form for either toxicology and/or first in human studies.

6.3 The Quality Target Product Profile and Its Impact on QbD

6.3.1 The Importance of the QTPP in Identifying pCQAs

The QTPP summarizes the key features of a protein therapeutic that are essential to ensure marketability. While the QTPP is sometimes not established at the outset of product development, it is condition *sine qua non* to guide the preformulation and formulation strategies. A summary of the elements that constitute the QTPP are

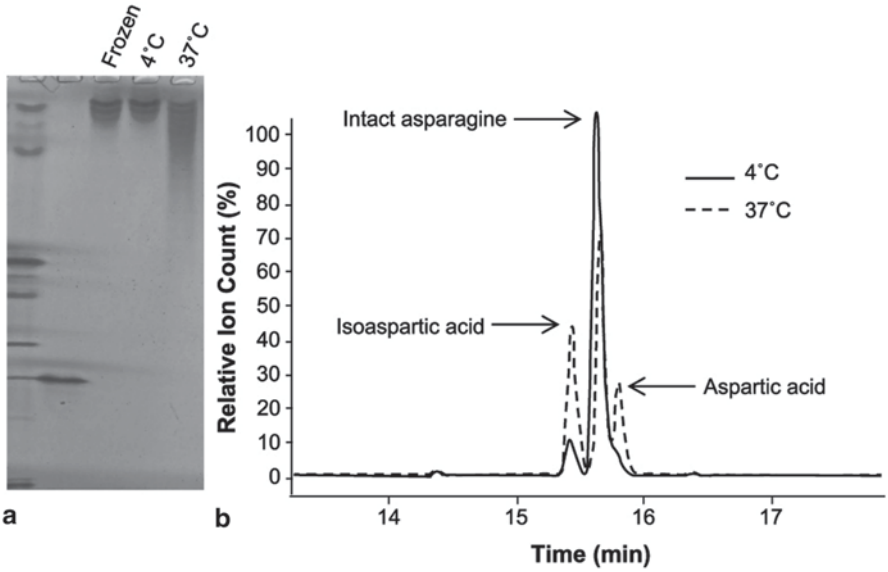

Fig. 6.7 Chemical instability of mAb-C after storage at pH 6 for 4 weeks at 4 °C and 37 °C; **a** Isoelectric focusing gel, **b** reverse phase peptide map chromatograms showing peptides identified by mass spectrometry analysis

Table 6.2 Apparent manufacturability evaluation summary

	Structural stability				Chemical stability		
	mAb-A	mAb-B	mAb-C		mAb-A	mAb-B	mAb-C
Thermal stability	O	–	+		O	O	O
Low pH	O	–	O		O	O	O
Shear	O	O	O		O	O	O
F/T	O	O	O		O	O	O
Temperature	O	–	O		+	+	—

(O) No change with respect to controls, (+) Desirable, (–) undesirable, and (—) very undesirable when compared to control and prior knowledge
mAb monoclonal antibody, *F/T* freeze thaw

shown in Table 6.3. One of the most important aspects of the QTPP with regard to QbD and early preformulation is the route and method of administration; with the challenge being that the dosing strategy may be not fully finalized.

If the QTPP features an intravenous (IV) infusion as the route of administration, compatibility of the protein with IV bag materials, such as polyethylene or polyvinyl chloride, is important to investigate. The stability of the protein can also be greatly affected when mixed with the IV administration diluents, such as saline or dextrose. The protein concentration within the IV bag may have to be variable to account for different doses in the patient population. Such changes in concentration can affect the physical stability of a molecule. Additionally, if an in-line filter is used, the stability of protein as it is infused across this filter needs to be confirmed.

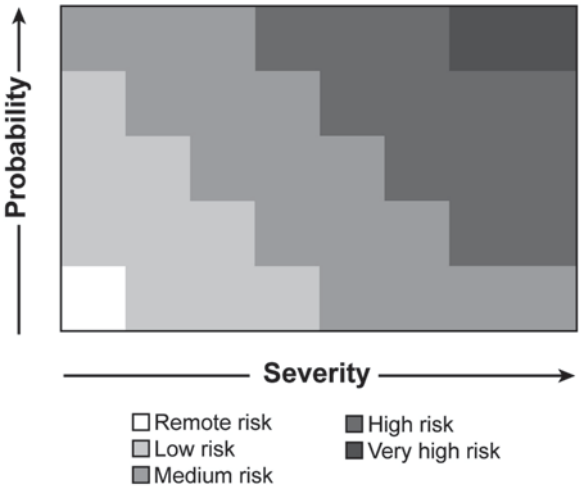

Fig. 6.8 Risk analysis of critical product attributes

Table 6.3 Prototype target product profile

Product characteristic	Target
Dosage form	Liquid, single use
Route of administration	Subcutaneous, single injection
Protein content per vial	100 mg
Presentation	100 mg/mL
Expected dose range in phase I clinical trial	No greater than 100 mg
Appearance	Clear solution, free of visible particles and meets USP criteria for subvisible particles
Impurities, degradants	Below safety threshold
Excipients	Compliance with USA, European, and Japanese pharmacopoeia
Stability	≥24 months of shelf life at 5 ± 3 °C
Biocompatibility	Acceptable toleration upon infusion

SC administration provides an additional set of considerations that need to be accounted for during development. Briefly, because of the limits on injection volume, stability at high concentration becomes a much greater priority for proteins intended for SC injections. Such high concentrations can affect degradations related to physical stability, particularly aggregation, self-association, and particulate formation. Additionally, viscosity and rheological properties need to be considered early in formulation development to ensure that the resulting product is "syringeable" and can be administered within the allowable limits of force to push the product through the syringe (Jezek et al. 2011).

Additional considerations for SC injections are necessary if the QTPP requires the use of a prefilled syringe. While compatibility with the materials in

the syringe is certainly a factor for SC injections, it becomes a greater priority for prefilled syringes when the product will be stored in contact with the device for extended periods of time. Not only must the protein be stable at the contact surfaces between the product and the device, but it must be stable in the presence of such entities as silicon oil or tungsten, which are often found at residual levels in prefilled syringes.

With respect to all routes of administration, it is imperative that the QTPP for such an administration be defined early so that their specific considerations can be included in the QbD protocol for formulation development. As QTPPs are documents that are updated or change during the course of clinical development, it is critical that the formulation scientist is kept informed of changes to the QTPP in conjunction with other departments, including clinical and marketing.

6.3.2 Unique Challenges to QbD Presented by Drug Delivery Systems

Most of the liquid formulations for therapeutic proteins are either IV or SC. They are manufactured in a vial or convenient devices such as prefilled syringes (Overcashier et al. 2006; Liu W et al. 2010). In some instances, there is a need to combine the protein liquid formulation with a matrix or delivery system for a long-term sustained release or localized delivery therapies (Manabe et al. 2004). One example of this approach is the loading of liquid bone morphogenetic protein-2 onto a collagen sponge delivery matrix (Friess et al. 1999a; Friess et al. 1999b). Other materials have been investigated for optimal delivery of proteins (Geiger 2001; Haidar et al. 2009), including PLGA nanofibers (Sahoo et al. 2009), and other natural biomaterials such as hydrogels (Van Tomme et al. 2008), and silk lyogels for the potential local delivery of antibodies (Guziewicz et al. 2011).

If a product is regulated as a device or combination product, then the federal regulations governing design controls (21 CFR 820.30) apply (Code of Federal Regulations 2012). Although the regulations apply strictly to biological/medical devices, the same elements encountered in QbD are intrinsic elements of the design controls regulations. Thus, a formulator could benefit from becoming familiar with the concepts of design controls. This is relevant if a liquid formulation will be paired with a delivery matrix, for optimal local retention, where the final product may be regulated as a device or combination product and be subjected to design controls. If the interactions between the protein to be delivered and the delivery system are important for local retention, then dissecting the variables in the delivery matrix and the target protein is necessary for optimization of the intended product. This is particularly important if the active component is composed of different isoforms that could show differential binding to the delivery matrix (Morin et al. 2006) that in turn could affect potency.

6.3.2.1 Selecting the Proper Delivery Approach

Protein stability as it relates to the in vivo environment is usually of concern to the formulator only if there are gross incompatibilities, and this can be checked by dialysis into phosphate buffered saline for example. Such environments as they relate to the typical routes of administration are relatively mild, and proteins often have clearance rates such that any potential safety concerns due to protein degradation in vivo are addressed in toxicology studies. These considerations become significantly more important when drug delivery devices, particularly those intended for long-term sustained release, are introduced and their impact becomes much more prominent. Drug delivery devices are often desirable because they provide an option for local, instead of systemic delivery. However, local environments in the body can feature extreme conditions, such as low pH or elevated temperature. Since protein stability is an area in which the formulator has expertise, their input can be critical to guiding the QbD considerations of administering a protein therapeutic with a delivery device.

For instance, the parameters describing the length of time needed for sustained release should be part of the QTPP. The formulator can then assess the protein stability over this course of time under conditions similar to those that will be encountered in vivo. Elevated temperature is one such condition. While stability at 37 °C is often used to perform accelerated stability studies, when sustained release with a device is involved, this temperature will mimic the environment the protein will be exposed to in the body. When performing studies for a sustained release delivery, it is necessary to choose molecules to move to development that will have little or no degradation at elevated temperatures for the time period needed for delivery.

Early knowledge of protein degradation with respect to the in vivo environment can guide the selection of a delivery system (Jiskoot et al. 2012). For instance, if the protein is stable under in vivo conditions, a hydrophilic matrix may be appropriate for delivery. Release from these matrices is diffusion controlled which means that the protein will be interacting with the in vivo environment for a significant portion of time as it permeates the delivery device. If the protein is unstable, then a biodegradable matrix is a more appropriate device where the protein can be sequestered from the in vivo environment until the matrix breaks down and the protein is released (Sinha and Trehan 2003; Giteau et al. 2008). In these cases, it is important to have appropriate stabilization strategies for the protein in biodegradable matrix as the protein will be retained in that matrix for extended times and may react with the degradation products of the matrix. It is also important to understand the unit operations that will be employed to manufacture the protein-loaded matrix as protein instability during these processes may affect its biological properties.

6.3.2.2 Assessing Stability of a Protein in Combination with a Delivery System

While early knowledge of protein stability can help guide the selection of drug delivery, other considerations need to be taken into account when assessing the manufacturability of a protein in combination with a delivery device. Foremost is whether the protein is stable and remains intact during the construction of the device. Incorporation of a protein into a delivery device can entail steps that place extreme stress on the protein. For example, the construction of microspheres requires the protein to be subjected to an emulsion with an organic solvent (Li X et al. 2000). In addition to being subjected to this organic component, extreme shear stress is often required to form the emulsion. To form hydrogels, proteins need to undergo crosslinking (Choh et al. 2011). Any of these manipulations may lead to protein degradation. However, in early development, many of these conditions can be mimicked and studied in order to get a leading indication as to the compatibility of the protein with the manufacturing procedure.

In addition to assessing the ability of the protein to survive the incorporation procedures, an understanding of the interactions between the protein and matrix during administration are crucial in development. Due to the high doses and low volumes often needed for drug delivery, proteins will often need to be highly concentrated (>100 mg/mL) in the device. While these concentrations are often feasible in solution by adjusting formulation variables, it must be determined whether they are attainable within the delivery device of interest. Additionally, the breakdown products of the device and their interaction with the protein must be considered (Sinha and Trehan 2003; Giteau et al. 2008). For instance, lactic acid is a byproduct of PLGA (poly[lactic-co-glycolic acid]) degradation, the presence of which can lead to a decrease of the pH within the protein containment areas in the microspheres (Zhu et al. 2000; Sinha and Trehan 2003; Giteau et al. 2008) and may impact local protein stability and patient via pain. By considering these possible degradation mechanisms early, they can be added to the QbD testing approach and enable a more robust drug delivery device.

6.4 Role of DOE and Robotics in Preformulation Development

6.4.1 Design of Experiments

Coupling formal risk assessment with a DOE program is a powerful method to test the effect of various input parameters (e.g., pH, excipient concentration, etc.) that can influence the stability of the protein. As each unit operation has multiple input parameters that may influence the output variables (e.g., protein quality, subvisible

and visible particulates, etc.), it is time consuming and cost prohibitive to test each of the input factors in a univariate fashion. DOE makes it possible to study multiple factors simultaneously to see if there are interactions between the many input parameters that influence product quality. Scientists use prior knowledge, experience, and risk management to rank the effect of key variables and process parameters and investigate the subset in a multivariate fashion using either a full factorial or a partial factorial DOE study. The results from early DOE studies can help identify important parameters that influence the CQAs of the protein of interest and help build a design space for that unit operation (e.g., freeze/thaw process, formulation robustness, etc.).

Typically DOE and QbD strategies in formulation development are applied toward later phases of development to save resources and cost. Early formulation development can certainly benefit from a broad-based multivariate DOE study that looks at several main effects and interaction parameters in a partial or full factorial design. This helps set the stage for improving knowledge space for a molecule. Sample preparation and miniaturized analytical analysis for the above stated multivariate studies, especially in a high-throughput (HTP) format, would be useful in the early stages of formulation development when very little information of the molecule is available. Various analytical and biophysical techniques for determining thermostability, viscosity, and protein–protein interactions when added to molecule assessment in a HTP format can be invaluable for development of therapeutic proteins from discovery research through clinical development and into the commercial world.

In one example, protein stability challenges at high concentrations were noticed during early formulation development of mAb-X for an immunology indication. The QTPP for this molecule required a target formulation of 150 mg/mL that is stable for at least 24 months at 2–8 °C. mAb-X appeared quite stable at a lower concentration of 10 mg/mL under previously known platform formulation components. However, the same formulation conditions were not suitable for stabilizing mAb-X at higher concentrations as it formed insoluble aggregates in only a few months under refrigerated storage. A new study was undertaken in which various formulations at different solution pH, buffer species/concentrations, ionic strength, and surfactants were thermally stressed and stored for up to 4 weeks. The results from this study revealed that mAb-X at 150 mg/mL is most soluble in high-ionic strength solutions. Similar to early stage development of mAb-X, a screening study was conducted for another mAb-Y at 40 °C using various formulation conditions (high and low protein concentration, surfactant and tonicifier concentrations, pH and ionic strengths) to analyze for chemical and physical stability in a HTP format. Results from the initial DOE screening studies identified main and interaction effects between pH and ionic strength on the SEC main peak (monomer). SEC data (% loss in main peak) is shown in Fig. 6.9 and indicates that mAb-Y is sensitive to high salt concentrations at lower pH. Low pH and high salt concentration also led to more chemical changes as characterized by ion-exchange-HPLC (data not shown). Acceptable formulations with good physical and chemical stability for this mAb were obtained in the pH range of 5.0–5.5 at high salt conditions. pH and ionic

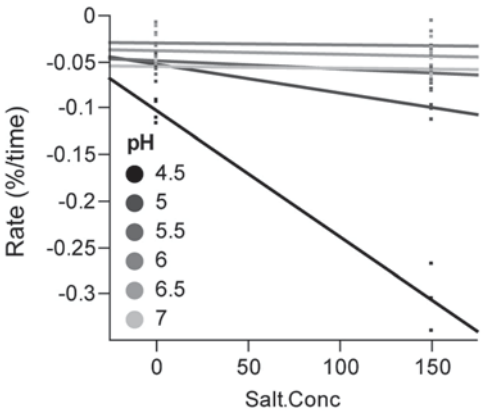

Fig. 6.9 Rate of loss of monomer from the initial design of experiments screening studies for mAb-B

strength sensitivity of mAb-Y could be a potential limiting factor if a mAb has to be delivered in a IV bag containing 0.9% saline. Screening for formulations that prevent such effects at an early stage of development using a robust DOE methodology and a prediction profiler could eliminate challenges in clinical trials and during various downstream processing steps and can add value during early clinical development.

6.4.2 High-Throughput Screening and Biophysical Approaches in DOE and QbD

Protein solubility and viscosity are critical attributes affecting drug stability and manufacturability making it important to understand these parameters while developing high-concentration protein formulations. The use of high-throughput screening (HTS) methods for protein solubility using UV-visible spectroscopy to test formulation parameters for IgG1 mAb was recently reported by Gibson et al. (2011). The PEG_{midpt} values (the weight% polyethylene glycol) in solution required to decrease the protein concentration by 50%) were compared to apparent protein solubility (in the absence of PEG) for various formulations and found to be a practical experimental tool to apply during early clinical development to rapidly screen appropriate formulations. Similarly, He et al. (2010b) used extrinsic fluorescence in a 96-well format to screen for IgG aggregation. It can be envisioned that several other analytical and biophysical techniques can be utilized in the future in a HT format to help determine protein solubility using various DOE principles.

Screening for formulations that reduce high-solution viscosity has become common as often high concentrations are required for administration. Testing in a HTS format using small volumes is valuable as both material and resource requirements in early development are limited. A multivariate analysis for factors influencing viscosity such as ionic strength, protein concentration, buffer composition, and pH

would be useful. A DOE approach with HT formulation screening that identified main factors affecting a IgG2 mAb thermostability and solution viscosity was reported (He et al. 2011). He et al. (2010a) employed dynamic light scattering (DLS) measurements of diffusion coefficients using small volumes of protein samples in a 384-well plate reader. Data generated from viscosity measurements were correlated to the DLS data (He et al. 2010a) and show the applicability of this method to screen for formulation variables using a HTS format.

He et al. (2011) also report the applicability of differential scanning fluorimetry, instead of the traditionally used DSC, in determining the thermostability of mAbs. The effect of several factors as well as the interactions between factors on both the thermostability and viscosity were evaluated using a 3^4 full factorial design (81 conditions) and multivariable regression analysis. Full and reduced models considering main effects of factors and two-way interactions were analyzed using JMP® software. Different mAb formulation variables that achieve optimum thermostability and viscosity were screened and can be easily viewed using the contour plot and profiler. The authors were able to apply prediction formulas using results from this DOE to determine formulations that can help meet predetermined values (e.g., low viscosity, high thermostability). The study (He et al. 2010a) exemplifies how a formulation design space can be explored with different factors using a combination of HTS and statistical methods and provides a pathway to use the concepts of QbD and DOE approaches to formulation development.

A similar DOE-based approach for formulation development of mAb-Z for an oncology indication was undertaken. mAb-Z has a QTPP of 30 mg/mL in a liquid formulation that needed to be stable for at least 24 months at 2–8 °C. A preliminary scan of the primary sequence of mAb-Z indicated the traditional hot spots, such as aspartate isomerization, asparagine deamidation, and methionine oxidation. Using prior knowledge for mAb formulation development, several formulation components such as tonicifiers (e.g., sucrose), surfactants (e.g., Polysorbate 20), buffer (histidine acetate) and pH (5.5–6.5) were evaluated for mAb-Z. A knowledge space screening study with mAb-Z was performed using HTS on robotic system. This study, run in duplicate, (Table 6.4) utilized a randomized, two-level, five-factor full factorial DOE design ($2^{5=}32$) for a total of 64 samples. Samples were stored for up to 4 weeks at 40 °C and analyzed over several time points using assays listed in Table 6.5. Data analysis (Fig. 6.10) from the knowledge space study indicated that pH and concentration of buffer species were the important criteria that affected mAb-Z stability under stressed conditions. Following the knowledge space design, phase III formulation selection, and further risk ranking and filtering, a fractional factorial design (e.g., 2^{4-1} or 2^{3-2}) with fewer parameters can be designed to determine the robustness of the selected formulation. Risk ranking and filtering tools as applicable to late stage/commercial formulation development are discussed in Chap. 4 of this book.

Higher dosing requirements in a limited injection volume have intensified the need to develop new techniques to understand intraprotein and interprotein interactions in highly concentrated protein solutions, especially because self-association of proteins under such conditions are crucial to appreciate the underlying

Table 6.4 Formulation excipients and study limits for mAb-Z in the knowledge space design

Formulation component		Target	Study range	Study limits	
				Low	High
Buffer	Histidine acetate	20 mM	±75%	5 mM	35 mM
pH	–	6.0	±0.5	5.5	6.5
Concentration	mAb-Z	30 mg/mL	±17%	25 mg/mL	35 mg/mL
Tonicifier	Sucrose	120 mM	±75%	30 mM	210 mM
Surfactant	Polysorbate20	0.02%	±95%	0.001%	0.039%

mAb monoclonal antibody

Table 6.5 Stability schedule and analysis methods used for knowledge space analysis

	Time (months)				
	0	0.25	0.5	1	3
Temperature (°C)					
5	X			X	X
25			X	X	X
40		X	X	X	
Analysis					
SEC	X	X	X	X	X
IEC	X	X	X	X	X
Turbidity	X	X	X	X	X
pH	X			X	X
UV-Vis spectroscopy	X	X	X	X	X
Osmolality	X			X	X

IEC ion exchange chromatography, *SEC* size exclusion chromatography, *UV-Vis* ultraviolet-visible

solution viscosity (Scherer et al. 2010). The recent study by Yadav et al. (2010) has brought attention to developing orthogonal screening methods for mAbs that have potential for self-association, aggregation, precipitation, and/or high viscosity and their crucial role for successful protein therapeutic development. It has also been demonstrated that the aggregation propensity of a mAb is closely correlated to the second virial coefficient (B_2), k_D and k_s (diffusion and sedimentation coefficients) (Saluja et al. 2010). The second virial coefficient is a parameter known to be valuable in understanding protein–protein interactions; this study also suggests that k_D can be used as a HTP predictor of protein aggregation. Zhao et al. (2010) have recently published formulation development of mAbs using a robotic system and a high-throughput laboratory. It is the first publication that demonstrates end-to-end formulation screening, from sample preparation to comprehensive sample analysis. Many analytical techniques including UV-Vis spectroscopy, DLS, various

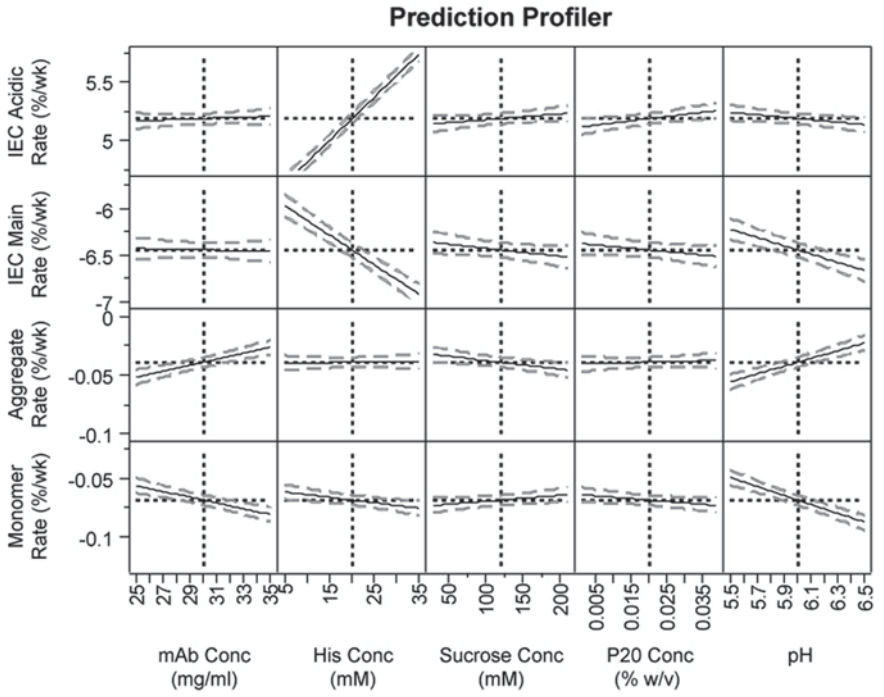

Fig. 6.10 Prediction profiler from the knowledge space study (40 °C, 4 weeks) for mAb-Z showing the impact of buffer concentration on charge variants and that of pH on aggregates and charge variants

chromatographic methods, and turbidity were performed for optimized formulation screens, and the best formulations were selected in a relatively short amount of time. The study demonstrates the value of a HTP laboratory in preformulation development of proteins, especially mAbs. Overall, a HTP approach combined with DOE can add significant value in the development of robust liquid formulations for therapeutic proteins using QbD principles.

6.5 Liquid Dosage Forms Development: Design Space, Optimization and Control Strategy

The key feature during the optimization phase of the liquid formulation is the creation of a dosage form that mitigates the impact of the degradations/instabilities identified during the preformulation step with the goal of long shelf life. In the particular case of developing formulations for unstable therapeutic enzymes, the risk analysis should also include the benefits of developing a freeze-dried formulation as a parallel track to a liquid formulation. The rules for rational design

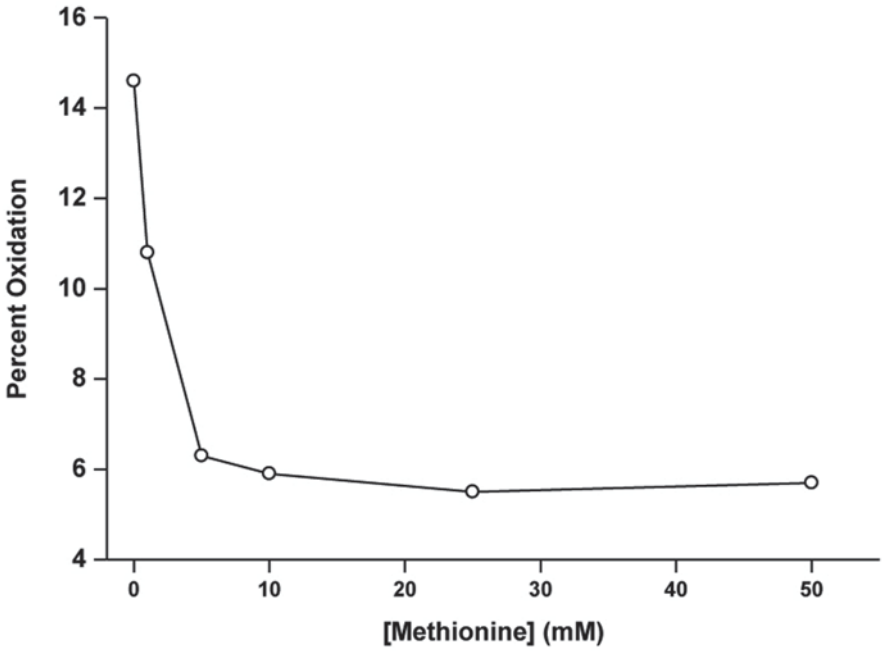

Fig. 6.11 Identification of the optimal concentration of methionine to use as scavenger of potential oxidation. Methionine oxidation after 9 months at 37 °C with varying methionine concentrations

of liquid formulations are less well defined than freeze-dried formulations; for instance, chemical stability is harder to control in liquid formulations. Even for mAbs, where a large amount of information has been accumulated, they will often differ in behavior under platform conditions. Thus, working within the particular knowledge space identified for a given protein therapeutic candidate, the formulator can begin to select buffers and excipients that will maintain stability for a period longer than 24 months under refrigerated conditions (5 ± 3 °C) for a liquid formulation and longer than 36 months for a freeze-dried formulation. These studies will typically include the selection of the buffer and excipients that can provide cryoprotection, isotonicity and protection from shear-induced denaturation. Other excipients such as anti-oxidants or chelating agents may be included based on the risk analysis of CQAs. Figure 6.11 shows identification of the optimal concentration of methionine to use as scavenger for potential oxidation in an mAb. During this step, the identification of different container closure options is investigated to avoid potential issues of compatibility. By this time, many of the analytical assays needed to assess stability are qualified by the analytic development group, allowing the formulator to set up stability studies under refrigerated and properly selected accelerated conditions (Perez-Ramirez et al. 2010). It should be pointed out that the use of Arrhenius kinetic models for the prediction of shelf life of large multi-domain liquid protein drug products is of limited value since the kinetics may not be strictly linear and other reactions may also become rate limiting. Thus,

obtaining the results of the stability studies under recommended and accelerated temperature and other stress conditions requires time. Those results are compared with the "fingerprint" (model) created from the output of the preformulation research to update and complete the stability risk assessment.

Ultimately, formulation development requires extrapolation of small-scale studies to large-scale production or manufacturing. This scale-up should be tested at real scale to ensure that the assumptions made during early QbD hold at the larger scale. This is particularly important during manufacturing, where additional levels of complexity including a large-scale process, various manufacturing sites, and differences in raw materials and equipment employed can introduce new challenges and variables that could be unaccounted for during the identification of the initial development space. Hence, the optimization of the development space is a continuum. There is always a balance that needs to be obtained between optimizing the formulation early in development and initiation of the first in human clinical studies. Given that the development space will continue to evolve as a project moves through different phases of development, it should be understood that a formulation for toxicology and first in human studies, such as phase I/II clinical studies, may be different than the one used for the pivotal studies and commercialization. Furthermore, as more information is collected on the first in human studies, the focus on the formulation will move to identifying the CPPs and the refinement of control strategies.

6.6 Summary

A systematic work process has been defined that strengthens the critical interface between discovery research and formulation development for the early identification of suitable protein candidates to be moved into formulation development. This predevelopment risk-based approach is essential for (i) understanding the solution behavior of therapeutic proteins, (ii) identify pCQAs, (iii) optimizing liquid formulations earlier, and (iv) aligning with the QbD requirements, namely ICH Q8(R2), Q9, and Q10 (ICH 2005; ICH 2008; ICH 2009). Proper preformulation research should also help in defining a well-characterized multidimensional window of operation (knowledge space) for further liquid formulation optimization as well as some of the key parameters for early process development.

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Chapter 7
Application of QbD Principles to Late-Stage Formulation Development for Biological Liquid Products

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7.1 Introduction

Protein formulation is a complex process during which the physical properties (e.g., solubility and aggregation) and the chemical stability of proteins (e.g., oxidation, deamidation, isomerization, etc.) need to be evaluated (Wang et al. 2007). Maintaining a protein drug in its active state typically requires preventing changes in its physical and chemical properties for the duration of its shelf life. The minimal shelf life for a sustainable product due to supply chain and marketing restrictions is usually between 18 and 24 months. A number of factors such as solution pH, buffer type, protein concentration, sugars, preservatives, salts, and surfactants are evaluated during formulation development. In addition to these factors, knowledge of certain intrinsic properties of the protein such as its primary structure, melting point, and viscosity is helpful. Formulation scientists utilize all available information on a certain class of molecules, various decision-tree methodologies, trial and error or combinatorial approaches to obtain a successful formulation for viable product development. While platform formulations are frequently used in early phases of clinical development, a robust formulation is needed for phase III and market. Robust protein formulation development is the keystone for all biopharmaceuticals. An inadequate market formulation can contribute to adverse safety events, poor efficacy, or reduced bioavailability, in addition to a host of other challenges, including poor manufacturability, during process development.

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Typically during early platform formulation development, the focus is on screening different formulation compositions based on prior knowledge for the molecule class to demonstrate adequate stability for early clinical supplies. Accurate protein solubility predictions and/or testing even during early research/preclinical phase can lead to successful formulation development for clinical candidates and avoid long-term stability concerns. Perez-Ramirez et al. discuss various approaches for early developmentability assessment for proteins and mAbs in greater detail in Chap 6. A major drawback in several current screening methods is the requirement for large amounts of proteins for testing. Platform formulations save time and resources for early-to-clinic and first-in-human studies (Warne 2011), however, very little information is available about the behavior of the protein in this formulation when the molecule transfers over to late-stage drug-product development (clinical phase III and market). Late-stage formulation development involves optimizing and characterizing the formulation by screening excipient ranges to define the optimum combination. Also, the performance during the process unit operations and with the primary packaging or device plays a major role. As quality target product profiles get updated throughout the clinical development of the molecule, late-stage formulation development may involve reformulation for pivotal phase III studies, in order to increase concentration, reduce viscosity, or introduce new primary packaging (e.g., prefilled syringes (PFSs)) or other devices for new routes of administration or different dose presentations.

Formulation development of a monoclonal antibody (mAb) presents distinct challenges that may not be associated with other therapeutic proteins (Shire 2009). mAbs are typically administered in higher doses (~10–200 mg) for the treatment of immunological and allergic disorders or oncology applications. One of the key concerns that has garnered attention for protein biopharmaceuticals is the generation of aggregates especially under high-concentration formulation conditions and the likely increase in detrimental immunogenic responses that follows aggregate formation (Maas et al. 2007; Jiskoot et al. 2012).

Sometimes a liquid subcutaneous (SC) formulation is desirable to ensure user convenience. To administer such a high dose with acceptable dose volumes (~1.0 mL) through SC route, mAbs need to be formulated over a concentration range from 100 to 200 mg/mL or even higher depending on the indication (Shire 2009). The high protein concentration often results in a dramatic increase in the viscosity of the solution as reported for MAb1 (Liu et al. 2005). At 25 °C, Liu et al. (2005) reported that MAb1 at 125 mg/mL is about 60 times more viscous than buffer alone. The authors went on to show that reversible self-association was responsible for the observed high solution viscosity in the case of MAb1. High solution viscosity leads to difficulties while pumping, filling, filtration, and product recovery from vessel and it even hampers the injectability of the formulation. Knowledge of solution viscosity and underlying mechanisms to decrease it are vital for pharmaceutical development and to propose a viable target product profile and device if necessary.

7.2 Incorporating QbD Elements into Drug Product Development

Quality by Design (QbD) is a science- and risk-based approach to drug product development and provides confidence that a target product profile of desired quality is attained and well understood. This chapter deals with late-stage formulation development and the role of QbD in developing a robust marketable formulation. Results from characterization studies carried out using either a liquid drug product (mAb-1 and mAb-2) filled into glass vials and administered by intravenous (IV) dosing or a product in a PFS (mAb-3) for SC delivery are used to exemplify the QbD concepts in drug product development. Prior platform knowledge and risk assessments are used to identify critical quality attributes (CQAs), key process parameters, and critical process parameters (CPPs) and to develop control strategies and design experimental studies to define the multivariate manufacturing space. The desired manufacturing space also takes into account manufacturing capability whereby robust process performance can be achieved while ensuring product quality. An important element of the QbD approach is the development of laboratory-scale models to perform multivariate experimental studies. These models require justification or demonstration that they are representative of the full-scale operations and provide relatively rapid feedback for further formulation development if necessary. In a standard approach, utilization of these types of laboratory models in multivariate studies was limited. In general, much of what was discussed and reduced to practice in a standard program is now formally assessed, documented, and rendered more robust.

The approach presented in this chapter leverages scientific understanding of products and processes, risk assessments, and rational experimental design to deliver processes that are consistent with QbD philosophy yet do not require excessive incremental effort. Results generated using these approaches will not only strengthen data packages to support specifications and manufacturing ranges and streamline testing but will hopefully simplify implementation of postapproval changes and technology transfers.

Risk Analysis and Assessment Tools. While this chapter will not provide an in-depth analysis of each risk assessment tool available, some of the tools that have been applied in a QbD design for formulation are highlighted. The ICH guideline Q9 (ICH 2005) shows a variety of risk assessment tools in its Annex I. The different tools were briefly described and a potential area of use was recommended. Also, some published QbD mock exemplary modules used different risk assessment tools; for example, failure mode, effects, and criticality analysis (EFPIA 2010) was conducted to identify CPPs. In the A-Mab case study presented by the CMC—Biotech Working Group (2009), risk matrices, risk ranking and filtering (RRF) tools, fault tree analysis, and preliminary hazard analysis (PHA) served for the ranking of CQAs and CPPs. Ng and Rajagopalan (2009) have reported on the use of the Ishikawa (Fishbone) diagram to demonstrate the factors that were assessed during formulation development. All potential variables that can impact various CQAs are listed and analyzed. Furthermore, failure mode and effects analysis (FMEA), which is a risk assessment tool for evaluating, prioritizing, and documenting potential failures of process and product performance, can be used to rank the parameters that

Table 7.1 Typical univariate formulation screening studies[a]

pH	Tonicifier concentration (mM)	Surfactant concentration (% w/v)	Protein concentration (mg/mL)	Buffer concentration (mM)
5.5	240	0.005	25	20
5.5	240	0.02	25	20
5.5	240	0.04	25	20

[a] Stress studies involve freeze/thaw, agitation, and elevated temperature screening

affect quality attributes. A risk priority number (RPN) score is reported that is calculated based on the severity of operational parameter excursion (S), frequency of occurrence of the excursion (O), and ease or difficulty of detection of the excursion (D). These three aspects are each given a numeric value, usually between 1 and 10 (with 10 being highest risk). An RPN score ($=S\times O\times D$) has been used in prioritizing activities and plans for risk management and has been applied in various process development studies (Singh et al. 2009).

7.2.1 Liquid Fill in a Glass Vial

7.2.1.1 Formulation Development—Traditional Approach

Formulation development at Genentech, including drug product unit operations, was traditionally carried out in a univariate fashion. During traditional formulation development, target formulation conditions are typically used to verify process acceptable ranges. Early in development, manufacturability assessment screens are used to aid in molecule selection. Typically the molecule is selected based on reduced hotspots for oxidation or deamidation or for lower viscosity attributes. Additionally, there is no formal risk assessment done prior to formulation development. Many companies have implemented a platform for phase I and II formulation and processing. Minimal formulation screening for excipients based on historical knowledge and performance of a class of molecules in precommercial setting is undertaken. Selection of a formulation parameter is conducted by varying its concentration while keeping everything else at target conditions—for example, varying surfactant concentration over the specification range while keeping pH and other components at target (Table 7.1 and Fig. 7.1, Study designs 1, 2, and 3). While these studies provide information on the individual formulation component, no analysis could be obtained for any interaction with other important formulation attributes—for example, interactions of polysorbate 20 with pH. Similarly, selection of pH, buffer components, tonicifier, stabilizers, etc., are also carried out in a univariate fashion and justified in various regulatory submissions.

Stability studies are performed at various storage time points (Table 7.2), as are in-use studies to support clinical trials. For phase III/commercial formulation and processing development, additional studies are performed to assess sensitivities to

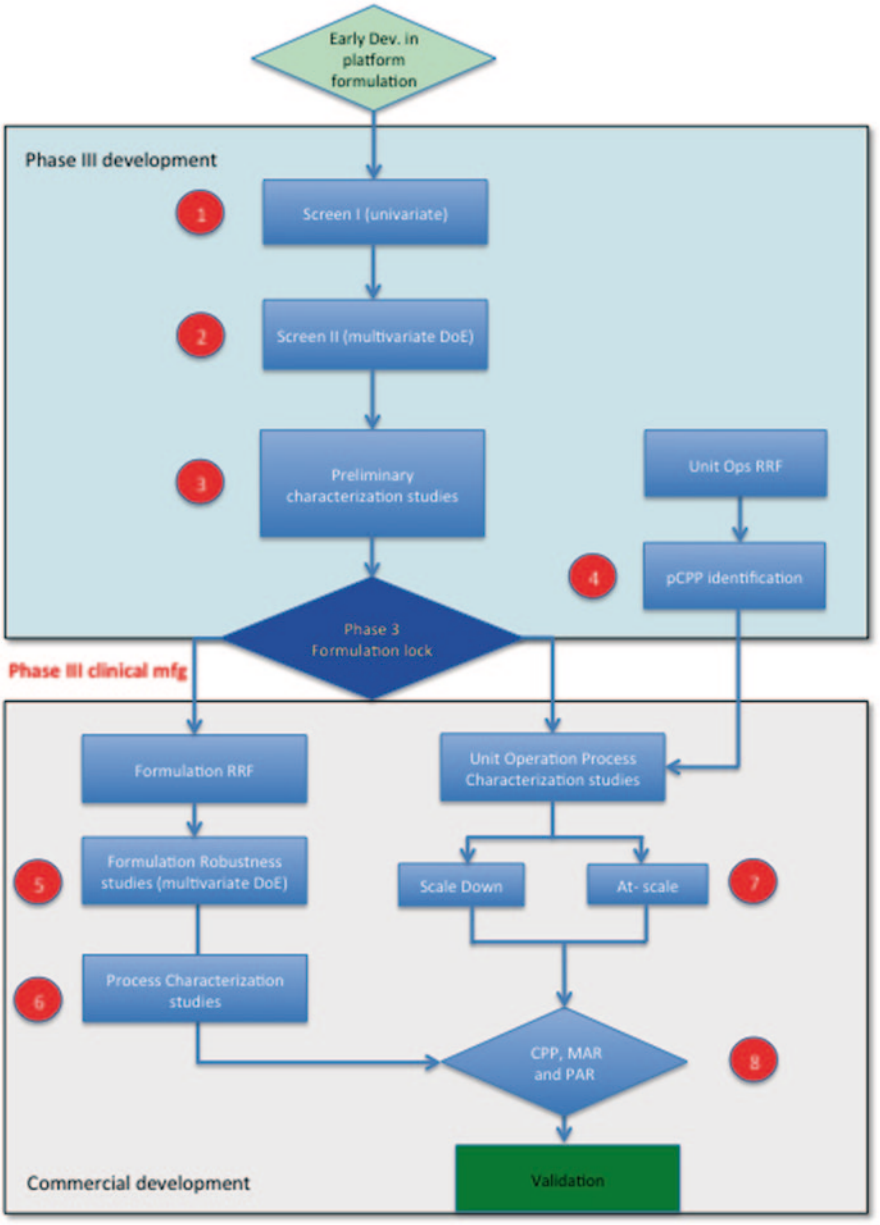

Fig. 7.1 Development of a phase III/commercial mAb liquid formulation with QbD elements. Study design numbers *1–8* (*circles*) are referenced in the text and facilitate incorporation of QbD elements into traditional development studies

Table 7.2 Typical storage conditions for univariate formulation screening studies

Storage conditions		Time (months)[a]						
Temperature (°C)	% RH	0	0.25	0.5	0.75	1	3	6
–20	–	X[b]	–	–	–	X	X	X[b]
5	–	X[b]	–	–	–	X	X	X[b]
25	60%	X[b]	–	X	–	X	X	X[b]
40	75%	X[b]	X	X	X	X[b]	–	–

RH relative humidity

[a] Time points may extend beyond 6 months up to expiration at –20 and 5°C

[b] Stress includes agitation studies to ensure surfactant degradation does not impede product development. Sample analysis included stability indicating assays such as size exclusion chromatography, ion exchange chromatography, potency, and other assays such as clarity, opalescence, and coloration, subvisible particles, peptide mapping, etc

protein concentration, excipient quality, freeze/thaw conditions, containers and contact surfaces, etc., and to fine tune excipient concentrations and ranges to ensure robustness. If the product is lyophilized, this processing step is specifically studied. If a device is required, additional studies are included during development. Lyophilization and device development studies are out of scope for this chapter and will be discussed elsewhere. Typically, several univariate (Fig. 7.1, Study design 1) and/or multivariate studies (Fig. 7.1, Study design 2) are conducted in order to understand the molecule. Preliminary characterization studies such as freeze/thaw stability, in-use stability, and primary container compatibility are carried out before nominating a phase III formulation (Fig. 7.1, Study design 3). As the molecule progresses through development, several process characterization (PC) studies are undertaken. Results from these characterization studies typically feed into process validation studies that are done either at registration or commercial scale using one to three lots with limited variation of parameter ranges. Few drug product operations such as filtration and lyophilization have a linear scale-down model and studies at large scale were very carefully selected due to material limitations.

7.2.1.2 Formulation Development with QbD Approach Including Analytical Sampling Plan

As a part of traditional formulation development work, a series of univariate studies were performed to identify the lead formulation. Figure 7.1 provides an overview of the phase III formulation and process development. Additionally, Fig. 7.1 also depicts how QbD elements can be embedded in the traditional development. Study designs 1, 2, and 3 indicate traditional development typically undertaken for phase III formulation development as discussed in Sect. 1.2.1.1. In order to support a QbD program, the unit operations RRF using previously determined tools is carried out to identify potential critical process parameters (pCPPs; Fig. 7.1, Study design 4).

Following CQA assessment, a formulation RRF is completed using the same RRF assessment tool that was used for the unit operations. This helps to initiate the formulation robustness studies (Fig. 7.1, Study design 5) and constitutes a new paradigm supporting the QbD program for biological drug product development. Further process characterization (PC) studies, such as impact of manufacturing stresses (e.g., mixing, freeze/thaw, vaporized hydrogen peroxide sterilization, etc.) are carried out to support the phase III and commercial formulation development (Fig. 7.1, Study design 6). Simultaneously, after the pCPP identification in the unit operation studies, several PC studies are carried out using worst-case processing conditions, and a target formulation either in a scale-down model or at scale (Fig. 7.1, Study design 7). Finally, all data collected thus far are used to identify CPPs, multivariate acceptable ranges (MARs), and proven acceptable ranges (PARs) for the phase III/commercial liquid formulation in a glass vial (Fig. 7.1, Study design 8).

Case Studies: mAb-1 is an IgG1 molecule in phase III development. An RRF analysis was performed (Martin-Moe et al. 2011) using tools given in the A-Mab case study. Results obtained from RRF for mAb-1 are shown in Table 7.3, and the corresponding design of experiments (DOE) is shown in Table 7.4. In the case of mAb-1, a PC study was designed to evaluate the impact of pH, protein concentration, concentration of surfactant, buffer, and tonicifier, as well as time and temperature on its CQAs. A multivariate study using partial factorial design with five factors and two levels ($2^{5-1} = 16$ formulations) was required to study formulation robustness as shown in Table 7.4. This design was performed at three temperatures (5, 25, and 40 °C) for drug product, analyzed using various analytical methods (e.g., ion-exchange high-performance liquid chromatography (IE-HPLC) and size-exclusion high-performance liquid chromatography (SE-HPLC)) and statistically evaluated by JMP® software. Figure 7.2 shows the prediction profiler of various formulation parameters and the impact of pH and time on acidic variants at 25 °C over 6 months as analyzed by IE-HPLC. As seen in Fig. 7.2, no parameter other than pH showed any statistical or practical significance, indicating that pH of the formulation should be tightly controlled during the formulation process.

A similar RRF was performed for another IgG1 mAb in clinical development, mAb-2. The RRF assessment was used to identify formulation components in mAb-2 drug substance and drug product that were important for maintaining product quality and to determine which formulation components were to be included in further multivariate and/or univariate robustness studies. Specifically, each formulation parameter was assigned a score for its potential main effects and potential interaction effects on product quality. The formulation parameters overall severity score was then calculated as a product of these scores, and this was used to determine if the formulation parameter needed further characterization. In cases where further characterization was warranted, the severity score indicated the type of robustness study required (e.g., univariate, multivariate); the results of this assessment are summarized in Table 7.5. The RRF analysis for the mAb-2 phase III and commercial drug substance and drug product formulation identified solution pH, surfactant concentration, and protein concentration as formulation parameters to be evaluated in multivariate robustness studies, whereas buffer and stabilizer concentrations can

Table 7.3 Formulation risk ranking and filtering for mAb-1. (Adapted from Martin-Moe et al.)

Formulation parameter	Formulation component	Proposed design space range		Typical control space range		Rationale for design space range		Main effect rank (M)	Rationale for main effect score	Interaction effect rank (I)	Potential interaction parameters	Rationale for interaction score	Severity score (M*I)	Recommended studies based on severity score
		Low	High	Low	High	Low	High							
Solution pH (5.3)	–	4.8	5.8	5.0	5.6	Insufficient chemical and/or physical stability	Insufficient chemical and/or physical stability	8	Physical and chemical degradation	4	mAb-1 concentration, Polysorbate 20	Chemical and/or physical degradation of formulation excipients and/or mAb-1; Moderate additive effect expected	32	Multivariate
Surfactant concentration (0.02%)	Polysorbate 20	0.01%	0.04%	0.01%	0.03%	Insufficient physical stability	Fatty acid chain degradation; oxidation via peroxides	8	Physical and chemical degradation	4	mAb-1 concentration, pH	Chemical and/or physical degradation of mAb-1; Moderate additive effect expected	32	Multivariate
Protein concentration (30 mg/mL)	mAb-1	27 mg/mL	33 mg/mL	27 mg/mL	33 mg/mL	Under dose patient	Over dose patient	4	Potential for insufficient efficacy and/or overdose; Upstream process ensures tight control	4	pH, excipients (e.g., buffer, surfactant)	Moderate additive effect expected	16	Multivariate or Univariate with justification

Table 7.3 (continued)

Formulation parameter	Formulation component	Proposed design space range		Typical control space range		Rationale for design space range		Main effect rank (M)	Rationale for main effect score	Interaction effect rank (I)	Potential interaction parameters	Rationale for interaction score	Severity score (M*I)	Recommended studies based on severity score
		Low	High	Low	High	Low	High							
Buffer species	Sodium acetate, glacial acetic acid	18 mM	22 mM	18 mM	22 mM	pH too high	pH too low	2	Upstream process ensures tight control	1	None expected	N/A		No study
Cryoprotectant (106 mM)	Trehalose dihydarate	74 mM	138 mM	85 mM	127 mM	Insufficient cryoprotection	Potential crystallization of trehalose	2	Potential for aggregation $n < 50$ mM or > 160 mM; Upstream process ensures tight control	1	None expected	N/A		No study

Table 7.4 mAb-1 Formulation design of experiments study design. (Adapted from Martin-Moe et al.)

Pattern	pH	Polysorbate 20 (%)	mAb-1 (mg/mL)	Sodium acetate concentration (% change from target)	Trehalose concentration (% change from target)
00000	5.3	0.02	30	0	0
−+++−	4.8	0.04	33	10	−30
−++−+	4.8	0.04	33	−10	30
−+−−−	4.8	0.04	27	−10	−30
+−−−−	5.8	0.01	27	−10	−30
−−+−−	4.8	0.01	33	−10	−30
++−−+	5.8	0.04	27	−10	30
+++−−	5.8	0.04	33	−10	−30
+−−++	5.8	0.01	27	10	30
+++++	5.8	0.04	33	10	30
−−−+−	4.8	0.01	27	10	−30
+−+−+	5.8	0.01	33	−10	30
−−+++	4.8	0.01	33	10	30
−+−++	4.8	0.04	27	10	30
+−++−	5.8	0.01	33	10	−30
++−+−	5.8	0.04	27	10	−30
−−−−+	4.8	0.01	27	−10	30
00000	5.3	0.02	30	0	0

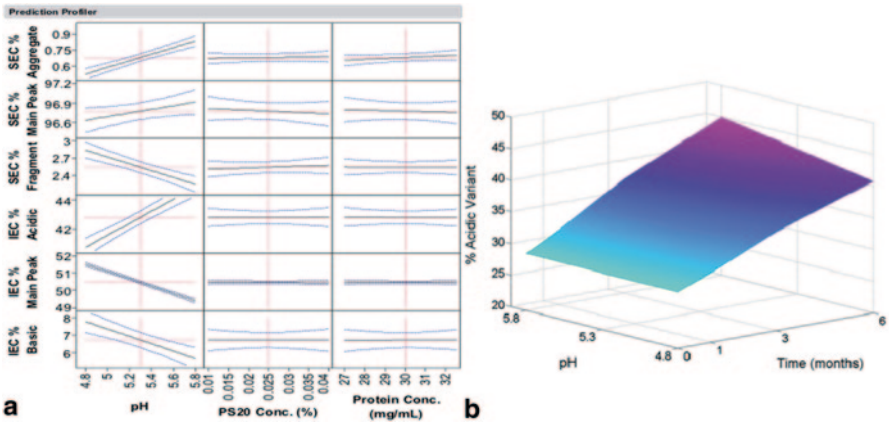

Fig. 7.2 **a**. Prediction profiler depicting the impact of formulation parameters (25 °C for 6 months) on presumptive CQAs for mAb-1. **b.** Effect of pH and time on acidic variants at 25 °C up to 6 months by IE-HPLC for mAb-1

Table 7.5 Categorization of formulation parameters based on risk ranking and filtering for mAb-2

Number of parameters in RRF evaluation	Parameters in multivariate studies	Parameters in univariate studies	Additional studies required
5	Solution pH Surfactant concentration Protein concentration	Buffer concentration[a] Stabilizer concentration[a]	None

RRF risk ranking and filtering

[a] Buffer and stabilizer concentrations were combined into one factor, osmolality, for the multivariate formulation robustness studies

Table 7.6 Target formulation and multivariate study ranges for mAb-2 drug substance and drug product

Parameter	Target	Target formulation range	Multivariate study range
mAb-2 concentration	30 mg/mL	27–33 mg/mL[a]	27–33 mg/mL
Buffer concentration	20 mM	18–22 mM[b]	10–30 mM
Solution pH	6.0	5.7–6.3[a]	5.7–6.3
Tonicifier concentration	120 mM	108–132 mM[b]	60–180 mM
Surfactant concentration (w/v)	0.02 %	0.01–.03 %[a]	0.005–0.04 %

[a] Target range based on specification acceptance criteria

[b] Target range based on manufacturing process limits

be examined in univariate studies. Previous univariate studies conducted at –20 °C and 2–8 °C for drug substance and drug product, respectively, showed that mAb-2 retained stability at the upper and lower limits of stabilizer (60–240 mM sucrose) in either 10 mM L-histidine chloride or 20 mM L-histidine acetate when the buffers were at the target pH of 6.0. Since the individual concentrations of the buffer and the stabilizer were not expected to have interaction effects with the remaining formulation parameters, these parameters were combined into one factor, osmolality, and included in the multivariate robustness studies. A multivariate two-level fractional factorial ($2^{4-1} = 8$ formulations plus two center points) stability study was conducted using pH, osmolality, protein concentration, and surfactant concentration as the formulation parameters. Target formulation and multivariate study ranges for mAb-2 drug substance and drug product are given in Table 7.6. The stability of mAb-2 in the robustness formulations was evaluated at the recommended (2–8 °C), accelerated (25 °C), and stressed (40 °C) storage conditions for drug product in glass vials, as well as at the recommended (–20 °C) and accelerated (2–8 °C) storage conditions for drug substance in stainless steel minicans.

Similar to mAb-1, samples were analyzed using various analytical methods (e.g., IE-HPLC and SE-HPLC) and statistically evaluated using JMP software. At

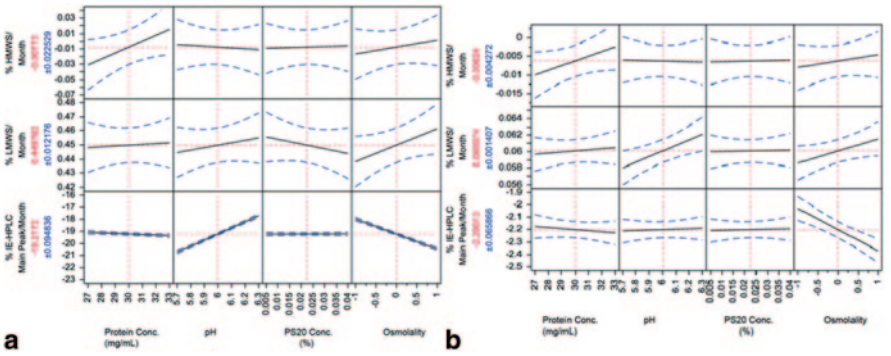

Fig. 7.3 Prediction profiler depicting the impact of formulation parameters on quality attributes for mAb-2 at (**a**) 40 °C for 1 month and (**b**) 25 °C for 6 months

accelerated (25 °C for up to 6 months) and stressed (40 °C for up to 1 month) storage conditions, all drug product formulations showed an increase in percent low molecular weight species (LMWS), no significant change in percent high molecular weight species, and a decrease in IE-HPLC percent main peak, which corresponded to an increase in acidic variants. The prediction profiler of various formulation parameters is shown in Fig. 7.3. The percent increase in LMWS was similar among all robustness formulations when stored at both 25 °C for up to 6 months and 40 °C for up to 1 month. Specifically, all formulations displayed either a 0.4% increase in LMWS when stored at 25 °C for up to 6 months or a 0.5% increase in LMWS when stored at 40 °C for up to 1 month. All other quality attributes studied including protein concentration as well as pH, and with the exception of color, did not change measurably from the initial time point when stored for the maximum amount of time at the accelerated and stressed conditions. However, some formulations developed a slightly more intense color when stored at 40 °C for 0.75 to 1 month. Subvisible particle data (2–25 μm) from drug product robustness formulations stored for up to 6 months at 25 °C, or for up to 1 month at 40 °C were also collected. Data for particles ≤ 10 μm are provided for information only. It was not possible to perform statistical analysis on the subvisible particle data for the drug product robustness formulations because of inherent challenges (Scherer et al. 2012) and variability of the method and the limited data set. As seen in the case for mAb-1, pH is the only parameter that showed any statistical significance for the charge variants. However, the effect of pH in this range (±0.3 units) is not deemed as being practically meaningful for this molecule based on various other biochemical and biophysical analysis, including PK and in vitro potency.

Overall evaluation of time points and sampling for mAb-2 led to approximately 2000 samples that would be analyzed to support formulation robustness studies. The value in adding this amount of work to development is in the ability for setting shelf-life specifications and perhaps in justifying removal of certain tests from the formal GMP stability studies. In addition, it should be noted that these samples would be tested over a 4-year period. It should also be pointed out that if a developer

does not lock a robust formulation, robustness studies at this stage in the process could generate results that necessitate a formulation change and would put the project timeline at risk. These are important considerations in designing these studies at a later stage of the project, making it necessary to drive QbD in early development as well (Chap. 7).

7.2.2 Prefilled Syringes (PFSs)

7.2.2.1 Incorporating QbD Elements into a Traditional Liquid in PFS Development

The starting point for the QbD development of a liquid in a PFS drug product was an already existing, highly concentrated protein formulation that was developed following a traditional development approach. At the time of development of this PFS drug product, only limited platform knowledge was available. The CQAs of the molecule were understood in the context of the technical and clinical development of a lyophilisate presentation of the molecule. Although this information provided a fundamental scientific understanding of the molecule, the highly concentrated liquid formulation, the primary container, and processing aspects required special attention during PC studies.

Figure 7.4 provides an overview on the formulation, primary container, and process development and how the QbD elements were embedded in the traditional development. The boxes framed in black show the traditional development studies and the boxes framed in red mark the QbD studies.

7.2.2.2 Traditional Development of the Commercial Formulation in the Primary Packaging (Case Study mAb-3)

The development of the commercial formulation started with the selection of the optimum pH of the molecule in Fig. 7.4, Study number 1. This was done very early in the formulation development by a pH screening. Subsequently, a lyophilizate in vial presentation was developed to serve early phase clinical supply needs. Shortly after, the commercial liquid in PFS development was initiated, first by excipient selection (Fig. 7.4, Study number 2) and second, excipient concentration range finding (Fig. 7.4, Study number 3). Noteworthy, Study numbers 2 and 3 in Fig. 7.4 were conducted as multivariate DOE investigations. Within the optimization study (Fig. 7.4, Study number 4), the PFS as primary packaging was introduced into the development of the liquid in PFS. Results from 1-year real-time stability and accelerated conditions were used to lock the commercial formulation. The final primary container was not yet selected. In order to lock the primary container additional scouting studies (Fig. 7.4, Study number 5) and different syringe qualities (Fig. 7.4, Study number 6) were evaluated, as well as processing at the production line. The

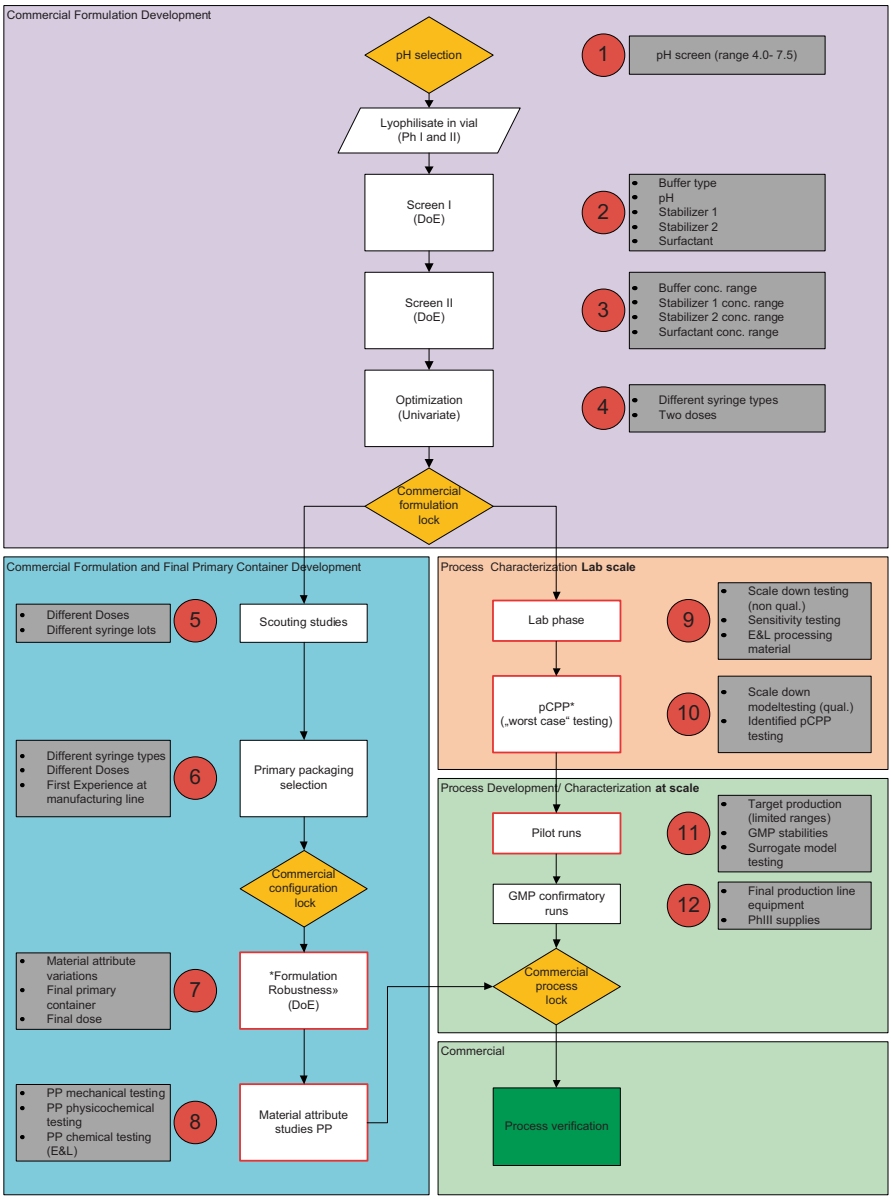

Fig. 7.4 Development of mAb-3 liquid in prefilled syringe (PFS) with QbD elements: studies performed for the liquid in PFS development (*white boxes*); information on the setup of the respective study (*gray boxes*); Study numbers 1–12 are referenced in the text and should facilitate the sequence of traditional studies (*white boxes with black frame*), and QbD studies (*white boxes with red frame*). *DOE* design of experiments, *RSD* response surface design, *pCPPs* potential critical process parameters, *PP* primary packaging, *E&L* extractables and leachables

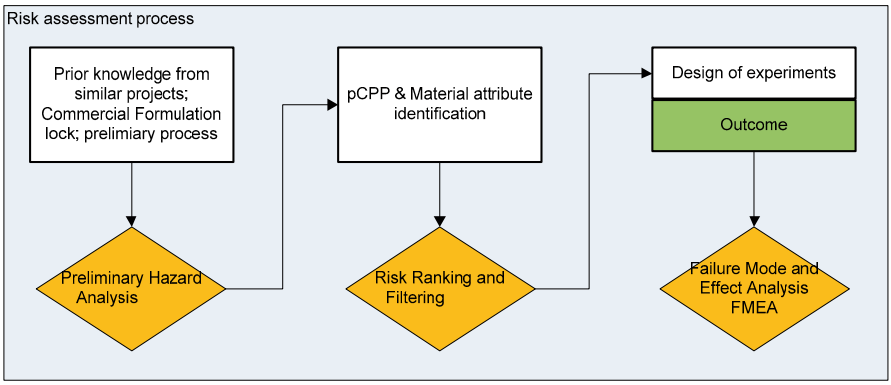

Fig. 7.5 Risk assessment process for the liquid in prefilled syringe (PFS) example (mAb-3)

liquid in PFS commercial configuration was locked, after sufficient stability data of the commercial formulation in the final primary container was available.

7.2.2.3 QbD Elements Embedded into the Traditional Approach

The initial identification of pCPPs and impact of material attributes was performed after the formulation lock and prior the configuration lock of the PFS. For this purpose a PHA was used. The PHA has been found to be most useful for the initial risk assessment prior to PC. In general, for a process risk assessment, an FMEA serves as a useful tool. But at this stage of the development with only limited platform experience, the control strategy cannot be fully established. Therefore, it was hardly possible to determine the detectability of process parameter variations in order to conduct an FMEA. The format of the PHA was kept as close as possible to the one used for an FMEA (i.e., using scores of 1–10 to rank severity and occurrence). The risk assessment update of the initial PHA could later easily transition into an FMEA by adding the factor "detectability" to the severity and occurrence (Fig. 7.5). In the PHA, a severity and occurrence score has been assigned to all pCPPs and material attributes. Based on prior knowledge of the molecule, development experience from comparable molecules, and literature, a severity and occurrence ranking was assessed over the intended commercial formulation, primary container, and manufacturing process. A criticality risk priority number (criticality RPN), was generated by multiplying severity and occurrence (both scale of 10). The criticality RPN was split in two categories of low (1–16) and major (17–100) risk. All pCPPs and material attributes with a severity score ≥ 9 or an overall criticality RPN of ≥ 17 were selected for investigation in laboratory experiments. It was found essential that scientific and technical experts from development as well as from commercial manufacturing contributed in order to obtain a meaningful risk ranking. Based on the ranking of the pCPPs and material attributes, the required experimental plan for PC of the commercial manufacturing process was defined.

A similar risk assessment approach using the PHA has been used for the QbD mock example of the module 2 for the "Sakura tablet" (NIHS, Japan 2013) and has been described in the A-Mab case study (CMC-Biotech Working Group 2009).

For the given case of a liquid in PFS, the material attributes were ranked according their criticality RPNs using a PHA. For this assessment, excipient quality (impurities and degradation products) and concentration ranges in the formulation, primary packaging container quality, product contact material quality, as well as the leachables and extractables from the primary container, and the product contact materials were included. The PHA to rank the pCPPs and material attributes was found to be useful in identifying material attributes that needed further investigation.

7.2.2.4 Formulation Robustness

After the identification of pCPPs and the material attributes by the PHA, the setup of the QbD studies was defined (Fig. 7.4). In order to define the experimental design of the "formulation robustness", an RRF tool was used. The ranking via the RRF tool was supported by data generated during the formulation development (until formulation lock) for each single formulation parameter on the analytical output. The RRF tool was found to be very useful to come up with a comprehensive rationale for multivariate or univariate experiments assessing the individual formulation excipients. The PHA and the RRF tools have been described in the A-Mab case study (CMC-Biotech Working Group 2009). The RRF for the "formulation robustness" of the liquid in PFS resulted in three factors (formulation parameters) that were investigated in a multivariate study and two factors in univariate studies (see Table 7.7).

Finally, in the "formulation robustness" (Fig. 7.4, Study number 7), three formulation parameters within tight ranges were addressed in a multivariate study design. The excipients concentration ranges have been defined upon the intended specification, the variation in compounding and the variation of the analytical method (excipient assay). The details on the design of the "formulation robustness" study are given in Table 7.8. A stability program was set up covering the intended drug product shelf life at real time, accelerated and stress (40 °C) conditions in order to allow a timely identification of potential additional studies that might be needed for refinement of the design space. The selected analytical methods were dependent on the nature and number of potential critical quality attributes (pCQAs) affected by material attributes (Table 7.9). A matrix showing the relationship between material attributes and the CQAs was helpful to set up the analytical program.

7.2.2.5 Primary Packaging

In addition to the material attributes related to the formulation excipients, those of the primary container also require attention. For the development of a liquid in PFS a number of interactions between primary packaging and the drug product have

Table 7.7 Factors to be investigated in the "formulation robustness"

Factor	Range	Rationale	Investigation
mAb-3 concentration	Target ± 15 %	To support specification settings	Univariate
pH	Target ± 0.3	Parameter of significant impact during formulation development	Multivariate
Buffer concentration	Target ± 10 %	Tested during formulation development	None
Stabilizer 1 concentration	Target ± 10 %	Tested during formulation development	None
Surfactant concentration	Target ± 0.02 % (w/v)	Parameter of significant impact during formulation development	Univariate
Stabilizer 2 concentration	Target ± 50 %	Parameter of significant impact during formulation development	Multivariate
Stabilizer 3 concentration	Target ± 5 %	Parameter of significant impact during formulation development	Multivariate

Table 7.8 Design of experiments for the "formulation robustness"

Experiment No.[a]	pH	Stabilizer 2 concentration	Stabilizer 3 concentration
1	–	–	–
2	–	+	–
3	–	–	+
4	–	+	+
5	+	–	–
6	+	+	–
7	+	–	+
8	+	+	+
9	0	0	0
10	0	0	0
11	0	0	0
12	Target formulation/+ 15 % protein concentration		
13	Target formulation/–15 % protein concentration		
14	Target formulation/+ 0.02 % surfactant concentration		
15	Target formulation/–0.02 % surfactant concentration		

– low range level, + high range level, 0 target level

[a] Experiment No. 1–11 is design of experiments; Experiment No. 12–15 is univariate extensions

Table 7.9 Spiking study for the evaluation of potential impact of material attributes from the primary container

Experiment no.	Spiking component	Concentration
1	Tungsten extract	Low
2	Tungsten extract	High
3	Negative tungsten control	No spiking
4	Siliconized syringe	High
5	Control siliconization	Target

been reported in recent years. For example, protein degradation and aggregation was reported to be induced by tungsten oxides at a different oxidation state present in the syringe as residue from the syringe-barrel forming process (Bee et al. 2009; Liu et al. 2010; Nashed-Samuel et al. 2011). In the case of tungsten oxides, different testing approaches were discussed in the literature. It was emphasized that for the purpose of conducting tungsten oxide spiking studies, some syringe suppliers provide extracts from tungsten pins used during the syringe-forming process. Recently, a study was reported comparing the effect of solution of various tungsten salts with those of a placebo extract from tungsten pins (Seidl et al. 2012). For the case of the investigated epoetin alfa in head-to-head studies, comparable aggregate formation was found using both methods of tungsten spiking.

Glass syringes contain silicone oil on the inner barrel surface in order to reduce break-loose and gliding force of the rubber stopper during the injection of the drug product. Silicone oil was discussed to potentially impact the drug product quality (Thirumangalathu et al. 2009). It was suggested that silicone oil emulsions spiked into protein solutions could mimic the situation found in a syringe and could lead to protein aggregation products. Some suppliers provide syringes with custom-made siliconization to investigate the sensitivity of protein solutions to the silicone oil of the inner syringe barrel.

Additionally, the epoxy glue used to fix the injection needle to the syringe glass barrel could lead to protein oxidation (Markovic 2006). In order to identify the material attributes of the primary packaging a thorough investigation of the interaction of the drug product with the primary container is required.

In the case discussed here, the main focus was on interactions of the protein with tungsten species at a different oxidation state and silicone oil concentration. These two material attributes have also been scored as potentially critical in the initial PHA. Spiking studies of tungsten extracts into the drug product in tungsten-free containers and “worst-case” siliconized syringes were done (Fig. 7.4, Study number 8). A negative control without spiking was investigated in parallel in a glass vial. A stability program was set up over the intended shelf-life at real-time conditions and accelerated testing in order to allow a timely identification of potential additional studies. The selected analytical methods were dependent on the nature and number of pCQAs affected by material attributes from the primary container.

7.2.2.6 Process Characterization

Figure 7.4 also describes the PC studies performed at laboratory scale (Study numbers 9 and 10) and at production scale (Study numbers 11 and 12) until the process lock for the liquid in PFS drug product. After the process lock, the validation at the launch site was performed. The QbD PC in down-scale models and at scale as well as process verification is not in the focus of this chapter.

7.3 Introduction to Control Strategy

In general, the desire and need to demonstrate control is no different in a QbD program than it is in any other program. The advantage of a QbD program is that formal risk-based identification of CQAs and CPPs allows mapping of control to critical elements. A demonstration of control in a QbD development plan is covered in the CMC-Biotech Working Group's (2009) document.

7.4 New Roadmap for Formulation Development

7.4.1 Suggested Timelines for QbD Approach in Late-Stage Development

QbD elements can be embedded into existing programs as discussed by Martin-Moe et al. (2011) While an important step in applying QbD is the development of small-scale models it is imperative that these are representative of full-scale operations. Process understanding using small-scale models needs to be built into timelines, at least for the first few molecules in a class (e.g., IgG1 mAbs). Once a thorough understanding has been recorded, it becomes easier to build on these laboratory models to justify at-scale operations (e.g., filtration, lyophilization cycle development, etc). In other cases, it may be necessary to use worst-case studies to justify small-scale models (e.g., freeze/thaw studies at small scale under worst-case surface area to volume interface). Similarly, elements that are new in the QbD realm, such as RRF, pCQA identification, and finally CPP identification along with final CQAs have to be incorporated at various stages of phase III development using results from phase III manufacturing lots as well as qualification lots. As QbD becomes the mainstay for biological drug product development, some critical elements such as pCQA identification and formulation knowledge space can also be built in to early development as discussed by Perez-Ramirez et al. in Chap 6.

7.4.2 Potential Benefits of a QbD Approach to Drug Product Formulation Development

For a typical liquid process, movement within a formulation parameter design space is not typically desired due to the requirement of meeting a label composition claim. Rather, robustness studies are designed to assure the product meets its quality target product profile (QTPP) throughout the tolerance ranges around the nominal label claims. Embedding QbD elements into a drug product development and validation program keeps the overall process and documentation streamlined. Formalizing a risk-based approach allows documented use of prior knowledge and results in stronger development rationale and eliminates unnecessary experiments. There is initial investment in developing small-scale models for all processing steps, and there are additional robustness studies that may be needed. Additional robustness studies have a dual purpose of supporting design space definition and specification setting. It also may be possible to significantly reduce robustness studies as a platform design space is repeated for new molecules (a prior knowledge feed-back loop), by performing worst-case testing of edges rather than full or partial DOE exercises. A QbD program may ultimately provide development relief, especially for a molecule class portfolio (e.g., IgG1s), while strengthening the support of ranges and specifications for a particular drug product process.

Submissions that are more standardized, where the end-to-end logic is easier to follow, and the supportive information provided with such submissions add confidence in the overall process and product control strategy. QbD submissions may be longer but stronger submissions, and there may be less need for supplemental submissions during the lifecycle of a product, thus providing regulatory relief. This could occur through the use of QbD coupled with expanded change protocols or comparability protocols to reduce the number or type of submissions. An example could be for technology transfers of a drug product process from one site to another as discussed by Lim et al. in Chap. 27. The same work and testing would be performed for the company's evaluation but instead of a PAS, a CBE30 may be acceptable.

It is also possible with a QbD approach to proactively define acceptable deviations and excursions and provide some quality relief with respect to investigations. For example, if there is a temperature excursion at a point in the process that has been captured in the acceptable formulation space, then it may become minor in nature because the lack of impact to product quality has been studied during formulation space definition. Finally, an enhanced rigorous and standardized process could result in more predictable approval timelines and inspection success, and it may result in greater opportunities for flexibility and postapproval process optimization as well and positively impact new or improved drug product availability and associated cost for companies, regulatory agencies, and patients.

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Chapter 8
Application of QbD Principles for Lyophilized Formulation Development

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8.1 Introduction

With the significant emergence of biopharmaceuticals in the past two decades, lyophilization has become a method of choice for stabilizing many of the protein therapeutics. Lyophilization is a process involving removal of solvent (water in most cases) which results in an elegant final drug product ready to be reconstituted at the time of use. When a product does not exhibit sufficient stability in aqueous solution, the product needs to be produced in a stable, solid form. It is well known that the stability normally increases upon removal of water due to the restricted mobility of the reacting species. Lyophilization reduces the rates of degradation, by several orders of magnitude and improves storage stability for molecules with physical and chemical instabilities.

Lyophilization has been the method of choice for products intended for parenteral administration because of the following advantages:

- Low-temperature process, and hence is expected to cause less thermal degradation compared to a "high temperature" process, such as spray drying
- Does not involve terminal sterilization step and maintains sterility and "particle free" characteristics of the product much easier than other processes
- Offers a method of controlling residual moisture content and head space gas composition for products sensitive to residual water and vial headspace gas composition such as oxygen
- Ease of scale-up and reasonable process yields

The final characteristics of the lyophilized drug product depend on many factors including the formulation, the lyophilization process, and container-closure size and

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geometry. The formulation influences not only the stability of the protein but also the design and performance of the lyophilization process, and therefore careful selection of excipients provides significant benefits. For biopharmaceutical applications, the selection of excipients available for stabilization of proteins that also contribute positively to the performance of the lyophilization process is somewhat limited. Use of sugars such as sucrose or trehalose as a cryo- and lyophilization-protectant is usually necessary, and several excellent literature references (Pikal 1990; Carpenter et al. 1997, 2002) can be found describing many of the considerations a formulator should keep in mind, such as optimal protein-to-sugar (Cleland et al. 2001) ratio required for protein stability. Crystallizing excipients such as mannitol and glycine are commonly used as bulking agent for low protein concentration formulations providing elegant cake structure when used at appropriate levels (Jameel et al. 2009; Lu and Pikal 2004). Overall, the formulator often limits the total solids content of the formulation to under 10–12 % w/v to keep the lyophilization process relatively efficient. The use of surfactants such as polysorbate 20 and 80 has also been extensively studied, and can potentially play a role in influencing both protein stability (Kerwin 2008; Bam et al. 1998) and reconstitution time (Webb et al. 2002; Wang et al. 2009).

As defined in ICH Q8(R2), the goal of Quality by Design (QbD) is to assure drug product quality (that is, to ensure patient safety and drug product efficacy). QbD embodies a systematic approach to gain product and process knowledge, and involves:

- Adoption of good design practices during product and process design (for example, prior experience, DoE studies, identifying interactive effects, understanding edges of failure) to identify the factors that affect product performance and to build the knowledge base.
- Systematic risk assessment as described in International Conference on Harmonisation (ICH) Q9 to identify critical quality attributes (CQAs) that affect safety and efficacy based on the severity of their impact, probability of occurrence, and detectability.
- Risk management by identification of appropriate control strategies which may include either accepting the risks if low, or further making the product and process changes robust to reduce risks to acceptable levels.

This chapter describes a mock case study to illustrate the application of principles of QbD as described in ICH Q8 in the development of a lyophilized formulation. The clinical and commercial requirements of the drug product are described by the "Target Product Profile (TPP)," from which the quality target product profile (QTPP) for the drug product is defined. The study presented below is a *mock* case description that makes several assumptions:

1. The protein drug is a monoclonal antibody (mAb) called mAb X which is very similar to mAbs Y and Z for which a significant amount of prior platform formulation knowledge has been generated. As needed, this prior knowledge is leveraged.
2. A lyophilized product is developed for mAb X because prior efforts in developing a liquid formulation indicated that the protein was unlikely to provide 2–3 years of shelf life. During this effort, significant amount of knowledge has been generated to identify the buffer species, stabilizing excipients, surfactants, the physical and chemical degradation pathways, and this prior knowledge is leveraged.

3. Potential CQAs for the drug substance, raw materials, and container/closure have been previously identified for mAb X and are consistent with prior knowledge generated with mAbs Y and Z, which will be useful during the initial risk assessment.

8.2 Overview of Qbd Strategy for Lyophilized Drug Product Development

Once a container-closure is selected based on the dose, the quality of the final lyophilized drug product depends on the formulation composition and the lyophilization cycle parameters. Pharmaceutical development studies can be carried out to establish a knowledge space and design space in which one could envision a variety of formulation excipients and concentrations over a range of lyophilization process conditions that can result in acceptable product quality. While the generated knowledge would be immensely helpful, it would be unreasonable to expect regulatory relief in making formulation composition changes post-approval based solely on this knowledge, even when the changes are within an established design space, because of the potentially high impact on local tolerability, pharmacokinetics, and efficacy. The way to interpret formulation design space would be to evaluate and understand the impact of formulation parameters (that is, pH, excipients and their levels) on drug product quality which would later enable defining the acceptable variability within the drug substance manufacturing process. Therefore, it makes logical sense to take an approach wherein the product design (formulation composition) and process design (lyophilization process) are evaluated as two distinct steps; however, all lyophilization process design considerations are taken into account for product design. The objective of the formulation design is to identify appropriate excipients, optimize their proportions, and chose the final drug product configuration that

1. Supports stability of the drug as it is processed through all unit operations of manufacturing and finally lyophilized.
2. Maintains the stability of the drug product in the final presentation during the shelf life and supports the safety and efficacy of the drug as it is administered to the patients.

This requires scientific knowledge and understanding of the physicochemical characteristics of mAb X (that is, protein), excipients, raw materials, container-closure, variability and interactions of these parameters with each other, and how these elements enable or disable to meet the target product profile (TPP).

The formulation composition is first selected based on the QTPP, as described in Table 8.1, and prior knowledge of the protein characteristics (Fig. 8.1). The protein at this stage can be lyophilized using a conservative lyophilization cycle, and the resulting product is thoroughly characterized. An initial risk assessment is then conducted to identify pCQAs (potential critical quality attributes) that impact either the

Table 8.1 Quality target product profile (QTPP)

Product attribute	Acceptable profile	Comments
Target indication	Oncology—treatment of leukemia	
Dosage form	Sterile lyophilized powder for reconstitution with WFI	
Final presentation	Vial configuration, 20 mL with 20 mm stopper	Type-1 glass vial, elastomeric stopper
Route of administration	IV infusion	Represents worst case for lyophilization with a large fill volume in a vial
Dose (protein)	125 mg per vial	
Dose range	50–250 mg	≤350 mg per week
Dose frequency	Every 2 weeks (preferred)	Every 1 week is acceptable
Setting for use	Clinic-based administration	Assisted by medical personnel
Single or multidose	Single dose vial with elastomeric closure	
Volume per dose	Based on patient weight	
IV bag	50 mL or 100 mL bag	Mention type and size of the bag along with type of tubing: normal saline or dextrose
Target recon volume	5.5 mL	Nominal final volume 5.0 mL
Diluent (reconstitution medium)	Commercially available WFI	
Target recon time	< 10 min	
pH	6 ± 0.5	Anything in pH 5–7 range is acceptable/suitable
Osmolality	280–350 mOsm	Based upon USP isotonic solution desirable
Recommended storage conditions	Refrigerated (2–8ºC)	
Shelf-life	≥2 years at 2–8 °C	1 week at 2–8 °C following reconstitution and 8 h at RT
Main degradation route	Aggregation	
Secondary degradation route	Deamidation and oxidation	

lyophilization process and/or the final product quality attributes. Based on the initial risk assessment, further optimization of the formulation is then performed prior to selecting the desired formulation composition. Once the formulation is selected, a more traditional QbD approach can then be applied to understand the impact of variation in formulation parameters on the performance of lyophilization process and product quality attributes. This starts by conducting formal risk assessment, characterizing formulation and the lyophilization process, and ends with a proposed control strategy, all of which are the focus of the subsequent chapters.

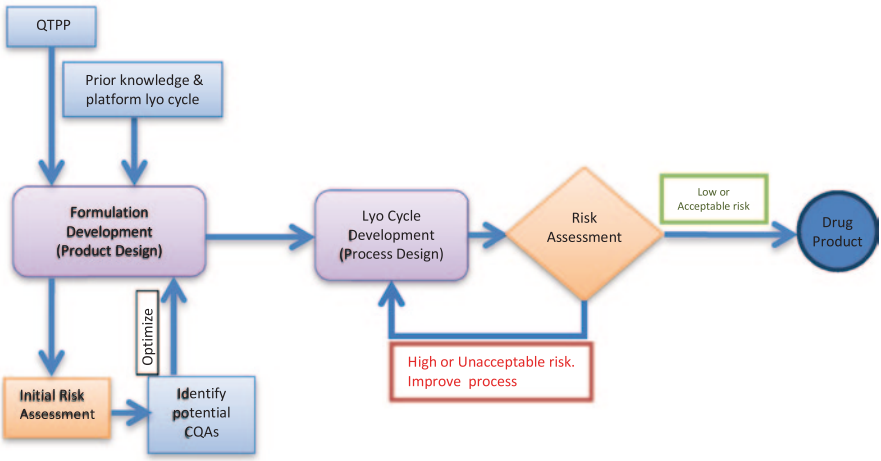

Fig. 8.1 Overview of QbD strategy for lyophilized drug product development

8.3 Initial Knowledge of mAb X

mAb X is an IgG1 antibody with a kappa light chain, with an approximate molecular weight of 146,000 daltons. Primary sequence analysis revealed no sequence liability to deamidation (NG), isomerization (DG), fragmentation (DP), or N-linked glycosylation (Asn-X-Ser) in the complementary determining regions (CDRs) of the molecule. There are no free cysteine residues present. Forced degradation studies showed that both deamidation in the Fc region (NG/PENNY sequence) and oxidation of methionine framework residues had minimal impact on potency.

8.4 Prior Knowledge from mAbs Y and Z

Pre-formulation studies on mAbs Y and Z which are similar to mAb X indicated marginal protein stability in liquid formulation due to rapid aggregation. Aggregates were determined to be noncovalent in nature, and a dependency on initial protein concentration was established. Exposure to freeze–thaw cycles also indicated loss of main native peak on high-performance size exclusion chromatography (HP-SEC) suggesting their sensitivity to the freeze–thaw process. Pre-formulation data of mAb X demonstrate comparable characteristics with Y and Z mAb molecules.

8.5 Preformulation Studies with mAb X

Previous knowledge from literature and experience with the design of the formulation for freeze drying of similar mAbs was used as a rationale for the determination of initial or working formulation and is summarized below.

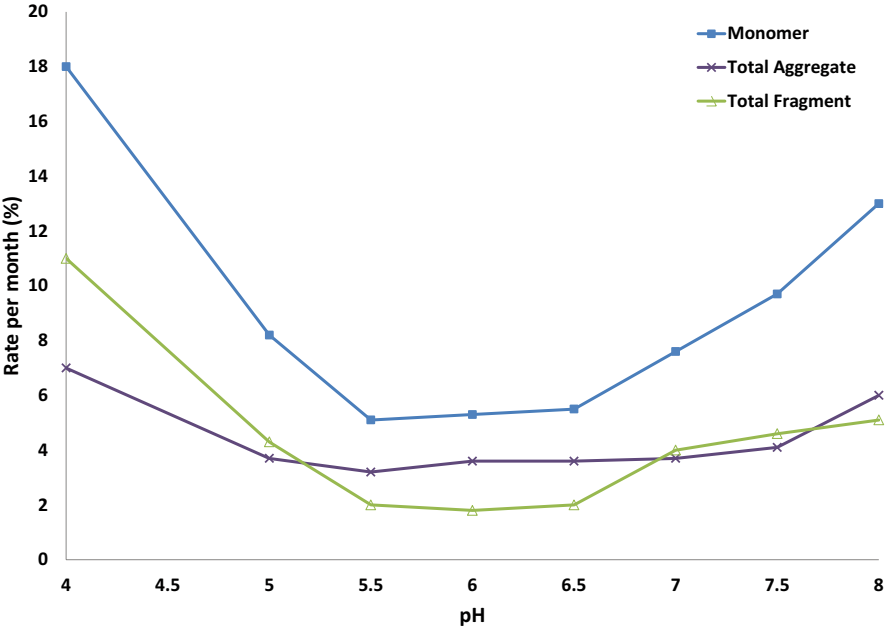

Fig. 8.2 Effect of pH on stability of mAb X at 40 °C/1 month using size exclusion chromatography

Monoclonal antibodies are commonly most stable to physical and chemical instability when formulated at slightly acidic pH, and many commercially marketed mAbs are formulated under such conditions. Furthermore, prior knowledge of the stability of mAbs Y and Z showed similar behavior. The stability of mAb X as a function of formulation pH and protein concentration was conducted to define suitable formulation conditions and identify the major modes of degradation. The stability of mAb X vs. formulation pH was evaluated at 40 °C for 1 month, 5 °C for 3 months, and the physical stability of the molecule was determined using HPSEC. As shown in Fig. 8.2, the physical stability of mAb X was optimal when formulated at pH 6.0, where the lowest combined rates of fragmentation and aggregation were observed, with an acceptable range from pH 5.5 to 6.5.

Furthermore, the long-term stability of mAb X was evaluated at 5 °C storage temperature for 6 months, which is shown in Fig. 8.3. At refrigerated conditions, mAb X also shows a slight pH dependency to stability but the lowest rate of monomer loss, 2.3 % per year (at pH 6.0), is still unacceptably high for a liquid formulation and would not result in a commercial drug product with the desired 2-year storage shelf life. Therefore, lyophilization is required for the formulation of mAb X.

The protein concentration dependence of physical stability of mAb X was also evaluated to determine a suitable protein concentration for optimal stability when stored as a liquid during manufacturing operations and also as a lyophilized drug product. As shown in Fig. 8.4, the rates of aggregation of mAb X increased with protein concentration as expected, but the rate of aggregation was much higher at 50 mg/mL compared to 25 mg/mL. Since the expected commercial dose of mAb X is expected to be greater than 500 mg, a protein concentration below 25 mg/mL was not considered commercially feasible. Therefore, a protein concentration of 25 mg/mL was selected for all future studies.

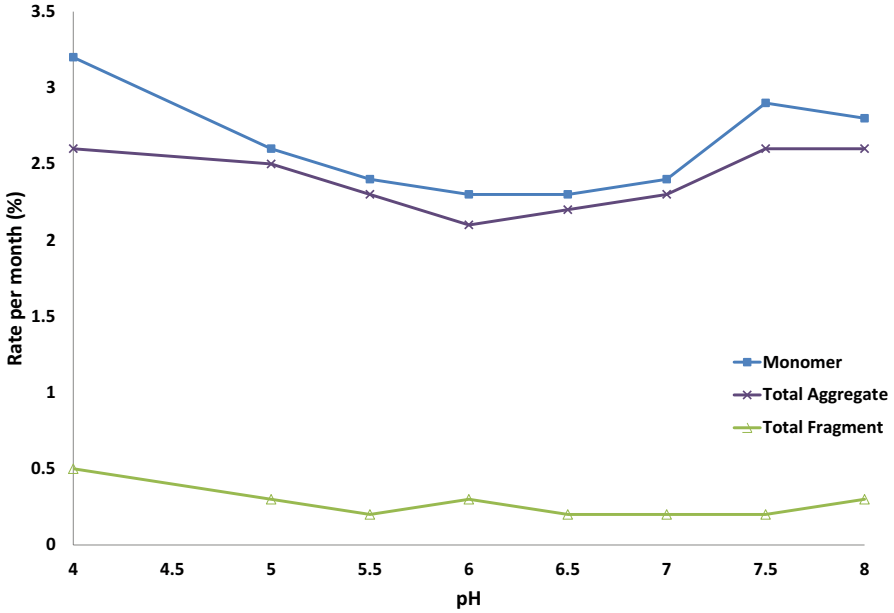

Fig. 8.3 Effect of pH on stability of mAb X at 5 °C/3 months using size exclusion chromatography

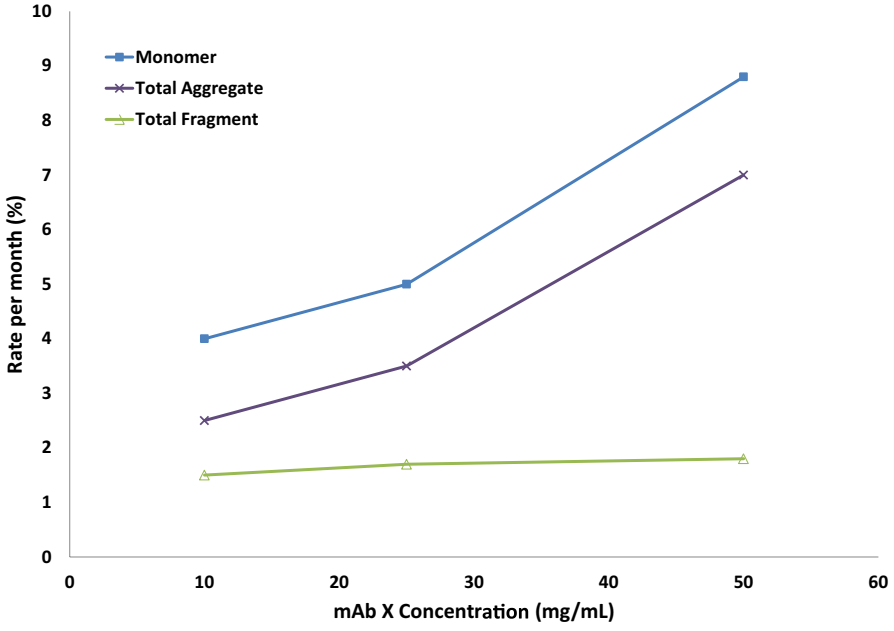

Fig. 8.4 Stability of mAb X at various concentrations at 40 °C, pH 6.0 using SEC

Based upon these pre-formulation studies and the defined QTPP of mAb X, a suitable formulation pH and protein concentration for a lyophilized formulation of mAb X was identified to be pH 6.0 and 25 mg/mL, respectively.

8.6 Candidate Excipient Selection Based on Prior Experience with Similar mAbs Y and Z

The application of QbD strategy towards the formulation development of mAb X involves the use of prior knowledge to define improved molecule characteristics and likely modes of degradation that are common to mAbs to enable a rational and risk-based selection of formulation excipients. Table 8.2 describes several parameters that can be applied towards the formulation approach of mAb X based upon prior knowledge of mAbs Y and Z.

Furthermore, prior formulation strategies for lyophilized proteins in general can be applied towards the selection and justification of excipients in the lyophilized

Table 8.2 Prior knowledge available from mAbs Y and Z

Formulation parameter	Prior Knowledge
mAb molecule	• Candidate selection based on physico-chemical stability assessment during pre-formulation • Pre-formulation data demonstrate comparable characteristics of mAb X with Y and Z justifying use of prior knowledge • Sequence optimization to prevent deamidation in the Fab (CDRs). • Known hotspots were engineered out of mAb-X based on knowledge of previous molecules. • pI appropriate for solubility within pH 5 to 7.5. • Thermal stability by DSC of mAb X is similar to that of mAbs Y and Z
Modes of instability	• Covalent degradation (Asp isomerization/Asn deamidation) is minimal in a pH range of 5 to 6 • Disulfide scrambling rates are higher at neutral pH • Aggregation can impact potency and may also have immunogenicity risks • Formation of fragments can impact potency • Sub-visible particle formation can be predictive of visible particles and is dependent on protein concentration (lower protein concentration is more likely to produce higher sub-visible particles)

formulation for mAb X. The selection of common excipients for lyophilized proteins and their justification is described in Table 8.3.

8.7 Formulation Development Studies

Based on the prior knowledge summarized in Tables 8.2 and 8.3, and extensive experience with previous antibodies in these formulations, three formulations were selected:

a. 10 MM histidine, 4% mannitol, 2.5% sucrose, 0.01% polysorbate 20, at pH 6.0

Table 8.3 Application of prior knowledge in the selection of excipients for mAb X

Formulation parameter	Prior knowledge
Formulation variables and their interactions	The effect of pH/buffer type on stability is the most important factor and is independent of the effect of excipients on stability Phosphate and acetate buffers resulted in pH shift during processing
Polysorbate 20	0.01% (w/v) Polysorbate 20 protects against interfacial instability and can mitigate adsorption/aggregation during freezing/thawing and other unit operations of manufacturing and transportation Potential for peroxide contaminants
Sucrose	Used as an amorphous bulking agent Known to behave as a cryo and lyoprotectant, stabilizes protein during processing and upon storage Commonly used as an osmolyte to provide isotonicity Impact on quality: hydrolysis and subsequent glycation of the protein at low pH and elevated temperatures, contamination with glucose
Mannitol	Commonly used as a crystalline bulking agent, mannitol has been used successfully in previous products to provide crystalline matrix and mechanical strength to the lyophilized cake Complete crystallization is important to appearance and stability of the product Potential for hydrate formation during lyophilization and storage that can affect stability (Yu et al. 1999)
Glycine	Commonly used as a crystalline bulking agent, glycine has been used successfully in previous products to provide crystalline matrix and mechanical strength to the lyophilized cake (Nail et al. 2002) Complete crystallization is important to appearance and stability of the product Potential for staying partially amorphous during lyophilization and storage that can affect stability
Excipient binding during protein concentration	The Donnan effect explains pH shift during UF/DF due to co-concentration and exclusion of excipients at high protein concentrations
pH	mAbs Y and Z demonstrated optimal stability between pH 4–6
Buffer	Histidine is known to have good buffering capacity in pH range 5–7 and was successfully used for mAbs Y and Z

Table 8.4 Thermal characteristics of selected formulations

Formulation	Glass transition temperature, Tg' (°C)	Collapse temperature (°C)
A	−21	−18
B	−22	−19
C	−25	−22

b. 10 MM histidine, 2.5% mannitol, 2.5% sucrose, 0.01% polysorbate 20, at pH 6.0
c. 10 MM histidine, 8% sucrose, 0.01% polysorbate 20, at pH 6.0

The thermal characteristics of these formulations as a function of cooling and heating were studied using freeze drying microscopy and modulated DSC and the results of these studies are summarized in Table 8.4.

Mannitol was included as a common crystallizing bulking agent and sucrose as an amorphous excipient that is expected to promote stability of the protein during freeze thaw as well as freeze-drying.

All three formulations were lyophilized in a single run using the conservative lyophilization cycle shown in Table 8.5. Formulations were frozen at a shelf temperature ramp rate of 0.5 °C/min to −40 °C and held for an additional 2 h. Primary drying was carried out at a shelf temperature of −25 °C for 82 h at a chamber pressure of 65 microns. Secondary drying was carried out at a shelf temperature of 40 °C for 6 h, respectively, keeping the pressure unchanged.

After lyophilization the vials were examined for visual appearance, cake characteristics, residual moisture, reconstitution time, and post reconstitution stability using several assays. The results are summarized in Table 8.6. The vials for each formulation looked elegant and contained acceptable cakes with no visible signs of collapse or meltback. Formulation C produced cakes that showed slight pullback which is characteristic of sugar-only formulations (Rambhatla et al. 2005) and was not considered to be a defect. Residual moisture was measured by Karl Fischer and was below 1.5% w/w in all three formulations. Stability of each formulation was evaluated under accelerated temperatures of 25, 40, and 55 °C and monitored as a function of time for 3 months. Initial characterization of the freeze dried solid on

Table 8.5 Conservative platform lyophilization cycle

STEP	Ramp rate (°C/min)	Temperature (°C)	Pressure (mTorr)	Hold time (hr:min)
Loading	N/A	20	N/A	0:15
Freezing	0.5	−40	N/A	2:00
Annealing	0.5	−16	N/A	2:00
Freezing	0.5	−40	N/A	2:00
Primary drying	0.3	−25	100	82:00
Secondary drying	0.1	40	100	6:00
Unloading	1	5	100	0:15

Table 8.6 Summary of stability evaluation at 3 months

Attribute	Formulation A				Formulation B				Formulation C			
	Initial	25° C	40° C	55° C	Initial	25° C	40° C	55° C	Initial	25° C	40° C	55° C
Lyophilized Drug Product												
Cake Appearance①	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++
Moisture (% w/w)	0.2	0.2	0.9	1.2	0.9	0.9	1.2	2.5	1.6	1.6	1.8	1.9
Tg midpoint (°C)	95	95	84	79	90	90	85	70	80	80	79	74
Reconstituted Drug Product												
Reconstitution Time range (min, n = 3)	2-4	2-4	3-6	4-12	6-10	6-10	8-14	8-20	5-8	5-8	5-15	8-20
Post-recon Appearance②	CCPF	CCPF	CCPF	CCPF	OCPF	OCPF	OCPF	OCPF	CCPF	CCPF	CCPF	CCPF
Relative Change in subvisibles (>2 µm)③		+	NT	NT		++	NT	NT		+++	NT	NT
Loss in Purity (% Monomer by SEC)		1.0	3.0	11		1.2	3.7	15		1.1	3.0	10
Loss in Purity (% Main Charged Peak by		0.0	1.5	6.4		0.0	2.0	8.2		0.0	2.5	6.8

IEX)												
Loss in Purity (% oxidized by RP-HPLC)		0.0	3.0	8.0		0.0	3.3	9.2		0.0	2.8	6.5
Potency (% of Reference Standard)	98	98	90	85	98	95	90	80	98	98	95	85
Shelf Life (years at 5°C) based on Modeling	5.0				3.0				5.0			

① ++ = elegant mechanically strong cake with slight pullback; +++ = elegant mechanically strong cake with no pullback

② CCPF = clear, colorless, particle-free;OCPF = opalescent, colorless, particle-free

③ + = small increase; ++ = moderate increase; +++ = significant increase; NT = not tested

DSC indicated glass transition above 60 °C, and therefore Arrhenius square root of time kinetics was applied to predict the storage stability at desired shelf temperature (Wang et al. 2010).

In addition, the above formulations were also subjected to five cycles of freezing and thawing by sequential cycling between 5 to –40° C, and changes in purity, subvisible particles, and potency were evaluated (Table 8.7).

Based on freeze–thaw and overall results after 3 months of accelerated lyophilized drug product stability, Formulation A was selected as drug product formulation because of freeze–thaw stability, faster reconstitution time, more esthetic cake appearance and mechanical strength, desirable post-reconstitution visual appearance, lower subvisible particles and longer predicted shelf life. The drug substance formulation was same as the drug product formulation: 10 mM histidine, 4 % mannitol, 2.5 % sucrose, and 0.01 % polysorbate 20 at pH 6.0.

Table 8.7 Freeze-thaw characteristics of formulations

Formulation	Change relative to unfrozen controls			
	Visual appearance	HPSEC purity (% monomer)	Subvisible particles	Potency
A	No change	No change	No change	No change
B	Increased opalescence	No change	Slight increase	No change
C	No change	No change	Slight increase	No change

Table 8.8 Initial risk scoring methodology

Relationship criteria between cause (parameter) and effect (quality attribute)	Score
Not possible or likely (no known or reported relationship in literature and consistent with prior knowledge consistent)	1
Possible but not likely (reported in literature as possible, but not likely with mAb X based on prior knowledge with mAbs Y and Z)	4
Possible and likely (strong likelihood of possible relation based on literature and confirmed based on prior knowledge with mAbs Y and Z)	7
Established or highly likely (established relationship or strong possibility based on prior knowledge with mAbs Y and Z)	10

8.7.1 Initial Risk Assessment

The initial risk assessment for the formulation involved a cause-and-effect analysis of all theoretical parameters which are likely to have an impact on the drug product quality attributes, either directly or indirectly through their effect on the lyophilization process. The product or process parameters (cause) and the product quality attributes (effects) were selected based on prior knowledge with mAbs Y and Z. The risk assessment utilized a simple scoring approach as shown in Table 8.8.

For the initial risk assessment (Table 8.9), an arbitrary cutoff score of 60 was used based on prior experience with mAbs Y and Z. Attributes with scores above 60 were considered to be potential CQAs. These include cake appearance, reconstitution time, aggregation rate, residual moisture, subvisible particles, and potency. Four parameters with scores above 60 were identified as potential critical product or process parameters for further optimization. These included protein, mannitol, sucrose, and polysorbate-20 concentration, pH, and the lyophilization process.

8.7.2 Optimization of Sucrose and Mannitol Concentration

The QTPP defined the formulations to be isotonic, and because mannitol and sucrose influenced osmolality the most, an increase in one would necessitate a decrease in the other, leading them to be treated as a covariate parameter for additional studies. A multivariate DOE study should be conducted at this stage to further optimize the ratio of sucrose and mannitol; however, for the sake of simplicity, we will assume that the selected composition is optimal based on prior knowledge using the similar mAbs Y and Z.

8.7.3 Optimization of Polysorbate 20 Concentration

Further optimization of polysorbate 20 concentration was conducted as polysorbate 20 can impact reconstitution time, and product characteristics including subvisible particles

Table 8.9 Initial risk assessment of product or process parameters and quality attributes of drug product (cause and effect analysis)

Quality attributes parameters	Appearance (lyophilized)	Appearance post-reconstitution	Reconstitution time	Residual moisture	Degradation (aggregate formation)	Degradation (deamidation)	Degradation (fragment formation)	Degradation (subvisible particles)	Degradation mannitol hydrate formation	Potency	pH measured	Endotoxin	Sterility or container/closure Integrity	Viscosity and syringeability	Dose accuracy	Score
Protein concentration	7	4	7	1	4	1	4	7	1	7	4	4	1	4	7	62
pH	1	4	1	1	10	10	4	4	1	7	10	1	1	4	1	60
Mannitol concentration	10	7	7	4	7	1	1	7	7	7	1	4	1	4	1	69
Sucrose concentration	10	7	10	1	10	1	1	10	7	7	1	4	1	7	1	84
Polysorbate-20 concentration	4	4	10	1	10	1	1	10	1	7	1	4	1	4	1	63
Buffer concentration	4	4	1	1	1	4	1	1	1	1	10	4	1	1	1	36
Fill volume	7	1	4	4	1	1	1	4	4	1	1	4	1	1	4	51
Vial size	7	1	1	4	1	1	1	4	4	1	1	4	1	1	1	45

Table 8.9 (continued)

Quality attributes parameters	Appearance (lyophilized)	Appearance post-reconstitution	Reconstitution time	Residual moisture	Degradation (aggregate formation)	Degradation (deamidation)	Degradation (fragment formation)	Degradation (subvisible particles)	Degradation mannitol hydrate formation	Potency	pH measured	Endotoxin	Sterility or container/closure Integrity	Viscosity and syringeability	Dose accuracy	Score
Stopper/closure type	4	1	4	4	1	1	1	4	1	1	1	1	1	1	1	24
Stopper/closure formulation	1	1	1	4	1	1	1	1	1	1	4	1	1	1	1	21
Headspace gas	1	1	4	4	1	1	1	1	1	1	1	1	1	1	1	21
Drug substance formulation process	1	4	1	1	7	7	4	7	1	7	4	4	1	1	7	54
Raw material purity	4	4	1	1	4	4	4	4	1	1	4	4	4	1	1	39
Fill speed	7	4	1	1	4	1	1	7	1	4	1	1	4	1	4	39
Filtration	1	4	1	1	4	1	1	7	1	1	1	4	10	1	4	39
Capping	1	1	1	4	1	1	1	1	1	1	1	1	7	1	1	24

Table 8.9 (continued)

Quality attributes parameters	Appearance (lyophilized)	Appearance post-reconstitution	Reconstitution time	Residual moisture	Degradation (aggregate formation)	Degradation (deamidation)	Degradation (fragment formation)	Degradation (subvisible particles)	Degradation mannitol hydrate formation	Potency	pH measured	Endotoxin	Sterility or container/closure Integrity	Viscosity and syringeability	Dose accuracy	Score
Lyophilization process	10	7	7	10	4	1	1	7	4	7	4	1	1	1	1	63
Score	80	53	74	62	70	38	29	83	26	62	53	47	41	35	41	

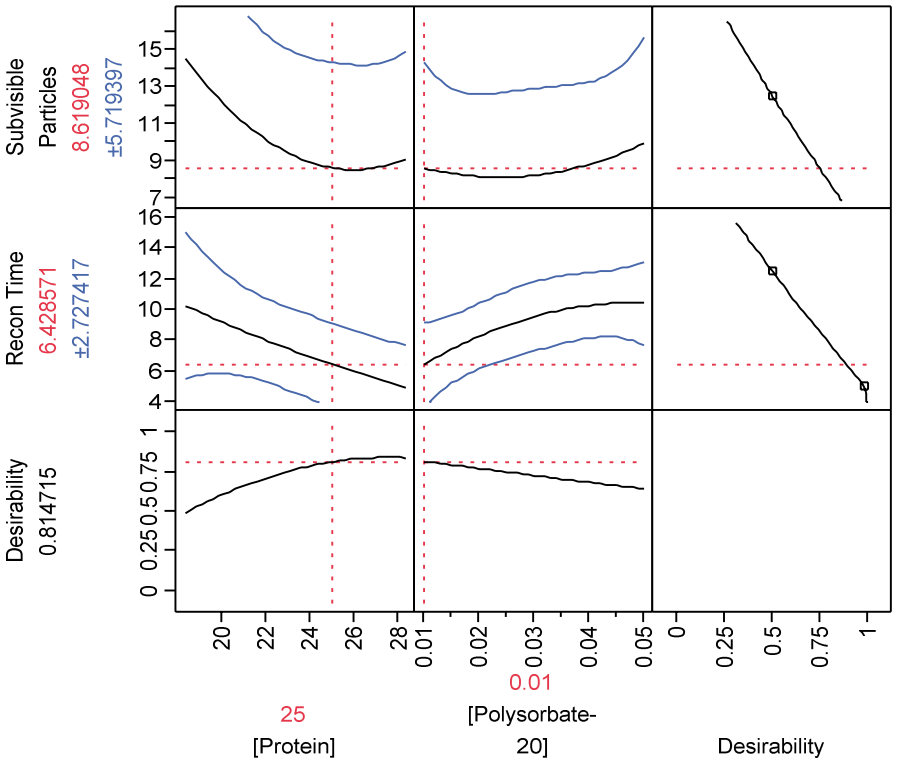

Fig. 8.5 Prediction profiler results for optimization of polysorbate 20 concentration

and visual appearance after reconstitution. Concentrations of polysorbate 20 ranging from 0.01 to 0.05% w/v were evaluated using a DOE approach in the narrow protein concentration range of 20 to 30 mg/mL. This protein concentration range was selected based on the assumption that the product concentration specification would be within 20% of the target (that is, 25 mg/mL). Since prior knowledge indicated that protein concentration affects subvisible particle formation, protein concentration was therefore assessed together with the polysorbate 20. A central composite design with two center points was utilized. Formulation A was lyophilized using the generic lyophilization cycle in the presence of 20 to 30 mg/mL protein and 0.01 to 0.05 mg/mL polysorbate 20. Upon reconstitution, the subvisible particles were measured and the reconstitution time was assessed. Figure 8.5 shows that the highest desirability (that is, the condition showing the lowest subvisible counts and reconstitution time) is obtained with a polysorbate 20 concentration of 0.01% w/v at the protein concentration target of 25 mg/mL.

8.7.4 Optimization of Protein Concentration and pH

Since the concentration of the protein was fixed at 25 mg/mL based on earlier considerations, further optimization of the protein concentration was not conducted. Also, based on previous studies (Fig. 8.2 and Fig. 8.3), a target pH of 6.0 was considered optimal.

8.7.5 Effect of Residual Water on Lyophilized Drug Product Stability

Typically the freeze-drying process is designed to ensure < 1 % residual water post lyophilization. However, the vials within a batch and between batches may have heterogeneity in drying profile which could translate to differences in residual water (Rambhatla and Pikal 2003; Rambhatla et al. 2004, 2006). Also, freeze-drying process scale-up is challenging and for the same cycle parameters moving from one freeze-dryer to another may lead to differences in residual water. Hence, understanding the impact of residual water on product quality is critical to define the acceptable limits that would potentially support identification of critical quality attributes and lyophilization process design space. Furthermore, this information can be used to assess the impact of process events that produce a batch with a higher than typical residual water content and to eventually set the drug product residual moisture specification.

Moisture equilibration studies were conducted to determine the impact of residual water on lyophilized drug product stability. Briefly saturated salt solutions of LiCl and $MgCl_2$ were used to equilibrate lyophilized vials at 11 % and 32 % relative humidity, respectively (Breen et al. 2001; Chang et al. 2005). The resulting residual water level after 24 h of equilibration and Tg (as measured by DSC) is shown in Table 8.10.

Post equilibration, all samples retained the cake structure, including samples with 3.5% residual water. Further, stability studies were performed at 40 and 5 °C for samples with low (0.5%) and high (3.5%) moisture to establish acceptable range for residual water. Primarily, loss of monomer by HPSEC was measured up to 4 weeks. At 40 °C, the rate of monomer loss for samples with 3.5% residual water was higher than samples with 0.5%; however, no difference was observed at 5 °C. This was in agreement with previous experience with mAbs Y and Z where no impact on stability was observed up to 4% residual water in similar formulations. Additionally, since the Tg is much above the intended storage temperature of 5 °C, minimal to no impact is expected on stability. Based on these studies, residual moisture does not appear to affect mAb X protein stability at concentrations up to 3.5%; however, it is identified as a potential CQA due to its undetermined effects on long-term stability, and

Table 8.10 Effect of residual water of Tg of the lyophilized drug product

Saturated salt solution in humidity chamber	Chamber humidity (% RH)	% Residual water measured in cake	Tg midpoint (°C)
Dry (post lyophilization)	Not applicable	0.5	92 ± 0.5
LiCl	11	1.4	72 ± 0.4
$MgCl_2$	32	3.5	50 ± 2

consistent with the prior knowledge and significant amount of literature relating an increase in residual moisture to faster physical and chemical degradation rates over time. Moreover, this study provides an excellent justification for identifying a somewhat higher upper limit of 3.5% residual moisture based on an understanding of the effect of moisture on degradation rates during accelerated stability.

8.7.6 Impact of Variation in Formulation Composition on the Freeze-drying Properties

The outcome of the formulation risk assessment was as expected based on prior knowledge. The highest ranked parameters (pH, mAb X, mannitol, sucrose, and polysorbate 20 concentration) from the risk assessment were screened as the main causes of impact on the freeze-drying properties (collapse temperature, eutectic melting temperature, and Tg') and CQAs (residual moisture, recon time, cake appearance, aggregation, and particle formation post reconstitution). The experimental design used was a fractional factorial in order to determine the critical formulation parameters for further characterization.

Characterization of the frozen system should include experiments where the formulation composition, including the pH, is systematically varied in order to determine the robustness of the formulation; that is, to determine whether relatively small changes in one or more formulation characteristics have a substantial influence on freeze-drying behavior and or on quality attributes of the freeze-dried product. The effect of variations in the excipient concentration on the freeze-drying properties was studied using the MDSC and freeze-drying microscopy (see Table 8.11).

Protein Concentration High protein concentration in the formulation is expected to increase the collapse temperature but could also inhibit complete crystallization of mannitol.

Table 8.11 Effect of variation of formulation parameters on freeze-drying properties

Formulation critical parameters	Tc	Tg'	Teu	Comments
High pH	−18	−21	−2	No change
Low pH	−18	−21	−1.8	
High protein	−17.5	−20.2	−2	Insignificant change
Low protein	−18.5	−21.2	−2	
High mannitol	−17	−20	−2.1	
Low mannitol	−18.5	−21.2	2	
High sucrose	−18	−21	−1.9	
Low sucrose	−17.5	−20.5	−2	
High PS 20	−18	−21	−2	No change
Low PS 20	−18	−21	−2	

Tg' glass transition temperature of maximally freeze concentrated solution
Tc collapse temperature
Teu eutectic temperature

Sucrose and Buffer Concentration High concentration of sucrose and buffer salts would inhibit the complete crystallization of mannitol, decrease the collapse temperature, and increase the potential for high residual moisture content and reconstitution time which in turn could impact storage stability of the dried cake leading to formation of aggregates, visible, and subvisible particles.

Weight Ratio of Sucrose-to-mannitol Typically a weight ratio of sucrose:mannitol of 1:1 to 1:2 is needed for complete crystallization of mannitol. Higher concentration of mannitol compared to sucrose favors complete crystallization of mannitol which in turn provides more mechanical strength and elegance to the cake. The opposite effect is also true, lower concentration of mannitol compared to sucrose results in incomplete crystallization of mannitol, lowers the collapse temperature which in turn results in inelegance and could potentially impact the storage stability.

Lower concentration of sucrose positively impacts the thermal characteristics (Tg'/Tc) of the formulation but anything lower than the minimum required to be an effective cryo- and lyophilization-protectant and be isosmolal will significantly impact the stability and isotonicity of the dried product.

PS 20 Concentration There is no deleterious effect of higher PS 20 levels on freeze-drying properties and storage stability but levels lower than the minimum required to protect the protein against interfacial instability and prevent from aggregation, formation of visible and subvisible particles can significantly impact the quality of the product.

Other Considerations Vial size and vial fill volume might be expected to influence the effective freezing and drying rate. The freezing rate would be expected to influence the morphology of ice and ability of mannitol to completely crystallize during the process. Inadequate heat transfer rates will cause inadequate drying rates during primary and secondary drying causing significant impacts on final product CQAs. All of these are discussed in the subsequent chapter on lyophilization process design and development.

Within the typical ranges of variation in formulation composition, Formulation A resulted in acceptable product quality attributes and stability without any significant impact on process performance. Identifying the edges of failures helps justify specifications [1] for drug product release and also for storage stability (pH, protein concentration, osmolality, PS 20, etc.).

8.8 Summary

While the application of QbD elements to a process is quite intuitive, application of QbD for product design (formulation development in this mock case study) is not as intuitive and is therefore seldom discussed in literature. This case study illustrates an approach for development of lyophilized formulation that is consistent with elements of QbD, while keeping in mind the entwined relationship of the formulation

[1] Specifications also take into consideration analytical method variability.

and the lyophilization process. As is commonly practiced in industry, the selection of formulation often precedes the development and optimization of the lyophilization process. Emphasis is placed on illustrating the elements of QbD such as (a) use of prior or platform knowledge or literature, (b) generation of knowledge in understanding the role of each formulation variable on the lyophilization cycle and the final product, with an eye on maintaining the desired and acceptable quality attributes that are important for maintaining safety and efficacy of the drug product, (c) risk assessment, which is illustrated through the use of a cause-and-effect matrix approach, and (d) risk management which is demonstrated through further formulation optimization.

Despite the expectation that application of QbD principles to formulation design would not likely result in any regulatory relief, it should be quite obvious that there are several additional benefits, including generation of robust product knowledge, a thorough understanding of critical attributes that impact product quality, and an advanced understanding of the risks and how to mitigate them. This knowledge can be immensely useful in assessing risks to product quality following changes to manufacturing processes, scales and sites, and in resolving manufacturing deviations.

Subsequent chapters provide a good illustrative application of QbD elements to lyophilization process design and overall risk assessment.

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Chapter 9
Drug Substance Frozen Storage and Thawing

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9.1 Introduction

Protein drug substances are often frozen for long-term storage and can be maintained in that state for a duration ranging from months to years. Freezing extends shelf life and provides flexibility for scheduling the final formulation and fill into the marketed product packaging. Depending on the nature and volume of the drug substance involved, various strategies have been used to conduct the freeze and thaw operations. The following discussion assumes that the drug substance is stable to freeze/thaw. Certain formulations, for example, those containing preservative agents, can induce product damage upon freezing and, therefore, cannot undergo this operation (Maa and Hsu 1996).

A common practice for freezing is to fill the drug substance into commercially available plastic bottles or carboys and place the containers in standard natural convection freezers, larger walk-in freezers or dedicated air blast freezers. More recently, disposable bag systems (bag inside protective shell, bag with support frame) have also been available from several vendors as an alternative to bottles, with a claim that bags, due to their geometry, can potentially provide more rapid and controlled freezing or thawing when paired with dedicated freeze-thaw hardware. For thawing, the containers are transferred to a cold room or simply left at ambient conditions. These low-capital cost solutions are suitable for handling modest drug substance batch sizes since each container is typically limited to less than 20 L in volume. However, many protein-based drugs require large administration doses to be efficacious; therefore, the volume of bulk drug substance to be processed is comparatively larger, on the order of hundreds to over a thousand liters per batch. For storing such batches using standard bottles, numerous containers would be needed, along with increased handling, tracking, and logistical complexity. In these

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instances, it would be more practical to use specialized equipment such as jacketed metal vessels and dedicated cooling skids for the freeze and thaw operations.

In this chapter, we concentrate on large-scale freeze/thaw operations performed in jacketed metal vessels for protein products. However, the concepts that are introduced can be easily applied to other systems. We demonstrate how knowledge of the fundamental physics of freeze and thaw combined with a systematic risk assessment strategy can help guide the protein drug product development process, leading to better process understanding. We begin with a general description of the freeze/thaw process and the stresses associated with freezing and thawing that may impact the quality attributes (QAs) of proteins. Next, we describe the key elements of the risk-based approach along with the risk assessment tools. The outcome of the assessment guides the development of at-scale and scale-down studies that test the sensitivity of the product to conditions experienced during manufacturing. Finally, we show how the study results are used to determine robust manufacturing operating ranges and to identify critical process parameters (CPPs).

9.2 General Description of Freeze/Thaw Processes

Irrespective of the scale involved, either milliliters or hundreds of liters, the freeze/thaw process can be a source of stress on the protein. A summary of the major physical changes encountered during the freezing and thawing steps and the resulting concerns to the stability of the drug product and process development are presented here. A detailed and illustrative discussion of freezing and thawing as applicable to biological products can be found elsewhere (Lam and Moore 2010).

9.2.1 Freezing Stress

During freezing (crystallization of water), the solute molecules are "cryoconcentrated" as they are rejected from the ice phase into the remaining intercrystalline liquid phase and can reach concentrations many times that of their starting levels. While in the "slushy" state, that is, after a significant amount of ice has formed but before the completion of the freezing operation, the temperature may still be sufficiently elevated as to permit significant motion of the solute molecules. Hence, in a protein drug substance, there may be some risk of protein aggregation, and in extreme cases, the high-concentration environment may induce protein precipitation. Depending on the composition of the solution, the temperature, the geometry of the container, and the rate of cooling, pockets of cryoconcentrate, which are denser than the starting solution, can migrate due to constitutional natural convection. This can result in an inhomogeneous distribution of solute about the frozen volume. The protein-to-excipient ratio may also vary throughout the solution.

While it is not possible to prevent the cryoconcentration phenomena at the microscopic scale, freezing at a more rapid rate can minimize the macroscopic

cryoconcentrate migration. Presumably, by lowering temperature rapidly, the cryoconcentrate pockets become less mobile and get trapped in the ice intercrystalline space. Hence, freezing methodologies that provide better heat transfer, such as the use of air blast freezers or the specialized freezing skid that circulates cooling fluid through jacketed vessels, are claimed to offer advantages over freezing in conventional freezers (Wilkins et al. 2001; Wisniewski 1998). For commercially relevant bulk sizes, several days may be required to complete the freeze operation using conventional freezers, whereas the enhanced convection methods can achieve the same result in less than 24 h. Therefore, during initial drug formulation development efforts, careful consideration should be given to actual manufacturing, storage, and cold chain transportation capabilities.

9.2.2 Thawing Stresses

The stresses involved during thaw are similar to those encountered for freeze, although they may be exacerbated due to the higher temperatures required to melt the ice in a timely fashion. There is a balance that must be determined between operational time and temperature of exposure. It is common practice to carry out the thaw step by moving the frozen containers from frozen storage to 5 °C, where they remain for many days until fully thawed. This strategy minimizes high temperature effects at the expense of longer exposure to cryoconcentration conditions. Alternatively, thaw can be completed in a few days or less, if the containers are allowed to remain at room temperature or by the use of one of the enhanced convection methods. In this case, the drug substance may be exposed to temperatures of 20 °C or more, but the operation can be optimized to rapidly cool the bulk to 5 °C as soon as all the ice has disappeared.

Unlike the freezing operation, where the liquid is initially homogeneous, thawing inherently involves multiple phases (ice and cryoconcentrate phases). A benefit of having reduced compositional variation in the frozen volume is the facilitation of mixing during thaw. It is easier to mix many small, dispersed cryoconcentrate regions than a single larger pool at the bottom of the container. At practical scales, mixing is achieved by mechanical means (e.g., container swirling, inversion, by stir bar or impeller, pumping). Here, the drug substance may be exposed to air–liquid interface stresses during mixing by container inversion or shear stresses during mixing by impeller or pumping. Therefore, physical stresses due to these additional post-thaw operations should also be considered.

9.3 Unit Operation Description

Due to relatively large batch sizes, portable jacketed metal freeze/thaw vessels (e.g., with capacities of 120 and 300 L) for long-term frozen storage of most drug substance bulks are typically used. These vessels are commercially available and can

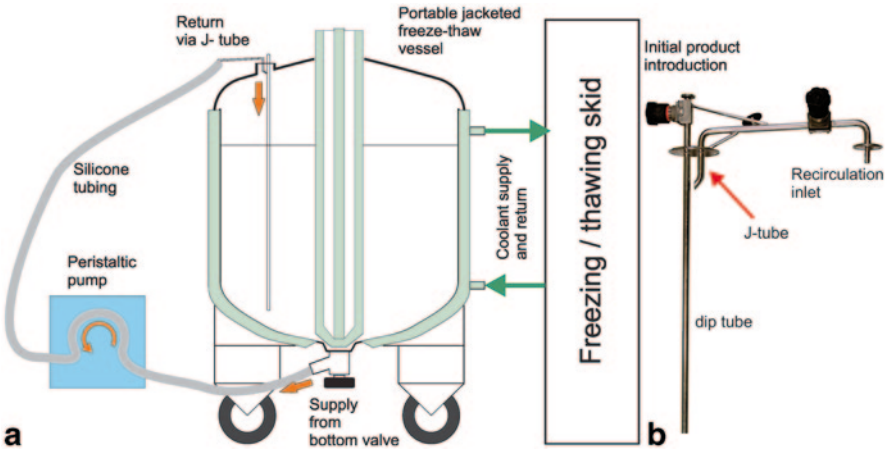

Fig. 9.1 Schematic of freeze/thaw unit operation. **a** Freeze/thaw vessel configured for pump recirculation mixing attached to skid during thaw operation. Freeze operation is similar, except the pump and tubing are not connected to the vessel. **b** Details of a dip tube assembly

be connected to cooling skids that circulate heat transfer fluid (HTF) through the vessel's jacket and internal coils. The skids can be used for both freezing and thawing, depending on the temperature control set point. For freezing, HTF temperatures are typically maintained between −55 and −45 °C. Under these conditions, 120-L and 300-L vessels filled to capacity can be frozen in about 14 and 18 h, respectively. Low HTF temperatures are utilized to hasten the freezing process. Once frozen, the vessels are detached from the skid and moved to a storage freezer, which is typically maintained at around −25 °C.

With the HTF temperature set at 22–25 °C, thawing the 120 and 300-L vessels can be completed within 9 and 12 h, respectively. Mixing of the vessel's contents is achieved by pumping the liquefied bulk from the bottom of the vessel at a modest flow rate (e.g., 1 L/min) and returning it at the top, allowing the fluid to run down the outside of a dip tube in order to minimize splashing. The mixing operation can be initiated after sufficient liquid is formed to allow for pumping (e.g., about 4–5 h after thaw is initiated); recirculation mixing is allowed to proceed until the end of thaw. Figure 9.1a shows a schematic of the system set up for a thawing operation and the components of a dip tube assembly is shown in Fig. 9.1b.

9.4 Risk-based Approach Applied to Freeze/Thaw Assessment

9.4.1 General Description of the Risk-based Approach

Martin-Moe (Martin-Moe 2010; Martin-Moe et al. 2011) describe a risk-based approach that can be applied to drug product process development. A risk assessment

is performed early in the development cycle to identify the potential critical quality attributes (pCQAs) of the protein. A separate risk assessment is then performed on each unit operation to gauge the impact of the process parameters on the identified pCQAs and other attributes. The outcome from this second risk assessment is the identification of potential critical process parameters (pCPPs) and the type of characterization studies needed to fully characterize the unit operation.

The studies are usually conducted in a multivariate manner, where the selected process parameters are simultaneously varied to the extremes of the operating limit. The results of these studies are then used to identify CPPs and robust multivariate acceptable ranges (MARs). These MARs are typically broader than standard manufacturing ranges.

In the following sections, these steps for this risk-based approach are described in greater detail, and case studies are provided to illustrate the process.

9.4.2 Identification of Potential Critical Quality Attributes (pCQAs)

The pCQAs of the molecule of interest are identified using a risk ranking and filtering (RRF) tool as described by Motchnik (2009). The pCQAs are usually determined early in the development cycle and are updated as more product knowledge is attained. The pCQAs need to be defined upfront since they are the criteria by which process impact on the protein is assessed. The RRF process considers two factors: (1) an impact value, which assesses the effect of the attribute on patient safety or efficacy, and (2) an uncertainty value, which assesses the degree of uncertainty of the impact value. The impact and uncertainty values are multiplied to yield a risk score. The location of the risk score in a predefined risk matrix determines whether the attribute is a pCQA. The quality target product profile, prior knowledge of the molecule or similar molecules, and clinical experience are factors considered for the scoring. Examples of pCQAs that pertain to a drug product are shown in Table 9.1.

Table 9.1 Examples of potential critical quality attributes (pCQAs) impacted by drug product unit operations

Potential critical quality attribute category	Examples
Product variants	Aggregates Fragments Charge variants Oxidation Subvisible particles
Composition and strength	pH Osmolality Protein concentration
Process impurities	Leachables Foreign particulates
Microbiological	Endotoxin Bioburden Sterility

9.4.3 Identification of Potential Critical Process Parameters (pCPPs) and Characterization Studies

The freeze/thaw operation subjects the drug substance to a variety of stresses including cryoconcentration, air–liquid interfacial interaction, mechanical shear, and exposure to elevated temperatures, as discussed above. The magnitude of the stresses experienced by the protein is dependent on the process operating conditions. An RRF assessment (McKnight 2010) is performed to gauge the impact of the freeze/thaw process parameters on process and product attributes. The RRF tool is used to determine which process parameters are enrolled in process characterization or validation studies and whether the study should be conducted in a univariate or multivariate manner. In a univariate study, only one process parameter is varied with the remaining parameters held at their target set point. In a multivariate study, multiple process parameters are varied simultaneously (e.g., design of experiments (DOE) study).

Risk assessment allows developers to leverage knowledge of the product, first principles understanding of the unit operation, and experiences gained from processing similar types of products in the past. The appropriate rationale is formally documented in the RRF report. Subject matter experts list the process parameters for the unit operation. Using the RRF tool, each process parameter is assessed for potential (1) main effect and (2) interaction effect with other process parameters against predefined responses. Two types of responses are considered: (1) product QA responses, which include pCQAs that can impact product safety, activity, or efficacy; and (2) non-CQA and process attributes, which are nonproduct quality outputs of a process and generally apply to process performance. The main effect score assesses the degree of impact of a given process parameter on all the responses, independent of the other process parameters. The interaction effect score assesses the potential that the interaction of two or more factors that are simultaneously varied results in a greater (or lesser) response than the sum of each factor varied individually. The assessment is based on operating ranges that are defined for each parameter. Score values are assigned for the main and interaction effects based on set criteria (see Table 9.2). The pCQAs are more heavily weighted than noncritical or process attributes. The severity score, which is the product of the main effect and interaction effect scores, is used to determine the study approach and the pCPPs. As depicted in Table 9.3, there are three options: (1) no additional study required, (2) univariate study, or (3) multivariate study. Process parameters may always be upgraded to a higher level study (i.e., univariate to multivariate), if appropriate. The RRF process is illustrated using the following case study for Protein X.

Tables 9.4 and 9.5 summarize the results of the freeze and thaw unit operation RRF exercise for Protein X. The main effect and interaction effect scores for each pCPP are listed for different quality and process attributes. The QAs of Protein X

Table 9.2 Criteria for the assignment of impact values for pCPP RRF

Impact value	Description	Definition
1	No impact	Effect causes variation in process output, which is not expected to be detectable (e.g., no effect or within assay variability)
2–4	Minor impact	Effect causes variation in process output, which is expected to be within acceptable range (2 for noncritical quality attribute)
4–8	Major impact	Effect causes variation in process output, which is expected to be outside acceptable range (4 for noncritical quality attribute)

Table 9.3 pCPP risk ranking filtering decision matrix

Severity score	Experimental strategy
≥32	Multivariate study
8−16	Multivariate, or univariate with justification
4	Univariate acceptable
≤2	No additional study required

that are potentially impacted by the freeze/thaw unit operations include product variant attributes (e.g., size variants, charge variants), filterability, and compositional attributes (e.g., protein concentration and osmolality). The relevant process attributes are completion of freeze/thaw and post-thaw filterability. The attributes are selected during a risk assessment and are product specific. The selection is based on previous knowledge of the protein by subject matter experts. Note that the impact of long-term storage of the frozen bulk is not part of the assessment or the study. For each pCPP, the maximum attribute risk priority number is used to classify the study type.

The pCPPs for the freeze unit operation are: freeze duration, bulk volume in the vessel, HTF temperature, and number of freeze/thaw cycles. The pCPPs for the thaw unit operation are bulk recirculation time, bulk recirculation start time, bulk volume in the vessel, recirculation flow rate, HTF temperature, and number of freeze/thaw cycles. For both freeze and thaw unit operations, the HTF flow rate was not considered as a process parameter since it is fixed by the equipment and cannot be modified.

For the thaw unit operation, all parameters should be enrolled in multivariate studies. For the freeze unit operation, all process parameters should be enrolled in multivariate studies except for number of freeze/thaw cycles. This parameter can be enrolled in a univariate study.

Table 9.4 Freeze unit operation: pCPP risk ranking and filtering exercise for Protein X

Process parameter	Affected attributes	Main effect rank (M)	Rationale for main score	Interaction effect rank (I)	Potential interaction parameters	Rationale for interaction score	Severity score ($M \times I$)
Freeze duration	Completion of freeze	8	Freeze time is formulation dependent. Adequate time is needed to completely freeze bulk	8	HTF temperature Bulk volume	HTF temperature and bulk volume will impact time needed to completely freeze bulk and freeze rate	64
	PV attributes	4	Inadequate freeze time has effect on product and process attributes since product may be in partially frozen state for extended time. Long durations are not expected to impact attributes since phase transition temperature of DS bulks are higher than freeze temperature	4			16
Bulk volume in vessel	Completion of freeze	8	Freeze time is dependent on bulk volume	8	HTF temperature Freeze cycle duration	High HTF temperature, extends freeze time. Low freeze cycle duration may result in incomplete freeze	64
	PV attributes	4	Batch volume impacts freeze rate. Larger batch size extends time product may be in cryo-concentrated state	4			16
HTF temperature	Completion of freeze	8	HTF temperature affects freeze rate	8	Bulk volume Freeze cycle duration	High product volume extends freeze time. Low freeze cycle duration may result in incomplete freeze	64
	PV attributes	4	High HTF temperature may lead to incomplete freeze or slow freeze exposing product to cryoconcentrated state for extended time	4			16

Table 9.4 (continued)

Process parameter	Affected attributes	Main effect rank (*M*)	Rationale for main score	Interaction effect rank (*I*)	Potential interaction parameters	Rationale for interaction score	Severity score ($M \times I$)
Number of freezes	Completion of freeze	2	Not impacted by number of freezes	2	NA	No interaction expected based on experience with previous products	4
	PV attributes	4	Multiple phase change cycles and longer exposure to thaw cycle temperatures may impact product attributes	2			8

DS drug substance, *HTF* heat transfer fluid, *NA* not applicable, *PV* product variant

Table 9.5 Thaw unit operation: pCPP risk ranking and filtering exercise for Protein X

Process parameter	Potential affected attributes	Main effect rank (M)	Rationale for main score	Interaction effect rank (I)	Potential interaction parameters	Rationale for interaction score	Severity score ($M \times I$)
Bulk recirculation time	Completion of thaw/ homogeneity	8	Homogeneity is affected by recirculation time. For some formulations, additional recirculation is sometimes needed for homogeneity	8	HTF temperature Recirculation rate Bulk volume Bulk recirculation Start time	High HTF temperature, recirculation rate, multiple F/T cycles at low volumes may impact PV attributes and filterability. Low HTF temperature, low recirculation rate, early recirculation start time, high bulk volume impact homogeneity	64
	PV attributes	4	Long recirculation time subjects protein to recirculation stresses and longer ambient temperature exposure. Past studies have shown minimal effect	4			16
	Filterability	4	Long recirculation time increases air/protein interaction, which may result in subvisible particle formation and filter fouling	4			16
Bulk recirculation start time	Homogeneity	8	There may not be sufficient amount of thawed liquid in the vessel if bulk recirculation is started prematurely. This may lead to air entrainment in the pump and inadequate mixing at the end of the operation.	8	HTF temperature Recirculation time Recirculation rate Bulk volume	Volume of product and HTF temperature affects the amount of bulk thawed at start. Recirculation parameters impact thaw rate	64

Table 9.5 (continued)

Process parameter	Potential affected attributes	Main effect rank (M)	Rationale for main score	Interaction effect rank (I)	Potential interaction parameters	Rationale for interaction score	Severity score ($M \times I$)
Bulk volume in vessel	Homogeneity	4	Large batch size is more difficult to thaw and make homogeneous	4	HTF temperature Recirculation rate Recirculation time Bulk recirculation Start time	High HTF temperatures, long recirculation time, fast recirculation rate, multiple F/T cycles may impact PV attributes and filterability. Low HTF temperature, short recirculation time, low recirculation rate, early recirculation start time, high bulk volume impact homogeneity	16
	PV attributes	4	Large batch size extends time product may be in partially frozen state. Low batch volume results in more turnovers and recirculation stresses. Low bulk volume subjects protein to longer ambient temperature exposure. Past studies have shown minimal effect	4			16
	Filterability	4	Low bulk volume increases potential for air/product interaction since liquid level in vessel is lower and drop distance during recirculation is greater	4			16
Recirculation flow rate	Homogeneity	8	May not thaw completely or achieve homogeneity if flow rate is too low	8	HTF temperature Recirculation time Bulk recirculation Start time Bulk volume Number of F/T cycles	High HTF temperature and recirculation time at low bulk volumes, multiple F/T cycles may impact product variant attributes and filterability. Low HTF temperature, short recirculation time, early recirculation start time, high bulk volume impact homogeneity	64
	PV attributes	4	High flow rate increases stresses from recirculation. Past studies have shown minimal effect	4			16
	Filterability	4	High flow rate increases air/product interaction	4			16

Table 9.5 (continued)

Process parameter	Potential affected attributes	Main effect rank (*M*)	Rationale for main score	Interaction effect rank (*I*)	Potential interaction parameters	Rationale for interaction score	Severity score ($M \times I$)
HTF temperature	Homogeneity	4	Thaw completion may be affected by low HTF	4	Recirculation time Bulk volume Bulk recirculation Start time Number of F/T cycles	High recirculation rate, long recirculation times at low bulk volumes, multiple F/T cycles may impact PV attributes and filterability. Short recirculation time, low recirculation rate, early recirculation start time, high bulk volume impact homogeneity	16
	PV attributes	4	High HTF temperature may impact product attributes. Past studies have shown minimal effect	4			8
	Filterability	1	No impact expected	1			1
Number of thaws	Homogeneity	2	No impact expected	2	Recirculation time Bulk volume Bulk recirculation Start time HTF temperature	Potential interacting variables affect exposure to recirculation stresses and ambient temperature, which affect PV attributes and filterability. No impact on homogeneity	4
	PV attributes	4	May result in moderate impact from multiple phase change cycles and exposure to ambient temperature. Past studies have shown minimal effect	4			16
	Filterability	4	May result in impact from exposure to multiple thaw cycles if not filtered between cycles	2			8

F/T freeze/thaw, *HTF* heat transfer fluid, *PV* product variant

9.5 Freeze/Thaw Characterization Studies

It is common practice to conduct the commercial freeze and thaw operations using generic cycle parameters that are independent of the product. The recipes may differ between manufacturing sites due to differences in equipment or practice, but for any given site, the operating conditions employed are identical for all products processed there. Therefore, the goal of the characterization studies is not to optimize the operation parameters but to demonstrate that the operating ranges of the process consistently maintains the CQAs of the protein. The physical stresses from the freeze/thaw process are well understood. The operation does not involve complex chemical or compositional modifications or reactions. This allows selection of these worst-case conditions based on scientific and first principle knowledge. Therefore, the approach taken is to perform worst-case multivariate studies to establish the manufacturing ranges in place of a DOE series of studies. If acceptable product quality is achieved under worst-case conditions, then operations within the tested range should be satisfactory. Multiples experiments may still be necessary since different QAs may have different worst-case conditions. With this approach, if impact to QAs is observed, then more studies may be needed to determine which parameters are critical.

There are generally two types of characterization studies that are needed, as depicted in Fig. 9.2. The first type of study, referred to as an operation condition verification study, assesses whether the operational conditions are sufficient to completely freeze a bulk, or to completely thaw and attain a homogeneous bulk. For the thaw

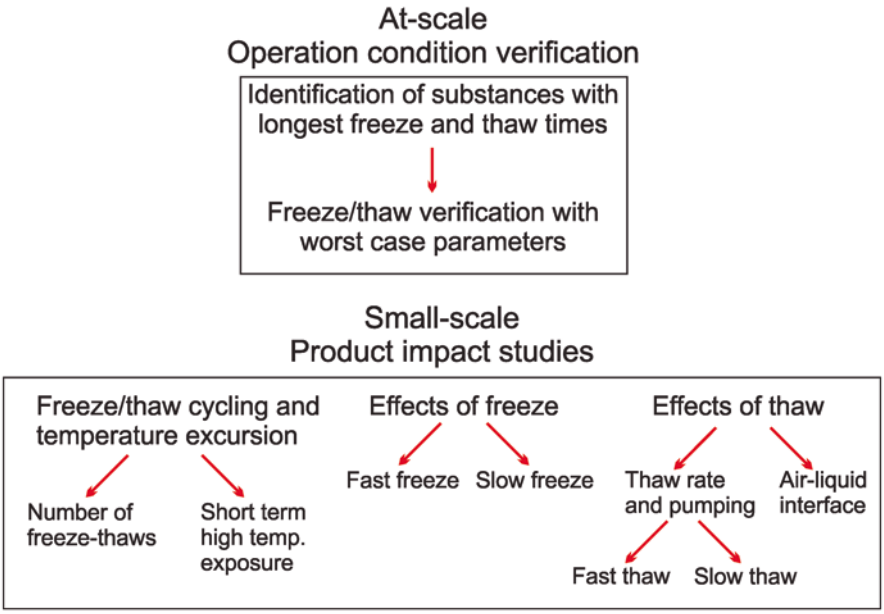

Fig. 9.2 Summary of at-scale and small-scale characterization studies

operation, only compositional critical quality attributes (CQAs), such as protein concentration or osmolality, apply. These studies are discussed in Sect. 9.8.1 below.

The second type of study, referred to as a product impact study, evaluates the impact of the freeze/thaw operation on the physical or chemical attributes of the drug substance and will be discussed in Sect. 9.8.2. Product variant attributes and filterability are assessed in this type of study. The worst-case processing conditions for the operation condition verification studies differ from those for the product impact studies. As is shown in later sections, it is more efficient to perform separate studies to assess the effect of the processing parameters on final process and QAs.

The worst-case studies provide lower or upper limits for each of the parameters. For example, the operation condition verification thaw study supports a lower limit for HTF temperature, while the product impact thaw studies support the upper limit for HTF temperature. The MAR range is then specified by the two types of studies.

9.6 Critical Process Parameter (CPP) Assessment

The worst-case freeze/thaw characterization studies are used to establish MARs for the process parameters and to identify CPPs based on the observed impact to the product's pCQAs. It is important to recognize that the CPP determination is restricted to the tested parameter MAR operating range. Operating outside these limits would warrant further investigation.

CPPs are determined based on a calculated impact ratio for each of the QAs tested (McKnight 2010). The impact ratio is a measure of the magnitude of the effect of a parameter on the pCQA relative to the allowable variation of that pCQA. It is defined as follows:

$$\text{Impact Ratio} = \frac{\text{Effect Magnitude}}{\text{Allowed CQA Variation}} = \frac{CQA_{\text{Stress}} - CQA_{\text{Control}}}{CQA_{\text{Mean}} - CQA_{\text{Spec}}},$$

where CQA_{Stress} is the resulting CQA test result for the stressed sample, CQA_{Control} is the resulting CQA test result for the un-stressed or control sample, CQA_{Mean} is the average value for the CQA from appropriate batches (typically GMP clinical batches or commercial batches if available), and CQA_{Spec} is the specification limit for the CQA. If desired, the specification limit can be narrowed to provide a safety margin.

Parameters that cause any CQA impact ratio to be greater than a prescribed value are CPPs. This value should be based on past knowledge, typical observed variations, and subject matter expert input. However, the value should be identified prior to initiating the study. If all the CQA impact ratios are less than the prescribed value, or the effect magnitudes are smaller than assay precision, then the parameters are not CPPs.

For attributes that are qualitative (e.g., appearance), parameters are considered CPPs if there is in an observed change to the pCQA. Otherwise, the parameters are considered non-CPPs.

9.7 Case Study

9.7.1 At-scale Operation Condition Verification

The actual time required to fully freeze or thaw and mix a given volume of drug substance is strongly dependent on the scale and geometry of the container and, to lesser extent, the composition of the liquid. In order to ensure the completeness of freeze or thaw operations, the use of at-scale vessels is required.

Freeze/thaw vessels are cleaned and sterilized in place prior to being filled with drug substance. The vessels are closed systems during normal operation to provide a protective barrier for the contents from microbial ingress. It is not possible to visually monitor the progress of the freeze or thaw processes, and temperature measurements of the vessel's contents can only be taken at one fixed location via the thermowell. Although the most relevant, it is not practical or economical to perform tests with actual drug substance bulks during every instance of skid commissioning, validation, or freeze/thaw cycle verification. This would necessitate introducing multiple temperature probes at various locations inside the vessel, thereby compromising vessel integrity. For these activities, it is acceptable to substitute an expendable substance that is either representative or performs "worse" (longer time required to freeze or thaw) than active material.

To determine an appropriate surrogate, a study was undertaken to compare the freezing and thawing times for several typical protein formulations, active and placebo, filled into 300-L freeze/thaw vessels at maximum capacity. Since each experiment was performed under the same conditions and using the same cooling skid, direct comparison between each case was possible (Table 9.6). From the tests, the freeze and thaw times were obtained visually, by using a small video camera inserted into the vessel and recording the progress of the operation. Freeze time is defined as the time required to fully solidify all the liquid; thaw time is defined as the time required to melt all the ice. Completeness of mixing after thaw was confirmed by sampling at the top, middle, and bottom of the vessel and testing for protein concentration (when applicable), osmolality, and refractive index.

It is worthwhile to note that deionized water, despite having the highest total latent heat requirement (solutes such as protein, salts, and other excipients do not contribute significantly to the heat load during freeze/thaw), freezes and thaws the fastest of all the solutions tested. Therefore, deionized water should not be used to set minimum operation times as the values obtained would lead to parameters that may not guarantee completeness of freeze and thaw for actual products. From these examples, it can be seen that the "worst-case" formulation that requires the longest time to freeze and thaw, is that of the placebo of Protein 2 (see Table 9.6). Therefore, Protein 2 placebo can be used as a surrogate to active material for purpose of equipment testing, validation, and parameter verification to ensure the completeness of freeze and thaw operations. Any new product formulation can then be tested at scale similarly and compared with prior data to verify the suitability of the process.

Table 9.6 Comparison of the freezing and thawing times for protein and placebo formulations

Product	Dissolved solids (g/L)	Freeze time (h)	Thaw time (h)	Post-thaw mixed
Protein 1, 25 mg/mL	46.5	10.7	8.9	Yes
Protein 1 placebo	21.5	10.6	9.7	Yes
Protein 2, 21 mg/mL	34	11.4	8.8	Yes
Protein 2, 55 mg/mL	68	11.7	8.8	Yes
Protein 2 placebo	13	12.1	11.1	Yes
Protein 3, 25 mg/mL	86.1	11.8	9.4	Yes
Protein 3 placebo	61.1	11.6	9.4	Yes
Deionized water	0	10.6	8.4	NA

NA not applicable

Provided that the results fall within the previously established worst-case then full-scale re-validation will not be necessary.

The multivariate freeze/thaw operation condition verification studies identified in the risk ranking step summarized in Tables 9.4 and 9.5 can be performed using Protein 2 placebo. These tests combine the worst-case equipment settings with the worst-case formulation. A full DOE study is not necessary since any other setting will not result in worst-case conditions (longest freeze or thaw times).

For the freeze operation, the following worst-case parameter conditions are tested:

- Maximum allowable fill volume
- HTF temperature at a set point of 5 °C above target
- This combines the highest thermal load with the lowest thermal driving force. The study ensures operation within the specified cycle time will lead to adequately frozen bulk

For the thaw operation, the following worst case parameter conditions are tested:

- Maximum allowable fill volume
- HTF temperature at 2 °C below target
- Recirculation mixing pump flow rate at 90 % of target for the standard recirculation time and the standard recirculation start time

This combines the highest thermal load with the lowest thermal driving force and lowest mixing rate.

Using these conditions, at-scale experiments were conducted in duplicate with actual manufacturing equipment. From visual and thermocouples data, it was confirmed that the freeze operation can be successfully completed, resulting in all locations within the vessel reaching well below −20 °C within 16 h. Likewise, thawing was confirmed to be complete by observing the lack of ice. The homogeneity was checked by taking samples at the top and bottom of the vessel and analyzing by measuring conductivity and pH.

The above results give confidence that freeze and thaw processes are robust and the operations can be successfully completed for all products when conducted under standard operating conditions.

9.8 Use of Small-Scale Models to Assess Unit-Operation Impact on Product Attributes

Conducting all tests at scale using actual product would require a large amount of material and be prohibitively expensive. In many situations, a small-scale model can provide sufficient information about the behavior of a drug substance as it is carried through the manufacturing unit operation. Furthermore, it is typically easier to operate, control, and manipulate conditions of the small-scale processes, which facilitate the collection of the required data. The freezing and thawing dynamics and resulting cryoconcentration behavior depend on both intrinsic scale-independent parameters such as HTF temperature and the material substance properties (e.g., heat capacities and thermal conductivities) and scale-dependent properties (such as vessel and product volumes, heat transfer surface area, and thaw recirculation conditions). Therefore, it is not possible to exactly mimic at-scale freezing/thawing dynamics using simple small-scale devices. For example, given two cylindrical containers of different volumes but of same aspect ratio, the smaller container will always freeze more rapidly than the larger one when exposed to the same freezing conditions. It is not possible to match both the freezing rate and the freezing temperature at the same time.

As discussed previously, optimization of the cycle process parameters is not the goal, so the small-scale models do not need to be an exact scale down, but rather, need to be able to mimic the stresses encountered during commercial operations. A bracketing strategy can be employed, whereby small-scale conditions are appropriately selected to be more extreme than those observed during normal at-scale processes. The assumption is that if the extreme conditions are shown to be satisfactory, then operation anywhere within the multivariate range will result in acceptable product quality. Experiments were carefully designed to address the concerns identified as part of the risk assessment activity.

For the freeze operation, the following parameters are enrolled in multivariate worst-case studies: freeze operation time, bulk volume in the vessel, HTF temperature, and number of freeze/thaw cycles. In the case of small-scale studies, the first three process parameters ultimately impact the drug substance freezing rate and the final frozen bulk temperature. Therefore, the effect of these parameters may be lumped together by performing univariate freeze rate studies. The last parameter, number of freeze/thaw cycles, is addressed in Sect. 9.8.2.3.

For the thaw operation, the following parameters are enrolled in multivariate worst-case studies: bulk volume in the vessel, HTF temperature, bulk recirculation time, recirculation flow rate, and number of freeze/thaw cycles. For small-scale studies, the first two parameters are accounted for by varying the rate of freezing

and thawing. Recirculation time and recirculation flow rate may be scaled and expressed as number of product turnovers. For the number of freeze/thaw cycles, two types of experiments are performed. For the first type of experiment, drug substance is subjected to multiple freeze thaw cycles under conditions of fast freezing and thawing rates but with no recirculation. For the second type of experiment, recirculation studies are performed with a single freeze/thaw cycle but with an extended number of turnovers to account for the maximum number of freeze/thaw cycles. Figure 9.2 gives an overview of the experiments discussed below.

9.8.1 Multiple Freeze/Thaw Cycles and Frozen Temperature Excursion

Several freeze/thaw vessels may be thawed and pooled to produce a single lot of drug product. In some instances, only a portion of the thawed contents in a particular freeze/thaw vessel is dispensed, requiring the remaining portion to be refrozen. Additionally, unforeseen manufacturing delays may necessitate refreezing of a thawed bulk. Therefore, the drug substance may be subjected to multiple freeze thaw cycles. Additionally, during storage and handling of the frozen bulk, the drug substance may deviate from the specified temperature conditions for a short period of time. This situation may lead to a partial thaw resulting in excipient crystallization or cold denaturation, which may impact the product.

Small-scale studies are performed to support the maximum number of freeze/thaw cycles allowed and short-term temperature excursions. These studies may be conducted separately or performed in series using sterilized mini-tanks (25–70-cc) that are constructed from the same metal alloy as the at-scale freeze/thaw vessels. Product volumes are selected to ensure that (1) the ratio of container surface area to drug substance volume and (2) the ratio of air surface area to product volume are both greater in the mini-tank compared with the freeze/thaw vessel. The freeze rate and thaw rate are faster than normally encountered at scale.

Protein X was subjected to a combined temperature excursion and multiple freeze/thaw cycle study. Filtered drug substance (20-mL samples) was aseptically added to sterilized 25-cc 316-L stainless steel mini-tanks which were then frozen and stored at −40 °C for 2 days. Subsequently, the mini-tanks were transferred to a −20 °C freezer for 2 days to mimic actual storage temperatures.

To assess the effect of temperature excursions, several mini-tanks were transferred to separate chambers maintained at −20 °C (control), −14 °C, and −8 °C for 2 weeks. At the conclusion of the study, the mini-tanks were completely thawed at 5 °C and an aliquot removed for testing.

The mini-tanks from the temperature excursion study were refrozen at −40 and −20 °C as previously described. The tanks were then transferred from the −20 °C chamber to a 5 °C chamber for at least 8 h to ensure complete thaw. The freeze/thaw process was repeated for a total of five cycles. Storage at frozen temperature

Table 9.7 Impact of frozen temperature excursion and multiple freeze/thaw cycles on Protein X

Sample storage temperature, duration, number of F/T cycles	Turbidity average 340–360 nm (AU)	Protein concentration (mg/mL)	Size exclusion chromatography (% monomer)
Initial time point ($T=0$)	0.10	49.7	99.3
−8 °C, 2 weeks	0.11	50.5	99.2
−8 °C, 2 weeks + 5 F/T cycles	0.09	50.1	99.2
−14 °C, 2 weeks	0.12	50.7	99.3
−14 °C, 2 weeks + 5 F/T cycles	0.10	49.9	99.3
−20 °C, 2 weeks	0.10	50.3	99.3
−20 °C, 2 weeks + 5 F/T cycles	0.10	49.6	99.3

F/T freeze/thaw

followed by thawing at 5 °C was considered one freeze/thaw cycle. Aliquots were removed for testing after the final freeze/thaw cycle.

The aliquots were tested for protein concentration, turbidity, and protein aggregates (see Table 9.7). The results show that differences between the starting material and any of the storage or cycling conditions were all within assay precision. Therefore, short-term storage of the drug substance above the recommended temperature of −20 °C followed by five freeze/thaw cycles did not impact the product based on these measured attributes.

9.8.2 Freezing

During the freezing process conducted at scale in freeze/thaw vessels (see above for description), the drug substance is exposed to a range of freezing rates. Here, we define freezing rate as the time between the onset of ice formation and when the temperature reaches −20 °C (the allowable upper limit for storage temperature). The regions adjacent to actively cooled surfaces such as the vessel walls and cooling coils freeze rapidly (within 0.25–0.50 h), while the warmest location (furthest from cooling surfaces) in the vessel may reach below −20 °C only after 12–18 h. Therefore, a small-scale model for this unit operation would consist of two parts, each capturing the extremes of the at-scale process:

1. To represent the "fast freezing" regions that are located near the jacketed walls and cooling coils of the vessel, experiments can be conducted using small volumes, where the sample temperature can be lowered to below −20 °C in less than 0.25 h after ice nucleation.
2. To represent the "slow freezing" regions that are located farthest away from cooling surfaces, experiments where the sample temperature is gradually lowered to less than −20 °C after ice nucleation, can be conducted. This is achieved by placing samples onto a lyophilizer shelf and using a slow temperature ramp, thereby maximizing the time spent in the "slushy" state.

Table 9.8 Impact of fast and slow freeze rates on Protein X

Freeze rate, sample description	Protein concentration (mg/mL)	Appearance[a]	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)
Fast freeze				
Control[b]	30.5	Color: ≤ B8 Clarity/opalescence: ≤ Ref I	0.07	99.7
Test	30.4	Color: ≤ B8 Clarity/opalescence: ≤ Ref I	0.08	99.7
Slow freeze				
Control[b]	30.4	Color: ≤ B8 Clarity/opalescence: ≤ Ref I	0.07	99.7
Test	30.4	Color: ≤ B8 Clarity/opalescence: ≤ Ref I	0.08	99.7

[a] Color, clarity/opalescence were evaluated using United States Pharmacopeial Convention and European Pharmacopoeia standards
[b] The control was prefrozen bulk

It is not necessary to explore more severe conditions such as flash freezing in liquid nitrogen or slow freezing over weeks' time, as these conditions are not achievable by a typical at-scale freeze process.

In the following example we apply the above strategy to test several protein formulations:

The fast freezing samples were prepared by filling 20-mL vials with 1 mL of the solution to be tested and loading the vials onto an aluminum plate (heat sink) pre-equilibrated in a −40 °C freezer. This arrangement allowed the contents of the shallow-filled vials to freeze and reach temperatures less than −20 °C within a few minutes.

Slow freeze samples were prepared by using 1-mL vials filled to 5 mL to provide a substantial fill height. The filled vials were then loaded onto a pilot-scale lyophilizer shelf and the temperature equilibrated at between −5 and −1 °C for 2–4 h to ensure that the liquid is slightly below its freezing point. Ice nucleation was induced in each sample, one at a time, by touching the bottom edge of the vial with a piece of dry ice. Immediately upon ice nucleation, the vial was returned to the lyophilizer shelf. Nucleation was performed to avoid supercooling and heterogeneous freezing of the product in the different vials. The cooling step, a linear ramp from the equilibration temperature down to −40 °C in 72 h, was then initiated.

After completion of freezing, the samples were transferred to a −20 °C freezer and stored for at least 2 days. The samples were then thawed and analyzed for protein concentration, appearance, turbidity, and by size exclusion chromatography for aggregates. The results for Protein X are summarized in Table 9.8. The control for this study was the prefrozen bulk.

Table 9.9 Impact of fast and slow freeze rates on Protein Y

Freeze rate, sample description	Protein concentration (mg/mL)	Appearance[a]	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)
Fast freeze				
Control[b]	5.9	Color: ≤ B8 Clarity/ opalescence: ≤ Ref I	0.00	99.7
Test	6.1	Clear, colorless	0.02	99.7
Slow freeze				
Control[b]	6.0	Clear, colorless	0.01	99.7
Test	6.0	Clear, colorless	0.00	99.7

[a] Color, clarity/opalescence were evaluated using United States Pharmacopeial Convention and European Pharmacopoeia standards
[b] The control was prefrozen bulk

Based on assay precision and compared with the control, no significant differences in any measured attributes for the fast or slow frozen Protein X bulk was observed. Since these studies show no impact on Protein X CQAs under these extreme, bracketed freezing conditions, the three freeze process parameters that control freeze rate (freeze operation time, bulk volume in the vessel, HTF temperature) are not CPPs for the tested operating range.

A similar freeze/thaw study was conducted for Protein Y (results summarized in Table 9.9). In this case, slightly higher turbidity was observed after fast freezing, which is attributed to formation of subvisible particulates. However, neither protein aggregation nor protein concentration was affected. The subvisible particulates are removed during filtration and do not impact the final filled drug product. No significant differences were observed in any of the measured attributes for slow freezing.

9.8.3 Thawing and Mixing

During thaw, in addition to thermal exposure, there are two other major types of stresses arising from recirculation mixing that can affect the product:

1. Pump and fluid flow stress of the solution though the pump and tubing: at the onset of recirculation, the thawed liquid is significantly more concentrated in solute than the initial prefreeze bulk since at these temperatures, the cryoconcentrates becomes mobile and pools at the bottom of the vessel. While this high concentration fluid "slug" is dissipated relatively rapidly, within the first 10–15 min of recirculation, stresses generated from mechanical pumping may still cause an increase in product aggregate levels. The processing parameters that affect the thaw rate and the persistence of the cryoconcentrates are bulk volume in the vessel, HTF temperature, bulk recirculation time, and recirculation flow rate. Though the number of freeze/thaw cycles do not affect thaw rate, the cumulative effect of multiple freeze/thaw cycles needs to be assessed.

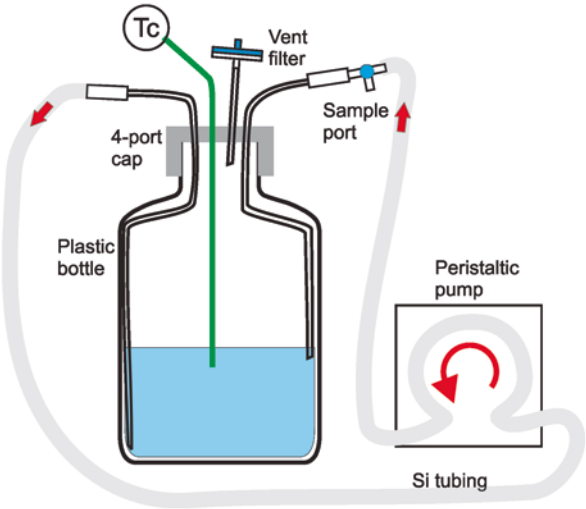

Fig. 9.3 Schematic of small-scale thaw recirculation apparatus. The bottle is constructed from clear polycarbonate. The dip tubes are either 316L stainless steel or Hastelloy C22. The return tubing is positioned below the liquid surface to reduce mixing and prolong thawing time. The peristaltic pump tubing is platinum-cured silicone. The thermocouple probe (T_c) is placed just below the liquid level

2. Increased air–liquid interface while the solution flows down the dip tube and impinges upon the liquid surface: surface sensitive products may be exposed to conditions that increase chances of denaturation and aggregation. The severity of the air–liquid interaction depends on the recirculation flow conditions (bulk recirculation time and recirculation flow rate) and the bulk volume in the vessel.

Following the same strategy as presented above, the at-scale unit operation is broken down into several small-scale models representing the extremes of conditions observed in the actual process.

Thawing and Pumping Stress To mimic the stresses from pumping, a system comprising a 500-mL bottle (reservoir), flexible tubing, and peristaltic pump was set up as depicted in Fig. 9.3. The components were chosen so as to permit steam sterilization. As in an at-scale freeze/thaw vessel, the thawed solution is withdrawn from the bottom and returned at the top by a peristaltic pump. However, in the small-scale apparatus, a dip tube is used as the outlet and the thawed fluid is returned below the liquid level, minimizing mixing. This lengthens the amount of time that the denser freeze concentrate is circulated through the pump and tubing. While the volumetric flow rate is lower than at-scale conditions, the corresponding shear rate in the tubing is significantly higher than at scale. The total recirculation time is calculated based on the flow rate so as to provide a greater number of turnover volume compared with the worst-case at-scale condition, and to also maximize exposure time to HTF temperatures. The maximum number of freeze/thaw cycles is included in this calculation. Once filled to half capacity with the test solution, the assembled appa-

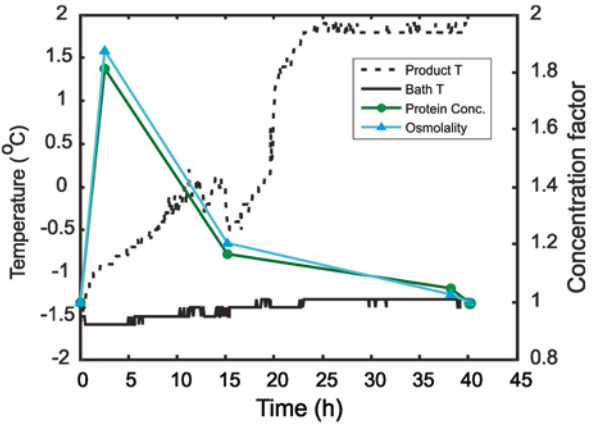

Fig. 9.4 Product temperature (*T*), protein concentration, and osmolality of liquid phase during slow thaw of Protein X

ratus is frozen. Thaw rates are controlled by placing the frozen bottle in a water bath (capable of both heating and cooling). Recirculation can be started after sufficient material has been thawed to permit pumping.

In the following examples, the samples were thawed at two different rates. Protein X was frozen in the bottles as previously described, and then allowed to thaw for 90 min at room temperature (to generate a sufficient amount of thawed liquid for recirculation) prior to immersion in the water bath and initiation of recirculation. The "slow thaw" was performed by setting the water bath to a temperature close to 0 °C, resulting in a thaw completion time of ~ 16–18 h (as confirmed visually by observing the presence of ice). The "fast thaw" was carried out with the water bath set to maximum allowable HTF thaw temperature, resulting in a thaw completion time of < 1 h. In either case, recirculation pumping remained enabled until the predetermined number of turnover volumes had been reached. Using this approach, we can capture the corresponding at-scale conditions prevalent at the beginning of thaw, when cold liquid with higher solute concentration is circulated, and at the end of thaw, when well-mixed liquid is exposed to higher temperatures. Typical temperature profiles for Protein X during the slow and fast thaws are shown in Figs. 9.4 and 9.5, respectively.

For both the slow- and fast-thaw experiments, the Protein X bulk was recirculated for 40 h, which corresponds to turnover volume that is 20 % greater than worst case at-scale condition. Intermediate samples were taken throughout the thaw process and analyzed for protein concentration and osmolality (see Figs. 9.4 and 9.5). The elevated protein concentration and osmolality levels at the early time points are due to cryoconcentration. For slow thaw, protein concentrations (and osmolality during the slow thaw rate) were above the initial levels even after all the ice was melted. The return outlet tubing was deliberately placed below the liquid surface, to cause inefficient mixing. Poor mixing prolongs the concentration effect, provid-

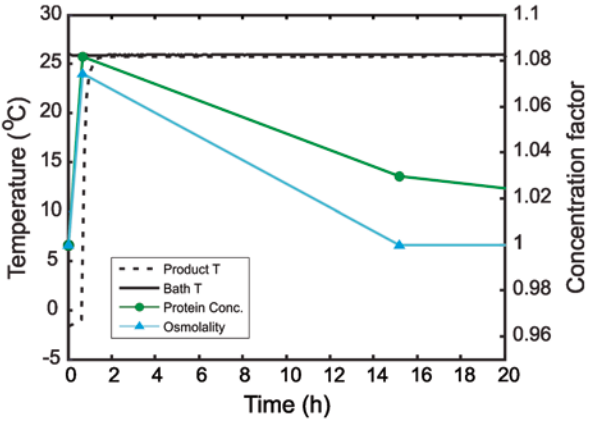

Fig. 9.5 Product temperature (T), protein concentration, and osmolality of liquid phase during fast thaw of Protein X

Table 9.10 Impact of thawing and recirculation stresses on Protein X thawed at slow and fast rates

Thaw rate, sample condition[a]	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)	Ion exchange chromatography (% main peak)
Slow thaw			
Pretest control ($T=0$)	0.08	99.7	67.2
Control (no recirculation)	0.07	99.7	67.2
Stressed (recirculated)	0.07	99.7	67.3
Fast thaw			
Pretest control ($T=0$)	0.07	99.7	67.3
Control (no recirculation)	0.07	99.7	67.0
Stressed (recirculated)	0.08	99.7	67.0

[a] Pretest control and control samples were exposed to the same temperature conditions as the stressed sample

ing worst-case conditions for the product. At the conclusion of the study, samples were taken and analyzed. The QAs for Protein X that are potentially affected by the small-scale thaw study are protein aggregation and deamidation. Results are shown in Table 9.10. A pretest control and a second control that did not undergo recirculation but was exposed to the same temperature bath conditions as the stressed samples were analyzed. Turbidity was measured to indicate particulate formation, which may affect filterability. Based on assay precision, no significant differences were observed in any of the measured attributes between the fast- and slow-thawed stressed samples and the two types of control samples.

For comparison purpose, a similar small-scale thaw study was performed for Protein Y. Results are shown in Table 9.11. In this case, the turbidity of the stressed

Table 9.11 Impact of thawing and recirculation stresses on Protein Y thawed at slow and fast rates

Thaw rate, sample condition[a]	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)
Slow thaw		
Pretest control ($T=0$)	0.00	99.7
Control (no recirculation)	0.01	NT
Stressed (recirculated)	0.06	99.7
Fast thaw		
Pretest control ($T=0$)	0.00	99.7
Control (no recirculation)	0.00	NT
Stressed (recirculated)	0.06	99.7

[a] Pretest control and control samples were exposed to the same temperature conditions as the stressed sample
NT not tested

sample increased compared with the controls, presumably due to a rise in subvisible particles, but no change in protein aggregation was observed by size exclusion chromatography. To determine whether the presence of these particulates would pose a filterability issue, the remaining Protein Y bulk from the fast- and slow-thaw experiments were combined and subjected to a small-scale filtration study (see Fig. 9.6) using Millipore Millex® GV 0.22 µm × 4.5 cm^2 filters. The two pools were combined to ensure that the worst-case at-scale product-volume-to-filter-surface-area ratio was tested. Filter fouling was observed as indicated by the plateauing of the filtration curve. The corresponding filtration profile of the nonstressed control was linear, showing no fouling was occurring. Postfiltration protein content analysis however showed no significant change in protein concentration, and turbidity

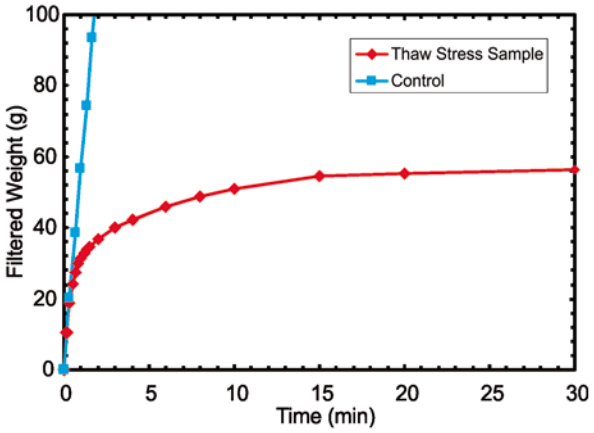

Fig. 9.6 Filtration rate profiles for the Protein Y stressed sample pooled from the fast- and slow-thaw experiments; Protein Y unstressed control

Table 9.12 Filtration results for pooled fast and slow thawed Protein Y bulk

Sample condition	Protein concentration (mg/mL)	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)
Pretest control ($T=0$)	6.6	−0.04	99.7
Prefiltration sample	6.6	0.06	99.7
Postfiltration sample	6.5	−0.02	99.7

was reduced to initial levels (see Table 9.12). Therefore, although stresses from the thaw unit operation may impact filterability of Protein Y, they do not affect postfiltered product QAs.

Air–Liquid Interface Stress The air–liquid interface stress generated during recirculation mixing is difficult to duplicate at reduced scale since the source and nature of the stress depends on the distance the liquid travels prior to impacting the liquid surface. However, it is possible to maintain the linear dimensions and geometry of the at-scale recirculation dip tube assembly while requiring substantially lower amounts of drug substance for testing as compared with an actual freeze/thaw vessel. The apparatus shown in Fig. 9.7 has a small footprint and permits tests to be per-

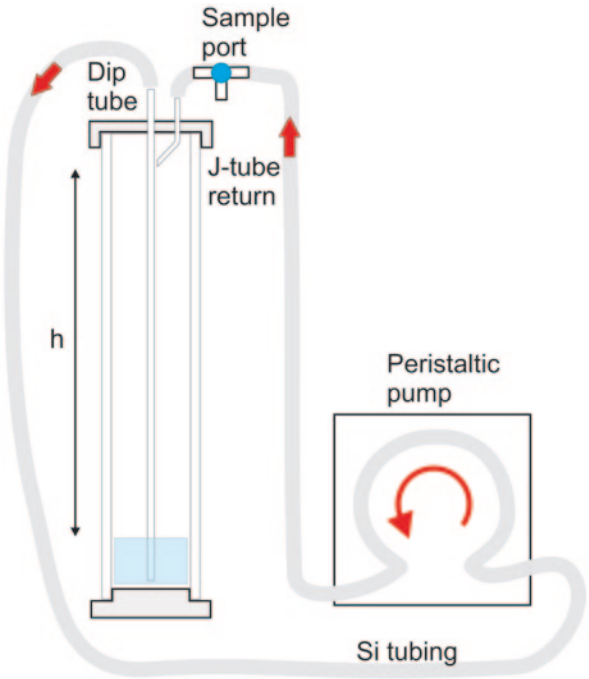

Fig. 9.7 Schematic of small-scale air–liquid recirculation apparatus. The cylinder is constructed from clear polycarbonate, the end caps are Delrin. The "drop" distance, *h*, is greater than the worst-case actual process as determined by the minimum fill volume

Table 9.13 Impact of air interface/recirculation stresses on Protein X bulk

Sample condition[a]	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)	Ion exchange chromatography (% main peak)
Pretest control ($T=0$)	0.07	99.7	67.3
Control (no recirculation)	0.07	99.7	67.3
Stressed (recirculated)	0.07	99.7	67.3

[a] Pretest control and control samples were exposed to the same temperature conditions as the stressed sample

formed with volumes as low as 0.7 L while still using the at-scale pump, tubing, and flow rate. The distance, *h*, at which the liquid is "dropped," is at least 10% greater than that encountered for the actual process under worst-case conditions (vessel at the minimum allowed fill volume).

Protein X bulk was recirculated for 2 h at room temperature at a 20% greater flow rate than target. These conditions result in turnover volumes that are 20% greater than the worst-case at-scale conditions. At the conclusion of the study, aliquots were removed for analysis. The QAs that are potentially affected by this small-scale recirculation study are protein aggregation and deamidation. The process parameter that is potentially affected is filterability so turbidity was measured as it is an indication of particulate formation that can impact filterability. Results are shown in Table 9.13. A pretest control and a second control that did not undergo recirculation but was exposed to the same temperature conditions as the stress samples were analyzed. Based on assay precision, no significant differences in any of the measured attributes between the stressed samples and the two types of control samples were observed.

A similar small-scale recirculation study was performed for Protein Y. Similar behavior was observed as with the fast- and slow-thaw stress studies described earlier. The turbidity of the stressed sample increased compared with the controls, but no change in protein aggregation was observed (see Table 9.14). The remaining Protein Y bulk was subjected to a small-scale filtration study (see Fig. 9.8) using Millipore Millex GV 0.22 μm × 4.5 cm^2 filters. Filter fouling was observed as indi-

Table 9.14 Impact of air interface/recirculation stresses on Protein Y bulk

Sample condition[a]	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)
Pretest control ($T=0$)	0.04	99.7
Control (no recirculation)	0.01	NT
Stressed (recirculated)	0.02	99.7

[a] Pretest control and control samples were exposed to the same temperature conditions as the stressed sample
NT not tested

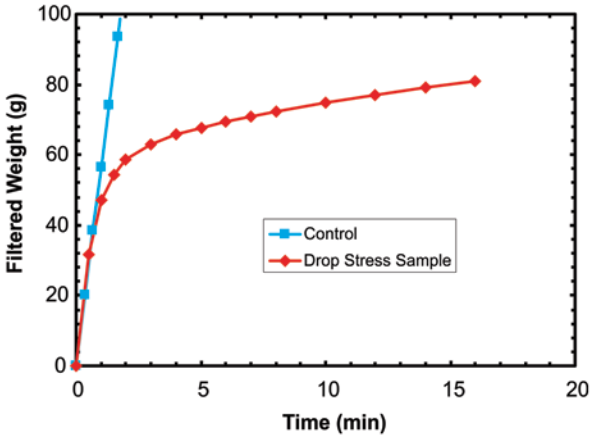

Fig. 9.8 Filtration rate profiles for Protein Y stressed sample from the recirculation drop thaw experiment; Protein Y unstressed control

Table 9.15 Filtration results for Protein Y bulk subjected to air/interface recirculation stresses study

Sample condition	Protein concentration (mg/mL)	Turbidity 340–360 nm (AU)	Size exclusion chromatography (% monomer)
Pretest control ($T=0$)	6.6	0.00	99.7
Prefiltration sample	6.6	0.02	99.7
Postfiltration sample	6.6	0.00	99.7

cated by the plateauing of the filtration curve. Fouling was less severe than the fast/slow thaw stress experiment (see Fig. 9.6). Postfiltration analysis however showed no significant change in protein concentration, and turbidity was reduced to initial levels (see Table 9.15). Therefore, although stresses from the thaw unit operation may impact filterability of Protein Y, they do not affect postfiltered product QAs.

9.9 Multivariate Acceptable Ranges and Critical Process Parameters (CPPs)

Several at-scale and small-scale multivariate worst-case studies were performed for Protein X to assess the impact of the freeze and thaw operations on the protein QAs. Minimal changes to pCQAs were observed for all studies. Therefore, robust MARs can be established for the freeze/thaw process for Protein X based on these study results. Tables 9.16 and 9.17 summarize the process parameter conditions used for

Table 9.16 Process parameter conditions and supported MAR ranges used from the freeze studies with Protein X

Parameter	Completion of freeze and thaw	Freeze rate characterization (fast freeze)	Freeze rate characterization (slow freeze)	Number of F/T cycles and temperature excursion	MAR
HTF temperature (°C)	Lower mfg limit	NA	NA	NA	≤ Lower mfg limit
Bulk volume (L)	Upper mfg limit	Supports mfg range[a]	Supports mfg range[a]	NA	Mfg range[a]
Freeze cycle duration (h)	Target	Freeze within 10 min	2 × Target	NA	1–2 × Target
Number of F/T cycles	NA	NA	NA	Upper mfg limit	≤ Upper mfg limit

F/T freeze/thaw, *HTF* heat transfer fluid, *MAR* multivariate acceptable range, *mfg* manufacturing, *NA* not applicable

[a] Study supports lower limits for bulk volume and greater volumes based on the time product is exposed to storage at ≥ −20 °C

the freeze studies and thaw studies, respectively. The final MAR ranges are also presented.

The at-scale thaw completion studies and the small-scale studies showed no impact on Protein X CQAs under these worst-case bracketed thaw conditions. Based on the impact ratio calculation, there are no CPPs identified. Study results also confirm that the conditions used for the small-scale models are sufficiently stressful so as to be able to identify products that can potentially be an issue during at-scale manufacturing. For example, in the case of Protein Y, the manufacturing process should be designed with consideration to filterability by possibly using oversized filters or enabling swapping of filter elements when fouled.

9.10 Conclusions

The quality risk management framework provides a systematic approach to freeze/thaw process development. A risk assessment was performed to determine pCQAs of the protein. A separate risk assessment identified pCPPs of the unit operation and the type of studies needed to characterize a given process. Worst-case small-scale and at-scale studies were performed to establish the MARs. Small-scale models were developed to mimic the stresses associated with freeze and thaw operations. Small-scale models offer convenience and substantial cost savings, as well as allow for the exploration of wider variable space than would be possible at scale. However, the models must be based on solid understanding of the fundamental physics

Table 9.17 Process parameter conditions used and supported MAR ranges from the thaw studies with Protein X

Parameter	Completion of freeze and thaw	Thaw and recirculation stress characterization (slow thaw)	Thaw and recirculation stress characterization (fast thaw)	Thaw and recirculation stress characterization (drop recirculation)	Number of F/T cycles and temperature excursion	MAR
Bulk recirculation time	Target	> 1.5 × target[a]	≤ 12[a]	≤ 12[a]	NA	1.0–1.5 × set point
Bulk recirculation flow rate	0.9 × set point	> 1.2 × target	> 1.2 × target	1.2 × target	NA	0.9–1.2 × set point
Bulk recirculation start time	Target	NA	NA	NA	NA	≥ Set point
Bulk volume	Upper mfg limit	< Lower mfg limit[b]	< Lower mfg limit[b]	< Lower mfg limit[b]	NA	Lower–upper mfg limit
HTF temperature	Lower mfg limit	<< Lower mfg limit	Upper mfg limit	Room temperature	NA	Lower–upper mfg limit
Number of F/T cycles	NA	> Upper mfg limit	> Upper mfg limit	> Upper mfg limit	Upper mfg limit	≤ Upper mfg limit

F/T freeze/thaw, *HTF* heat transfer fluid, *LPM* liters per minute, *MAR* multivariate acceptable range, *mfg* manufacturing, *NA* not applicable

[a] Support of upper limits on bulk recirculation time and number of F/T cycles are based on bulk volume turnovers and are proportional to the bulk volume being recirculated. Limits described here are calculated for the worst-case (minimum) bulk volume in each tank size

[b] Lower limits for bulk volume and number of F/T cycles are based on the number of turnovers during recirculation

involved in the at-scale process, and their limitations must be taken into account for the data to be meaningful. Finally, a tool is presented that identifies CPPs based on the impact to pCQAs. Following this risk-based approach, manufacturing ranges are established to ensure that the freeze/thaw process is robust and will consistently yield drug product with acceptable QAs.

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Chapter 10
Quality by Design as Applied to Drug Substance Formulation Using Ultrafiltration and Diafiltration

Joseph Edward Shultz, Herb Lutz and Suma Rao

10.1 Introduction

The concentration and formulation of a protein therapeutic drug into the desired formulation excipients is one of the most critical operations in the production of final drug product (DP) dosage forms. The main objectives of the protein drug substance (DS) formulation step is to concentrate the protein product to the desired final concentration, exchange the product into the desired formulation excipients, and exchange out residual excipients from the previous purification operations.

In general, the development of the ultrafiltration (UF) and diafiltration (DF) steps using tangential-flow filtration (TFF) has followed the same evolution as other process operations common to biopharmaceutical product production. Early biopharmaceutical processes were developed simply to enable the production of a product, using single factor experimental investigations. There was limited understanding of which and how process parameters affected the product quality. The focus was on enabling the rapid manufacture of DP to enable market registration. In addition, there certainly was limited understanding of process variation and quality variation. As the industry matured further, the concept of a well characterized product and process began to lead to design of experiment (DOE) strategies that utilized mainly single factor matrices of process parameters, but still had limited or no capability to understand interactions between process parameters. Some in the industry began to design for 2-factor interactions, when interactions were expected.

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In more recent practice, even before the official introduction of quality by Design (QbD) concepts, it has become more common to have processes developed with DOE strategies that are designed to understand multiple interactive factors. In many cases the interactions are complex enough that the only way to communicate the findings is through the use of contour plots. This leads to a means to analyze the data in a way that a robust operational space can be carved out of the data, which allows for predicable product quality within normal process variability. In fact, QbD is defined as designing quality in to the process, rather than testing the resulting quality that results from a process (ICH Q8 2009).

Although many in the industry are still evaluating or have abandoned their plans to file QbD biological license applications (BLAs), the concepts are still valuable and align well with the current trends of an "inspired process development strategy." This strategy moves beyond heuristically collected data and the use of contour data displays to the use of carefully designed experimental studies that enable the use of mechanistic models not just for understanding the experimental results, but to actually define a robust operational space (design space in QbD terms). In fact, an "inspired development strategy" utilizes these models early and throughout the process development activities to shape the design and direction of the process. This enhanced understanding, coupled with historical experience, can be combined to shape the operational space and steer the final process space away from conditions that will have an unacceptable effect on the final DP quality.

This chapter will outline the important critical quality attributes (CQAs) relevant to the UF and DF operations, common process parameters that may affect these CQAs, provide a discussion of how these parameters may affect a quality risk assessment, and provide examples of molecular characteristics, equipment, and facility factors that should be considered when designing these operations.

10.2 Important Attributes and Parameters Affecting the Outcome of the Formulation Operation

In order to achieve a proactive "inspired design," we must first assess what molecule and process attributes are important. We must also understand how one is affected by the other. The following section describes the objectives of a TFF operation in terms of a standard operation development sequence.

10.2.1 Critical Outcomes of the Operation

The main purpose of the formulation TFF operation is to bring the protein product to the final concentration and exchange the product into the desired formulation excipients. As such, the potential CQAs include:

1. Recovered retentate product concentration
2. Recovered retentate protein purity, often measured as aggregate or charged isoform content

3. Retentate buffer composition
4. Osmolality
5. pH

The key process related attributes include:

1. Product yield
2. Process time

10.2.2 Standard TFF Process Development

In a simplified process development scenario, we may consider the scale-up or transfer of a molecule to a TFF system that imposes no equipment constraints on the TFF process (facility and equipment limitations will be discussed in a later section). Variability arises from the protein feedstock stream, DF buffer, filters, and operating parameters for setup, process, and turnaround steps.

A minimum process flux is needed to limit the process time and the number of pump passes associated with aggregate formation. Cleaning ensures batch-to-batch flux consistency with a minimum filter permeability specification. Flux control is obtained using crossflow and TMP specifications.

The DF step is relied upon to establish the buffer composition of the final product. The initial feed solution must have maximum specifications on any buffer components that require reduction by DF. This can include leachable permeable components in the feed as a result of upstream operations. Any maximum and minimum component concentration levels in the final product should have corresponding maximum and minimum component concentration levels in the DF buffer. A minimum number of diavolumes is also required.

The recovery step is designed to limit holdup losses without incurring degradation or excessive retentate product dilution. Typically, a diafiltrate buffer flush is used. Specifications on a maximum buffer flow and maximum and minimum flush volume are required.

The cleaning and sanitization steps are relied upon to limit product carryover and bioburden in the system. Pre-cleaning limits filter leachables. Finally, the associated flushing step ensures that no cleaning or sanitization agents are carried over in the module to chemically degrade the protein product. These considerations require a minimum cleaner and sanitant concentration for a minimum time and minimum temperature and minimum postflush to be effective.

Ensuring good product yields requires integrity testing. A maximum integrity test airflow specification limits defects from poor installation or damaged filter modules.

Development of a UF step can be summarized as a series of selections based on vendor recommendations (EMD Millipore 1999), bioprocessor experience, and analysis of experimental results. Cassettes are the typical module of choice using membranes of either regenerated cellulose or modified polyethersulfone and a vendor recommended screen and crossflow. Typically, the membrane nominal molecular weight cutoff is selected to be approximately 20–33 % of the product protein molecular weight. The following sections describe a typical “base-case”

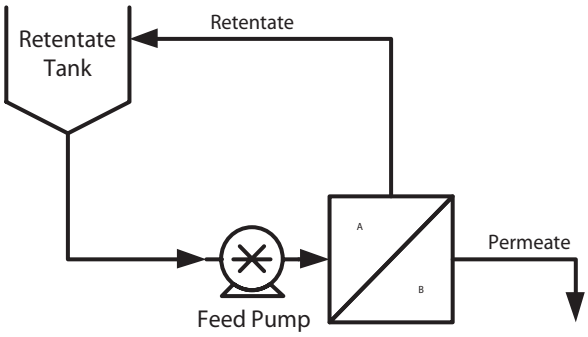

Fig. 10.1 Typical batch TFF system configuration. The typical TFF system configuration includes a holder that can accommodate a range of membrane modules, a pump to circulate the protein solution across the membranes, a restriction device to modulate the back-pressure on the membranes, and a holding (retentate) vessel

development scenario. Design exceptions, based on molecule or operational limitations, will be described in later discussion (Fig. 10.1).

10.3 Assessment of Crossflow Rate and TMP on Permeate Flux

A scale down test system is used to generate flux vs. transmembrane pressure (TMP) data and select a value at the "knee" of the curve, using low area membrane cartridges (see figure Fig. 10.2; EMD Millipore 1999). For example, a 160 kD antibody process may use a 30 kD regenerated cellulose cassette with a C screen run at 3 Lpm/m^2 feed flow and 20 psid TMP. TMP setpoints to the right of the knee yield diminishing flux gains for additional pressure force. Setpoints to the left of the curve are typically considered less optimal for permeate throughput, as the permeate flux rate decreases proportionally with decreased TMP.

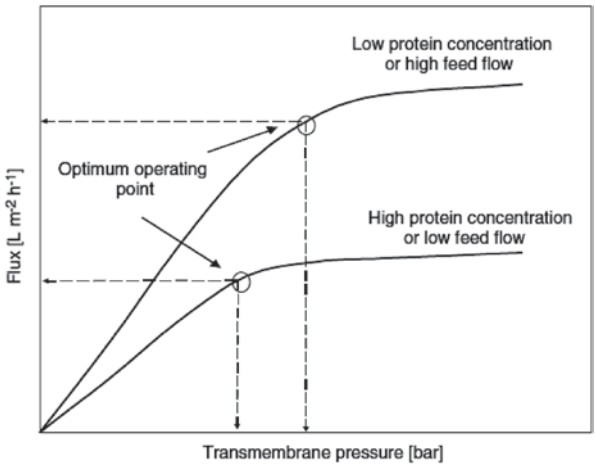

Fig. 10.2 Operational parameter effect on permeate flux rate. TFF permeate flux rates are affected by pressure, cross-flow rate, and protein concentration. The response is typically linearly proportional until the driving pressure or TMP becomes so great as to begin to foul the membrane pores, resulting in decreased flux performance. (Reproduced with permission from EMD Millipore)

10.4 Assessment of Protein Concentration on Process Performance

A scaled down concentration experiment is then used to generate flux vs. ln (protein concentration) data at the same feed flow and TMP. The x-intercept is ln (C_g), or "gel point concentration," and the slope is k or mass transfer coefficient. Protein processing operation is then given by:

1. An initial concentration step that initially increases the feed concentration to the optimal DF concentration of C_g/e
2. A constant volume DF at C_g/e, with typically 10 DF volumes to conservatively reduce the final process solute concentrations
3. A final concentration that increases the protein concentration from the DF concentration to greater than the desired final concentration (over-concentration). Typically, a "plug-flow" system recovery flush, into a recovery vessel, is used to provide high process yield

Membrane area requirements are determined by the measured average flux over this process (J_{ave}) as the amount of permeate volume generated per unit area and time. For the antibody example, a final formulation step might have a process pool feed concentration of 20 g/L, an optimum DF concentration at approximately 70 g/L, and a recovery concentration of 150 g/L that is diluted to a final concentration of 100 g/L during recovery. This processes 11 L of feed, generates 3.8 L of permeate per liter of feed at an average flux of 40 LMH. System loading is 220 g/m^2-h or 150–180 g/m^2-h with allowance for safety factor and process expansion.

10.5 Membrane Cleaning Assessment

Membrane permeability recovery is commonly used to verify the performance of a cleaning step, based on vendor recommendations (EMD Millipore 1991) or previous experience. The retentate product is tested to ensure it satisfies the critical outcomes. This is typically 65–80 % of the initial postcleaning clean water permeability measure (normalized water permeability (NWP)).

This approach to process development is efficient in quickly defining a process. However, it does not define process limits or account for special circumstances where performance does not match required outcomes and more development is needed.

10.6 Risk Assessment

General philosophies around the determination of CQAs and the options for assessing risk have been described in previous chapters (see Chap. ??). This chapter will discuss the potential attributes that may be important to consider for a TFF formulation operation.

Ideally a quality attribute assessment to determine potential CQAs takes place prior to selection of process operations and should be revisited at strategic points in the product lifecycle, as product knowledge and process understanding increase. Raw material attributes and process parameters are linked to the CQAs identified, and risk assessments are performed to evaluate their ability to affect selected CQAs. The risk assessment also identifies parameters that may impact process consistency and robustness. These parameters may not impact the CQAs but are important to ensure successful and reliable processing (step yield is a good example.)

CQAs potentially affected by UF/DF operations include:

- Protein concentration
- Protein aggregate levels
- pH
- Osmolality
- Excipient concentrations
- Residual process reagent levels

Initial risk assessments use prior knowledge and early development experience to determine what parameters should be evaluated during process development. Various risk assessment tools are available such as risk ranking and filtering, which utilize simple process impact and uncertainty scoring methodology to rank parameters.

For tangential filtration operations, parameters that might be included in the assessment include:

- Membrane loading
- Cross flow rate (feed and/or retentate)
- TMP
- Operating temperature
- Protein concentration during DF
- Final protein concentration
- Protein concentration during recovery
- DF and recovery/dilution buffer characteristics
- Volume of buffer exchanged during DF

Following process development/characterization, a more detailed risk assessment may be performed using the cumulative process understanding to assess the design space and assist with specifying the control strategy. A more thorough risk assessment, using a tool such as failure mode and effects analysis (FMEA), may be performed at this point to determine the control strategy going forward. This assessment considers the severity of the impact to product quality and process performance, the likelihood of occurrence that a parameter will exceed acceptable limits, and the ability to detect and/or correct a failure if it does. Process parameters are categorized accordingly based on the results of this risk assessment. The parameters evaluated are similar to the initial risk assessment, as are the CQAs and process attributes impacted.

The component concentrations in the recovered retentate product define the protein product quality and the critical outcomes described above. It is convenient to delineate these components as listed in Table 10.1. A distinction is made between buffer components in the feed that are to be diafiltered out, and buffer components in the diafiltrate that are intended to be in the final product. Impurity concentrations (i.e., aggregates, leachables, carryover, or cleaning agents) are typically characterized relative to the protein product concentration such as 1 % aggregates, meaning 1 g of aggregates per 100 g of protein product.

Factors that can potentially affect the component concentrations are conveniently identified using a mass balance (Bird 2002) where retentate concentration = retentate mass/retentate volume. This involves drawing a control volume around the UF processing skid and requiring the mass of each component to be conserved. Some mass is present in the control volume initially, mass is added through the product stream and buffer feeds, mass is removed through the recovered product and permeate, mass may remain behind at the end of the process, and some components may be consumed or formed by chemical reaction during the course of operation. As a result,

$$\text{Retentate mass} = \text{initial} - \text{final} + \text{product feed} + \text{buffer feed} - \text{permeate} - \text{reacted mass}$$

It may be noted that the final mass in the control volume may be adsorbed to surfaces or held up as unrecovered liquid. This enables one to identify the potential factors listed in Table 10.1. CQAs are a subset of this list that can be prioritized using risk analysis.

There are also process attributes that are important to an economically viable manufacturing process. Table 10.2 lists some of the attributes that may be of interest along with the factors that can affect them. Key process attributes (KPAs) are a subset of this list that have been prioritized using risk analysis.

Table 10.1 Factors affecting product quality attributes (final concentrations or critical outcomes)

Components	Factors affecting final concentrations
Protein product	Product losses (permeate, adsorption, holdup, and reacted), feed mass, final volume
Feed buffer	Feed mass, final volume, permeate loss
Diafiltrate buffer	Diafiltrate mass, final volume, permeate loss
Product aggregates	Feed mass, final volume, generation (pumping, foaming)
Leachables	Initial mass in filters and system, final volume, permeate loss
Batch carryover	Initial mass in system after cleaning, final volume
Cleaners/sanitizers	Initial mass in system after cleaning, final volume, permeate loss, reacted
Bioburden	Initial mass in system, mass added from environment, growth, holdup

Table 10.2 Factors affecting key process attributes (KPAs)

Key process attribute	Factors affecting attributes
Yield	Product losses (permeate, adsorption, holdup, and reacted)
Process time	Flux, filter area, feed volume, volume reduction, diafiltration
Operating cost	Filters with reuse, buffers, utilities, labor, QC
Capital cost	Skid, automation, validation
Robustness	Manufacturing process deviation costs
Footprint	Filter area, pumping requirements
Expandability	Pump turndown, pipe diameters, filter holder

10.7 Critical Process Parameters

While many factors can affect the CQAs, well designed processes minimize the contribution of many of these factors. The operational or process parameters that affect the CQAs are considered critical process parameters (CPPs). The recovered product protein concentration is determined by the process parameters of feed mass, final volume, and yield. Adsorption losses are mitigated by filter and system component selection. Inactivation losses are minimized by component selection and operation to minimize foaming and pump pass degradation. Product yield varies due to permeate losses associated with an integrity test airflow process parameter, and recovery losses associated with a recovery flush process parameter.

The recovered retentate aggregate concentration is determined by chemical degradation of the protein product. This can occur through damage incurred via cavitation in pumps or valves, or tank foaming where air bubbles may form and provide a hydrophobic surface for protein unfolding. While pumping aggregation is minimized by component selection and design, the operating process parameters that can affect outcomes include:

1. Longer processing time with increased pump passes
2. Higher flows causing more pump backpressure
3. Higher temperatures
4. Higher protein concentration can increase aggregation

Tank foaming aggregation is also minimized by component selection and design, but the following operating process parameters can affect outcomes:

1. Higher agitator speed
2. Smaller retentate volume
3. Higher retentate flows, and
4. Improper addition of feed to the retentate tank that leads to introduction to entrained gas in the liquid

Degradation induced by changing buffer conditions is more unusual and not considered further. The retentate solution can contain residual feed components, diafiltered -in components, leachables, and process carryover. Buffer component reten-

tion is determined by the filter and binding or association with the protein product and should be consistent from batch-to-batch. Residual feed and leachable permeable components depend on the process parameters of:

1. Initial concentration, and
2. Diavolumes

Diafiltered -in permeable components depend on the process parameters of:

1. Diafiltrate concentration, and
2. Diavolumes

Carryover will copurify with the protein product and depend on its initial concentration.

For a fixed membrane area, the process parameter, "process time," varies with the average flux through each of the concentration and DF steps, the amount of volume reduction and DF, and the feed volume. Process time varies with the operating process parameters of:

1. Volume reduction
2. DF volumes
3. Feed volume
4. Flux (through the parameters of TMP and feed flow)

Having identified the CQAs, and the associated process parameters that can affect them, a risk assessment can then be made to identify the CPPs. For each potential parameter and attribute interaction, the risk assessment involves assigning a relative rating (i.e., high, medium, and low) of (1) the relative severity of the impact on each attribute, (2) the relative likelihood of such an impact, and (3) the relative ability to detect the occurrence of a negative event. Examples or suggested baseline ratings that may be used to determine a net risk for the interaction is shown in Table 10.3.

The highest PQA risks arise from (1) product retentate concentration due to retentate mass, retentate volume, and recovery variations; and (2) aggregate retentate concentration due to formation by chemical reaction from low feed mass causing tank frothing, chemical carryover, and pumping damage from low flux, and high DF volumes. The highest process parameter risks arise from yield losses due to system integrity variation.

10.8 Molecule Characteristics and Their Impact on the TFF Operation

With regard to the development of a TFF operation, there are a number of molecular characteristics that are important to consider. These characteristics interact with the operational parameters and affect the optimal operation of the step. The following subsections describe the most important attributes and their effects.

Table 10.3 Example process parameters and likely risk assessment rankings

Variability source	Process parameter	Quality attribute	Process attribute	Severity	Likelihood	Detectability	Net risk
Feed	Feed protein mass	Protein concentration		High	Medium	High	High
		Aggregate concentration		Medium	Medium	High	High
			Time	Low	Low	High	Low
	Feed protein concentration	Aggregate concentration		Low	Low	High	Low
	Feed buffer concentration	Buffer concentration		Medium	Low	Low	Low
Buffer	Diafiltrate composition	Buffer concentration		Medium	Low	Low	Low
Filters	Average flux		Time	Low	Low	High	Low
Setup	Integrity		Yield	High	Medium	High	Medium
	Flush	Extractables concentration		High	Low	Low	Medium
Process	Average flux	Aggregate concentration		Medium	Low	Low	Medium
	Feed flow, TMP		Time	Low	Low	High	Low
	Buffer concentration		Time	Low	Low	High	Low
	Diavolumes	Buffer concentration		Low	Low	Med	Low
	Retentate volume	Protein concentration		High	Medium	High	High
	Diavolumes	Aggregate concentration		Medium	Low	Med	Medium
	Recovery	Protein concentration		High	Low	Med	Medium
	Recovery		Yield	Medium	Medium	High	Medium
Time	Cleaning	carry-over concentration		High	Low	High	Low

10.8.1 Protein Concentration

Typically, a protein therapeutic needs to be delivered as a DP at rather high concentrations. Concentrations of 25 mg/ml to as high as 183 mg/mL (Luo et al. 2006) have been reported in the literature. Higher protein concentration formulations allow dose volumes in the 1–1.5 mL range, as required for a subcutaneous injection. The product concentration is a CQA since the dose received by the patient is dependent on the product concentration in the device. Proteins typically vary in size and molecular weight, for example a protein concentration of 100 g/L for a 50 kD protein (e.g., fab, peptibody) translates to 2 mM and 150 kD (antibody) protein it corresponds to 0.66 mM. The protein size is a complex function of its amino acid composition, 3D structure, and the buffer (pH, ionic composition), the molecule is in. The buffer composition along with the protein charge and protein concentration determines the hydrodynamic radius of the protein, which plays a key role in the nonspecific electrostatic interactions (Burns and Zydney 2001).

In commercial TFF operations, most of the steps in the unit operation need to be performed at high concentrations, to enable fast processing times and manageable batch volumes. The DF concentration has an impact on processing time and buffer volumes required thereby translating to allowable batch size. As described earlier, the optimum DF concentration is C_g/e.

Typically, the product needs to be recovered from the TFF skid at a concentration that is higher than the targeted final product concentration, to allow for subsequent dilution from the sterile filtration step. To recover the product at the appropriate concentration, the recovery operation needs to be well designed. For a product concentration of approximately 50–70 g/L, recovery is a straightforward process. However, with the advent of very high protein formulations (concentrations > 120 g/L), recovery has become a significantly more complex challenge. Based on the ratio of batch volume to hold up volume, protein overconcentration factors up to 50% are expected. So for example at a 120 g/L protein concentration, the product would need to be concentrated to 180 g/L to ensure high yield recovery.

At higher protein concentrations, viscosity increases significantly (Shire et al. 2004). These high-viscosity solutions provide significant resistance to flow and result in very high feed pressures that are outside the validated pressure limits of existing skids. High viscosities limit the allowable overconcentration, thereby limiting the maximum achievable protein concentration, or lowering overall recovery by decreasing the ability to dilute to recover material that is being held up in the hold-up loops, both these outcomes are highly undesirable. Figure 10.3 describes the relationship of operational pressure as a response to protein concentration induced viscosity increases.

Certain excipients such as calcium salts and amino acids, have been seen to lower viscosity (Manning et al. 2010; He et al. 2010). The use of high temperature has also been used to reduce viscosity (Winter 2008). Viscosity typically increases exponentially with concentration (Monkos 1996) and a generalized Arrhenius equation is very often used to describe the relationship. Monkos et al. reported an approximately 50% reduction in viscosity of Bovine IgG as the temperature was

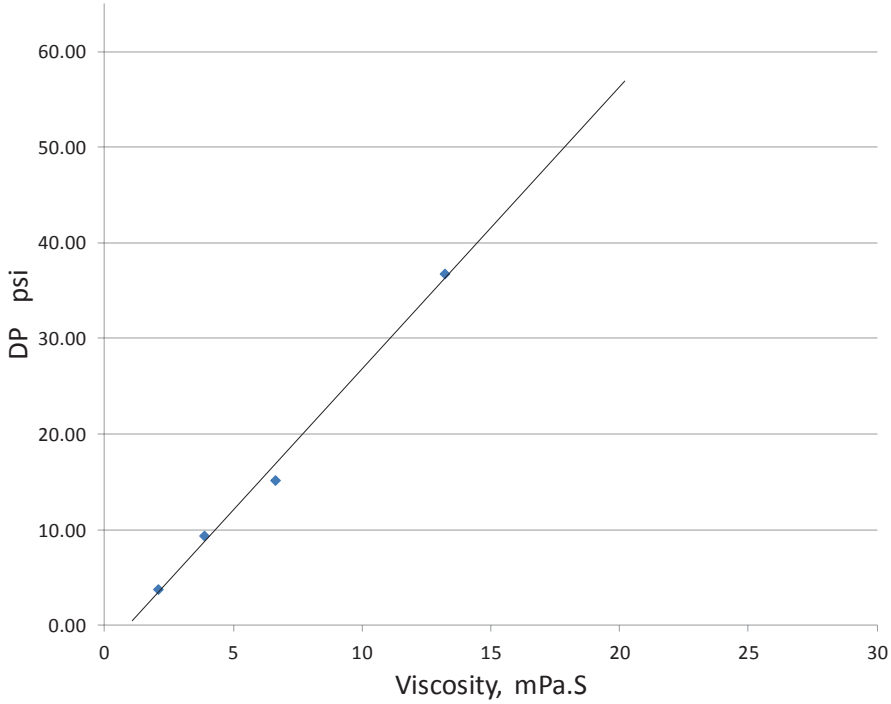

Fig. 10.3 Viscosity vs. normalized delta pressure. Protein concentration and molecular character can contribute to solution viscosity. This data describes the relationship of viscosity and resultant pressure across a TFF membrane cassette for an example monoclonal antibody when circulated at 120 L/h/m^2 of membrane (LMH) at 37 °C

increased from 5 to 50 °C (Monkos and Turczynski 1999). High-viscosity solutions in addition to causing high system pressures also result in considerably lowering the operational flux and thereby causing an overall increase in processing time. Therefore understanding the relationship of protein viscosity as function of protein concentration, excipient concentration and temperature is critical in determining overall design space. In addition, the process developer also needs to understand the relationship between batch size, hold-up volume, and the wash-out kinetics of the hold-up loop, where the high-viscosity product needs to be displaced by a lower viscosity buffer.

10.8.2 Protein Charge

At high protein concentrations, a significant portion of the volume in the solution is occupied by protein molecules. Stoner et al. report for a 100 g/L protein concentration a typical antibody occupies 7.5 % of the solution space (Stoner et al. 2004). In the TFF operation, the protein is selectively retained on the retentate side of

the membrane, resulting in a considerable solution volume that is now excluded due to the presence of a large number of protein molecules. This results in an unequal portioning of solutes in conditions where the operation is carried out in pH away from the isoelectric point (pI) of the molecule. These situations result in a solute imbalance between the retentate and permeate side commonly called as the Donnan effect. This phenomenon gets exacerbated in low ionic strength solutions. Protein–protein interactions resulting from electrostatic interactions, hydrophobic interactions (surface exposed patches), and interactions with formulation buffer components (i.e., salts, sugars amino acids exist and can effect on solute partitioning in a membrane system.) The overall protein surface charge is dependent on the amino acid sequence, the structure of the protein, its size, the buffer system the protein is in and specific and nonspecific interactions that may be present. Thus, the protein charge, the ionic species concentration, and the operation pH are related, careful consideration needs to be paid while designing the DF buffer to obtain the appropriated formulated to condition. During formulation development understanding the role of key excipients plays is important role in determining overall design space. Stoner et al. presented a few convincing cases studies to illustrate the ability to effectively design a DF buffer taking into account volumetric exclusion, Donnan effect and nonspecific interactions (Stoner et al. 2004).

10.8.3 Protein Solubility

In TFF operations, the protein is typically concentrated to the DF concentration from the preceding unit operation (e.g., a chromatographic column in which the protein is in a high ionic strength solution compared to the formulation buffer.) The protein is concentrated to the optimum DF concentration, as explained in the "The Impact of Equipment Constraints on Performance" section of the document. As the protein is concentrated, it begins to compete for solution space with the other ions in solution, the higher the ionic strength greater the competition. Protein solubility is also lowest at its isoelectric pH (Rupley 1968). Usually, on increasing protein concentration, ill-defined gels, which appear as protein precipitates, form (Middaugh and Volkin 1992). These tend to be nonequilibrium suspensions. During process development, one needs to map out the solubility space of the protein with respect to the initial buffer composition (last column buffer) and the final formulation buffer to ensure that the protein is always in aqueous state and not in a nonequilibrium suspension state.

10.8.4 Assays to Monitor Product Attributes

In order to monitor the important quality measures or CQAs in QbD terms, appropriate analytical methods are necessary. The following analytical methods may be useful in the characterization of these quality attributes during development and are grouped by product attribute:

10.9 Protein Concentration Determination

Spectrophotometry is the most commonly used method to determine protein concentration. A well defined assay needs to be in place and protein concentration needs to be monitored throughout the process by taking well mixed, point samples at critical stages of the UF, DF and recovery steps. High-performance liquid chromatography (HPLC) methods may also be useful, but are not typically used for highly purified samples, unless there are excipients or formulation additives that inhibit simple spectophotometry at 280 nm.

10.10 Excipient Concentration Determination

Excipients play a key role in determining protein characteristics such as charge, viscosity etc. assays need to be in place to ensure that the final product is in the appropriate formulated to condition. Often two groupings of excipients are monitored, the excipients that are present in the last purification step that are meant to be exchanged out of the final formulation and the excipients that are desired to be in the final DP formulation. Many of these excipient assays can be measured using USP methods, including colorimetric and HPLC methods.

10.11 Solution pH, Conductivity, and Osmolality

Solution pH and conductivity are often monitored as a part of the process to ensure sufficient buffer exchange and to ensure that the DP is in the appropriate buffer. Protein therapeutics are often targeted to be delivered in isotonic solutions and therefore the solution osmolality is often a quality parameter measured for DS release.

10.12 Particle Testing and Product Size Measures

Cavitation and air-liquid interfaces in pumps and across valves can generate protein particulates. Various methods of particle testing are employed to identify and describe particles in the DS. These may include HIAC methods for visible and subvisible particles.

Size exclusion chromatography and dynamic light-scattering techniques are commonly used to determine the relative protein size and dynamic radius, respectively, for subvisible particles. These methods are also useful to evaluate the presence of higher and lower order species.

10.13 The Impact of Equipment Constraints on Performance

As described earlier, the performance of a UF process depends on component selection, sizing, and integration (Lutz 2007). Component selection involves making sure that (1) all wetted surfaces are chemically compatible with process solutions (including cleaners and sanitants) and do not cause protein denaturing, (2) that there are no dead legs in the system that are difficult to flush out and can hold cleaners or carryover product from the previous step (Brunkow 1996), and (3) the feed pump and retentate tank mixer do not cause protein aggregation over their required range of operation (Virkar 1981; Maa 1996).

Component sizing involves ensuring that the:

1. Pump can deliver the required range of feed flows at the system backpressures
2. Piping elements are sized large enough to limit pressure drops at system flows
3. Components can handle the pressures required for operation
4. Retentate tank volume is large enough to hold the entire feed volume (or at least the volume at DF conditions if a fed-batch concentration is used)
5. Mixer can provide adequate mixing over the range of volumes in the retentate tank, and
6. Filter module holder is capable of holding the required membrane area

Component integration involves ensuring that:

1. The minimum working volume determined by retentate tank foaming is small enough to allow one to reach the desired final product concentration
2. No vacuums are formed in the piping system that cause degassing and protein degradation
3. The retentate line pressure drop between the filter outlet and tank meniscus is small enough so that one can run at the desired TMP and crossflow conditions

Some of these considerations apply to any system. However, one can also consider the scale-up or transfer of a molecule to existing systems, at different manufacturing sites or different firms. A particular system may be suitable to process one molecule but unsuitable for another molecule that has a larger feed volume and requires larger retentate tank, membrane area, and pumping system. In addition, some aspects of the system readily scale and some do not.

10.13.1 Implications of Equipment Constraints on the Design Space

The equipment constraints limit the design space further in order to keep the PQA within specifications. As described above, a given UF system is limited in the amount of material it can process. A minimum feed mass is needed to permit processing to the final concentration without falling below a minimum working volume. The minimum working volume is the point where the retentate tank level

falls below a minimum and protein degradation by frothing and/or poor mixing can occur. A maximum feed mass is needed so that the feed volume can fit into the retentate tank. If the transfer tank from the previous step is different from the UF retentate tank, one can run the initial concentration step as a fed-batch operation. This requires a maximum feed mass limited by the diafiltrate volume. Tank mixing must be adequate with the full tank.

A given UF system has limits on the module area it can hold. A minimum area is determined by the associated pumping package holdup volume. This volume limits the minimum working volume and the ability to reach the final concentration specifications. A maximum area is determined by the process holder and pumping restrictions. Process holder configurations of 6 levels high, incorporating 120 m^2 have been successfully employed. Pumps having maximum flowrate and crossflow rates, defined in terms of flow per unit membrane area, are restricted at higher areas. If a process requiring additional area is put into a smaller system, the process time will increase. This will impact the work flow between downstream processing operations and may cause a bottleneck. It may also create bioburden issues or increase pump pass induced aggregation.

A given UF system has pump limits. Proteins that are particularly sensitive to pumping damage may not be used with some pump designs or operating conditions involving high flowrates and high back pressure. As described above, the maximum pump flow, limits the maximum membrane area. If the pump is operated at a lower crossflow, the flux reduces and process time increases. For some processes, it is advisable to reduce the pump flow during the final concentration step to compensate for viscosity related pressure increases. The minimum pump flow can limit the achievable final concentration.

The integrated system pressure ratings and pressure drops can limit operation. For high-viscosity applications, the pump output/filter feed pressure can significantly increase. While cassettes are typically rated for continuous 80 psig pressures, systems may not allow equal pressure maximums. In addition, the system retentate line may have a high flow resistance causing elevated retentate pressures at the working flowrate. As a result, the system TMP increases to a minimum value. If the target process TMP specifications are lower than this limit, the skid cannot be used without modification.

10.13.2 Process Control

Keeping the process within the design space requires UF process control strategies. These strategies include specifications with monitoring systems and actions associated with action limits. The feed mass can be monitored by measuring titers and volumes and limiting UF processing if the mass falls outside the working range of the system. This could be done at the bioreactor with allowance for downstream losses. While a low feed mass could be qualified for pooling with another batch, this can introduce other complications.

Component concentrations in the feed and buffers need to be kept within specifications. Feed concentrations of protein product, aggregates, bioburden, and buffer

components can be measured directly and controlled through the previous step. The diafiltrate, flush, cleaning, and sanitizing solutions all need to be monitored and in control.

UF filter retention, integrity, permeability, and pressure drop needs to be kept within design specifications. Filter vendors qualify their manufacturing of membranes and modules for consistency. Filter vendors publish specifications for some of these properties and multiple lots can be tested for consistency. Vendor quality audits by biomanufacturers are a monitoring system to ensure that filter properties remain consistent. Maximum integrity test airflow specifications must be met to ensure no large diameter defects exist due to poor sealing or punctured membrane that can occur during shipping, handling, and operation.

Monitoring and controlling the hydraulic conditions within the filter that determine the flux can be accomplished in a variety of ways. It is convenient to consider (1) the TMP driving force and (2) the cross flow, as relatively orthogonal independent variables. Other choices such as P_{inlet} and P_{outlet} are sensitive to other variables and not as directly related to the fundamental forces at work within the filter module. Limit the filter TMP through feed, retentate, and permeate pressure monitoring and retentate valve control. While permeate and retentate pressures are sometimes assumed to be 0 psig, this is not always the case. The filter cross flow can be controlled through monitoring and control of pump feed flow, retentate flow, average flow, or filter ΔP. Of these options, it is recommended to use feed flow as least subject to variations due to filter ΔP or flux and measurement error. Control of process time, agitator speed, pumping flow rate, and pump backpressure can limit protein aggregation.

The concentration, DF, and recovery steps require monitoring and control. Measurement of retentate tank volume can be used as an endpoint for concentration steps. The DF step requires monitoring and control to ensure constant volume and sufficient solute wash out. The diafiltrate flowrate is controlled to keep the measured tank volume within specifications. The cumulative buffer or permeate volume is measured to ensure sufficient wash out. DF to reduce buffer ions can also employ monitoring of retentate or permeate ion concentration or conductivity directly and using this to control the DF step process time. This makes the process less sensitive to initial retentate and buffer specifications. Recovery flush out concentration monitoring and control over flush volumes and flow rates limit yield loss and product dilution during recovery.

Ensuring consistent processing and limiting batch-to-batch carryover is accomplished through monitoring and control of the cleaning process. If a minimum permeability is not restored, cleaning was not effective and filters may need replacement. Control of cleaning and sanitizing solution concentrations and operating conditions (time, temperature, and flow rates) ensure consistent performance. Control of flush flow rates and times limit carryover.

Some of these control strategies allow for process analytical controls (PAT) to change process operation based on in-process measurements as described above.

10.13.3 Continuous Process Validation

Over the course of operation, it is expected that batch consistency will improve and the likelihood of parameter variability will decrease. In addition, opportunities for improvement will occur that can change likelihoods, severities, and/or detectability of quality and process attributes. This will change the risk assessment and implementation of improvements will change process controls. It can be useful to update this analysis as part of annual process reviews.

10.14 Case Study Examples or Extrapolations from Previous Strategies

The base-case development strategy was discussed in prior sections. However, individual protein products exhibit a wide range of molecular behaviors and character that may require adjustments to the described development program. It is for this reason that a deeper understanding of molecule character and its interaction with operational and environmental factors is important prior to the start of process development. A selection of potential characteristics and their impact are described in this section.

10.14.1 Considerations Caused by pH or Salt Sensitivity

As was discussed above, protein product stability can be affected by solution conditions, such as, pH and salt concentration. Therefore, the stability of a given protein product may not allow for a direct transition from the final purification pool to the formulated DS. For example, the final purification pool may be at a pH that is higher than the pI of the protein product and the formulation pH may be below the pI. In cases where the protein is not be stable at or near the pI, it may not be feasible to directly diafilter the purified pool into the final formulation buffer, as this could cause the product to aggregate or precipitate from solution. Similar considerations would need to be made for products that were sensitive to salt concentration. This is where knowledge gained about molecule solubility and character, prior to development, is useful.

In one example of an “inspired development strategy” or QbD-like development program, an initial molecular assessment that evaluated the effect of rapid pH transitions across a range of pHs to assess the impact on product purity and the propensity to aggregate or self-associate was performed. The same program also completed an extensive solubility and purity assessment across a matrix of pH and salt conditions that identified areas of poor stability very early in the development program (data not published). This information was utilized to understand the mechanistic behavior of the protein in its environment to design a development program focused on pH and salt zones that led to a robust operating space. The re-

sulting process employed a rapid initial titration of the pool to a stable pH closer to the formulation buffer pH that avoided extended exposure of the product to pH and salt levels that were known to lead to product instability. This approach was scaled and demonstrated to be robust at multiple manufacturing-scales.

10.14.2 Considerations Caused by Molecules Prone to Aggregation

Aggregation is one of the major CQAs with regard to a TFF process. Therefore, a great deal of effort goes into controlling process parameters that may cause increased levels of aggregation or particulation. However, some molecules are more susceptible to self-association and this can result in not only poorer product quality, but also have operational impact. In one such example, a molecule was highly prone to self-association at gas-liquid interfaces. This manifested itself as a film on the surface of mixed tanks that were open to the air and in the form of increased soluble aggregate levels. This protein was also highly susceptible to pump and valve shear and cavitation. Knowing this characteristic of the protein led to the proactive development of process equipment that minimized air-liquid interfaces. Also, extensive work was done to identify pumping systems that minimized cavitation. Without prior knowledge of the molecular characteristics, process development and scaling would have been confounded by variable aggregation across scales and conditions.

10.15 Conclusions and Summary

Whether a particular organization intends to file a QbD application or not, the concept of developing a TFF formulation process with forethought and understanding results in efficient and robust process design. Having an understanding of the fundamental mechanistic effects of operational parameters on potential CQAs is valuable in developing a solid understanding of the process and supporting a wide robust operational space. By understanding the likely operational, facility, and equipment effectors and the common CQAs; process development can be focused while building quality into the process. No longer does the process define the quality, rather the quality is built into the process and the process of developing the process.

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Chapter 11
A QbD Approach in the Development and Scale-Up of Mixing Processes

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11.1 Introduction

"Pooling and mixing" (following the thawing in cases where multiple batches of "ready to fill bulk drug substance" are involved) or just mixing (in cases where the excipients are dissolved and mixed) is one of the critical steps of the unit operations of manufacturing. In both situations the desired outcome of this unit operation is to ensure that the resulting formulation is homogeneous, meets the specifications, and the product quality attributes are retained.

Mixing processes are complex, multifaceted in nature, and require understanding of fluid dynamics. The relationship between the design of the equipment and solution properties influences the process performance, which in turn impacts the product quality attributes (Harnby et al. 1992; Tatterson 1991, 1994; Wan et al. 2005). From a fluid dynamics or mechanism perspective, large liquid circulation loops developed in stirred vessels make mixing performance poor. To ensure effective mixing, the equipment and the mixing speed should be designed so that the fluid circulated by the impeller sweeps the entire vessel in a reasonable time. To avoid dead spots the velocity of fluid leaving the impeller must be sufficient to carry material into the most remote parts of the tank. The selected mixing speed in revolutions per minute (RPM) should develop turbulence in the fluid to ensure optimal mixing. Mixing can also be improved by changing the configuration of the system, hence, baffled tanks are recommended for larger batch sizes in manufacturing scale operation. Installing baffles destroys the vortices, produces greater turbulence, and promotes a flow pattern conducive for good mixing (Oldshue 1983; Paul et al. 2004). From equipment geometry perspective, for efficient mixing the impeller should be mounted below the geometric center of the vessel. In

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standard designs the impeller is located about one impeller diameter, or one third the tank diameter, above the bottom of the tank. All these factors are central to efficient mixing, which can be described as a combination of three physical processes: distribution,, dispersion, and diffusion (Brian et al. 1969; Levins and Glastonbury 1972a, b; Okamoto et al. 1981).

Different biomolecules display different levels of shear sensitivity, for example, monoclonal antibodies (mAbs) are known to be particularly sensitive to mechanical forces (Bee et al. 2009). Mixing is typically considered as a low shear rate operation in fill-finish operations, however, high shear rates are observed around baffles and at the impeller tip speed (Okamoto et al. 1981). The stirred vessels used in the mixing process must limit the intensity of shear while still providing adequate mixing and mass transfer. Additionally, if the vessel is sparged with air, shear damage can occur at much lower impeller speeds due to shear effects associated with bubbles (Maa and Hsu 1997).

Determining optimum operating conditions at production scale is not desirable as it is expensive and time consuming. Scale-down models are often used to carry out experiments under conditions mimicking large-scale environments to identify optimal operating conditions for productionscale. As long as the flow regime is the same in the small-scale and large-scale tanks, there is a better chance that results achieved in the small-scale unit will be reproducible in the larger system. These studies are often coupled with large-scale surrogate studies during development and confirmed later with product during qualification runs.

A well-designed mixing operation would develop from considering all of the above issues and variables characterizing mixing processes and physical properties of the fluid (e.g., density, viscosity, and surface tension) or their dimensionless combinations (e.g., the Reynolds number (Re), Power number (Ne), etc.), prior to determining optimal operational conditions. Potential scale-up issues should be identified for risk analysis and experiments designed to understand and mitigate issues. One key factor that should be understood is the effect of tank geometry on scale-up. Once a tank and suitable paired mixing equipment is selected, operating conditions should be defined. Ideally, this can be accomplished with a combination of well-planned lab-scale experiments that would target a similar performance at commercial scale as observed at lab scale coupled with mathematical models to predict operating conditions at commercial scale.

In this chapter, we will demonstrate through the mock case study how Quality by Design (QbD) elements could be applied to a complex mixing operation to accomplish the understanding to develop a well-designed mixing operation that is scalable and robust.

11.2 Definition of Target Process Profile

The mixing target process profile describes the process in two steps. In the first step a diluent or buffer is made where all excipients are mixed and in second step the diluent or buffer is mixed with the drug substance to produce the final drug product formulation at the specified active concentration.

Diluent/Buffer Preparation and Compounding The stainless steel diluent preparation tank is charged with water for injection (WFI) followed by the addition of the buffer species sodium acetate (excipient 1) and acetic acid (excipient 2), and mixed until dissolved. Next the sucrose (excipient 3) is added and dissolved. Separately, a 10% Polysorbate 80 (PS 80) stock solution is prepared by adding PS 80 into WFI in a glass bottle maintained at a temperature between 20 and 25 °C and stirred to achieve a 10% solution (w/w). The buffer solution is then supplemented with 10% PS 80 stock solution (excipient 4) to a final concentration of 0.1% (w/v) and mixed. The pH is tested online and adjusted if necessary to a final pH of 5.3. The final weight of the solution is adjusted with WFI and the diluent mixed. The expected batch size for this process is 30–200 L. The buffer or diluent is transferred through a 0.1 μm posidyne filter under filtered nitrogen pressure into a stainless steel vessel for endotoxin removal.

The drug substance prepared from the previous step is weighed into a compounding vessel. The drug substance may be from a single container or lot or pooled from multiple containers and lots. The diluent is added to the drug substance through a filter to complete the required final weight of the bulk drug product formulation. The pH, osmolality and the protein concentration are confirmed online concluding the compounding step.

Ready to Fill Bulk Drug Substance (BDS) In situations where the bulk drug substance does not need dilution and is ready to fill, the following procedure was followed. The content of each BDS container was pooled into a stainless steel vessel using a peristaltic pump. After the pooling of all bottles was finished, a bioburden reduction filtration using 0.22 μm PVDF filter was performed from the pooling vessel into a second sterilized vessel. The pooling vessel is equipped with a mixer, which allows for mixing of the BDS. During transfer of BDS from containers and mixing, measures were taken to minimize foaming. Samples of the BDS were drawn before and after the pooling/mixing and product quality attributes were tested.

11.3 Prior Knowledge

Compounding is a standard unit operation in the production of mAbs. Development information from previous products can be applied directly to X-mAb with only verification of applicability being required. This section summarizes the prior knowledge from development studies as well as from batches made for nonclinical and clinical studies for multiple marketed mAbs. The design space proposed for X-mAb in Table 11.1 is, in fact, identical to the equivalent design spaces approved for A-mAb, B-mAb, and C-mAb.

11.4 Initial Risk Assessment

An initial risk assessment was conducted using the cause and effect matrix to identify compounding and mixing parameters with the greatest impact on the overall quality and shelf life of X-mAb. The parameters that are normally evaluated relate

Table 11.1 Summary of prior knowledge for compounding and mixing design space for mAb's

Parameter	mAb product	Proven acceptable range		Experience and knowledge
		Clinical batch10–30 kg	Commercial batch 50–200 kg	
Buffer/Diluent/Placebo				
Mixing speed	A-mAb B-mAb C-mAb	30–40 RPM	60–120 RPM	Experience with clinical placebo/buffer manufacturing indicates that 10 min of mixing at 30–40 RPM is adequate to achieve homogeneity. Commercial manufacturing experience with similar mAbs at 50–200 kg scale indicates that the process ranges given here are suitable to dissolve the excipients completely. The mixing time required for complete dissolution in this operating range is <40 min
Mixing time		10–15 min	20–40 min	
Temperature		16–23 °C	15–25 °C	
Preparation of 10% polysorbate 80 stock solution				
Mixing speed	A-mAb B-mAb C-mAb		300–400 RPM	Prior knowledge and experience indicates that greater headspace volume corresponds to higher peroxide value. Less peroxide content is seen with lower air content in the headspace. Based upon this experience, a low headspace volume and nitrogen overfill with storage at controlled room temperature is recommended. Also, an opened bottle of PS 80 should be used within a campaign and within 2 weeks of opening. If not used completely by this time, it should be discarded
Mixing time			30–60 min	
Temperature			20–25 °C	
Fill volume			0.46–0.97 PS 80 volume/head Space volume ratio	
Head space			Air to nitrogen	
Storage			Held at RT with continuous mixing at 100 RPM for 8 h. For storage >8 h require filtration through 0.2 μm filter and 5 min of mixing prior to use	

Table 11.1 (continued)

Parameter	mAb product	Proven acceptable range		Experience and knowledge
		Clinical batch10–30 kg	Commercial batch 50–200 kg	
Dilution and/or pooling of bulk drug substance lots				
Mixing speed	A-mAb B-mAb C-mAb		60–120 RPM	Prior experience of dilution at 50–150 L scale indicated that these temperatures and mixing speed and time did not create splashing/foaming and no negative impact on the purity of the mAbs. This experience was gained in multiple tank and stirrer geometries
Mixing time			30 ± 5 min	
Temperature		2–8 °C	2–8 °C	
Formulated drug product hold times at 2–8 °C and RT in stainless steel containers				
Time (2–8 °C)	A-mAb B-mAb C-mAb	1 wk	1 wk	Prior experience shows that the impact of hold time is through the metals leached from the containers and any bioburden that may occur. mAbs are sensitive to metal induced oxidation, hence, the impact of metals should be studied for each mAb since this may be product specific. The maximum level of metals observed was 15 ppm Fe
Time (RT)	A-mAb B-mAb C-mAb	72 h	72 h	

to the processing conditions and equipment/material that the formulation can be subjected to during compounding and storage/handling. A cause and effect matrix consists of columns of quality attributes, and rows of parameters (compounding and handling/storage). Quantification of risk is calculated by multiplying the ranking of each quality attribute (the number at the top of each column) by the parameter ranking (the number in each row of the appropriate column). This is an assessment of the severity of the consequences of an adverse event in combination with the relative likelihood of that event happening. The functional risk prioritization will incorporate these critical quality attribute (CQA)/critical process parameter (CPP) attributes to develop the FMEA for the rigor of testing during the verification process. Scores are categorized "high," "medium," and "low." High >300, medium 200–300, low <200. Based on the scores from the initial risk assessment, the compounding parameters that scored highest were further examined in detail and characterized using experimental design, DOE. The results are summarized in the Table 11.2 where the highest scores are considered highest priority. The results from this initial risk assessment were then used to guide the process development and characterization studies.

11.5 Design Space—Process Characterization

The initial risk assessment identified the following four sets of studies for further detailed examination and creation of design space: (1) mixing and temperature during diluent preparation, (2) polysorbate preparation and handling (due to propensity to form peroxides), (3) mixing and handling during the dilution of the active product, and (4) hold times.

In this case study, dilution of X-mAb bulk drug substance with diluent/buffer to produce bulk drug product has been chosen to illustrate the mixing characterization study design to identify operating conditions at pilot scale (50 L). The use of models for scale-up coupled with the data obtained at 50 L scale are used to predict operating conditions at the 300 L scale. The specific outcome of this study was to identify the required mixing speed (RPM) and mixing time to produce a homogenous solution with no creation of foam or splashing and no adverse impact on the integrity or purity of the protein molecule. To achieve these, a bracketing approach or two extreme operating conditions were created and studied.

In one case the tank was filled to the height corresponding to minimum commercial batch size with X-mAb batch having protein concentration at the lower end of the specification (±15%) maintained at 20–25 °C, and the maximum mixing speed (RPM) required to achieve homogenous solution without the creation of vortex, foam, or splashing at room temperature (25 °C) was determined. This study defined the mixing speed range for the mixing process.

In the other case the tank was filled to a height corresponding to maximum commercial batch size with X-mAb batch having protein concentration at the higher end of the specification. This was maintained at 2–8 °C, and the minimum mixing

Table 11.2 Cause and effect matrix for compounding and mixing

Parameters	Quality attributes and rank (1–10)										Score	Experimental strategy
	Protein conc	Aggregate	Visible particle	SVP	pH	Osmo-lality	PS 80	Sucrose	Bio-burden	Sterility		
Excipient, water quality	1	7	5	7	1	1	9	9	9	1	428	Prior knowledge
Temp-DS dilution	1	7	7	7	1	1	1	1	9	1	330	Dev stability
Temp-diluent bulk DP	1	7	7	7	1	1	1	1	9	1	330	Dev stability
PS 80 handling	1	7	5	7	1	1	9	1	1	1	292	Prior knowledge
Mixing time-DS dilution	5	5	7	7	1	1	1	1	5	1	310	DOE
Excipient wt accuracy	1	5	1	5	5	7	9	9	1	1	362	DOE-dev
Mixing speed-DS dilution	5	7	7	7	1	1	1	1	1	1	290	DOE
Filter type diluent	1	1	9	1	1	1	9	1	9	1	206	Prior knowledge
Filter size diluent	1	1	9	1	1	1	9	1	9	1	206	Prior knowledge
Filtration flow rate/pump transfer rate-DS	1	1	9	1	1	1	9	1	9	1	206	Prior knowledge
Mixing time—dissolution	1	1	1	1	5	7	9	9	1	1	282	Prior knowledge
Mixing speed—dissolution	1	1	1	1	5	7	9	9	1	1	282	Prior knowledge
Hold time DS at RT	1	1	1	1	1	1	1	1	9	1	168	Stability
Hold time diluent at Rt	1	1	1	1	1	1	1	1	9	1	168	Stability
Hold time bulk DP at RT	1	1	1	1	1	1	1	1	9	1	168	Stability
DS weight accuracy	9	1	1	1	1	1	1	1	1	1	188	DOE-dev

Table 11.2 Cause and effect matrix for compounding and mixing

Parameters	Quality attributes and rank (1–10)										Score	Experimental strategy
	Protein conc	Aggregate	Visible particle	SVP	pH	Osmo-lality	PS 80	Sucrose	Bio-burden	Sterility		
Temp of diluent dissolution	1	1	1	1	7	1	1	1	5	1	168	Prior knowledge
pH meter accuracy	1	1	1	1	9	1	1	1	1	1	88	Calibration
Order of excipient addition	1	1	1	1	1	1	1	1	1	1	88	Prior knowledge
Impeller/mixer configura-tion-compounding tank	1	1	1	1	1	1	1	1	1	1	88	Modeling
Compounding tank size	1	1	1	1	1	1	1	1	1	1	88	Modeling
Diluent tank size	1	1	1	1	1	1	1	1	1	1	88	Prior knowledge

time (minutes) required to achieve a homogenous solution at 5 °C with the above defined mixing speed was determined. This study defined the mixing time range for the mixing process.

These mixing characterization studies with two different fill heights (min and max) were performed at a pilot scale in a 50 L tank and the mixing parameters (mixing speed and mixing time) were determined as a function of protein concentration (fluid viscosity and density). Based on this information the mixing characteristics of the pilot scale vessel, the Power number (Ne) and Reynolds number (Re) were determined and a correlation was developed between the Ne and Re for the pilot scale tank and mixer configuration.

Since the scale-up of the mixing process from pilot to commercial scale is between the mixer-vessel configurations of similar geometry, the fill heights (representing the minimum and maximum commercial batch size) required for pilot scale studies were obtained based on the following relationship coupled with the knowledge of dimensions of the commercial scale tank/mixer. The geometric similarity of the mixer-vessel configuration means that the D_{mixer}/T_{vessel} and the $H_{filling\ level}/T_{vessel}$—ratio are constant between small scale and larger scale.

Where,

D_{mixer} Diameter of the mixer
T_{vessel} Diameter of the vessel
$H_{filling\ level}$ Fill height

$$\frac{D_{mixer}}{T_{vessel}} = constant = 0.368$$

$$\frac{H_{filling\ level}}{T_{vessel}} = constant = 1.059$$

11.5.1 Characterization at Pilot Scale (50 L Vessel)

Mixing characterization studies were performed at pilot scale using the following input/operating parameters (Table 11.3) to identify the optimal output/performance parameters, the mixing speed, and time..

Table 11.3 Operating parameters of pilot scale studies

Process parameters	n = RPM various (e.g., 90)
Test fluid parameters	ρ = various (e.g.,1.03 g/mL)
	η = various (e.g., 2.5 mPas)
	ν = various (e.g., $2.43 \cdot 10^{-6} m^2/s$)
Vessel dimensions	T_{vessel} = 408 mm $H_{filling\ level}$ = 432 mm (Min) $H_{filling\ level}$ = 921 mm (Max) (V_{fluid} = 50 L)
Mixer dimensions	D_{mixer} = 150 mm
Temperature	5 °C and 25 °C

11.5.1.1 Methodology

The compounding tank was filled to the fill height representing the minimum commercial batch size with the test fluid and equilibrated to room temperature (25 °C). The mixer speed was gradually increased at regular intervals. At each increment of the mixing speed (RPM) the solution in the tank was visually inspected for vortexing, splashing and foaming and observations were recorded. The mixer speed (RPM) at which the solution starts foaming or vortexing was determined. Based on these observations the optimal mixing speed for the test solution was selected. This was repeated for test fluids with different protein concentrations (viscosities and densities) and optimal mixing speeds for each test fluid were determined. Samples were drawn from various locations and purity (aggregates) of the X-mAb was determined using SE-HPLC.

Using the optimal mixing speeds (RPM) identified in the above studies for each test fluid, the mixing studies were repeated at 5 °C with test fluid filled to the fill height representing the maximum commercial batch size. The optimal mixing time required to produce a homogenous solution was determined for each test fluid. Formation of a homogenous solution was determined by analyzing the protein concentration of the samples pulled at frequent intervals from various locations including dead spots during the mixing process. The integrity of the protein was also determined using SE-HPLC

11.5.1.2 Determination of Dimensionless Numbers

Newton (or Power) number and Re are commonly used to describe the mixing behavior of the liquid in the compounding tank (Gorsky and Nielsen 1992). Newton (or Power) number is defined as a ratio of resistance force to inertial force and is denoted by

$$Ne = \frac{P}{\rho . D_{mixer}^{5} . n^{3}}$$

where P is the mixer power in Watts that mainly influences the mechanical stress, which acts on dissolved molecule

and

P $2 \cdot \P \cdot n \cdot M_d$ (M_d is the torque on the mixer to maintain the speed and is measured)
ρ Density in kg/m^3
n Mixing speed in rpm

Re is the ratio of inertial forces to viscous forces and is given by the formula

$$Re = \frac{n . D_{mixer}^{2}}{v}$$

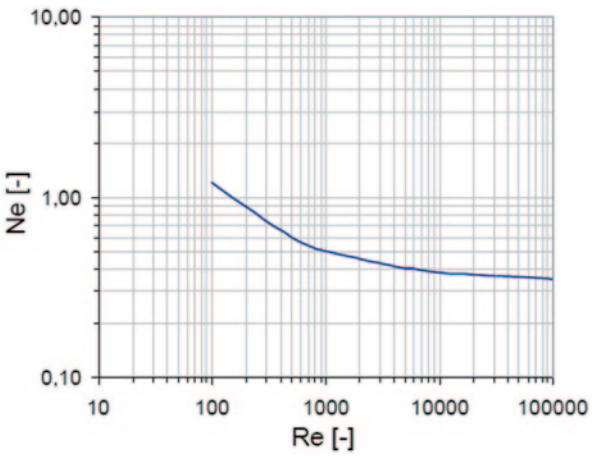

Fig. 11.1 Characterization of the power input of the impeller mixer

ν Kinematic viscosity in m^2/s

It will be shown below how these numbers will be utilized in the scaling up the mixing process.

Using the above equations, the Re number for each test fluid at a certain protein concentration (viscosity and density) or rotational speed "n," and the corresponding Ne number are calculated and a correlation (Fig. 11.1) is developed.

The intensity of mechanical impact on the product solution which is a product of mixer speed (n) and mixing time (Θ) is plotted against the Re number and a correlation (Fig. 11.2) is developed.

These two correlations serve as the basis for the scale-up of the mixing process.

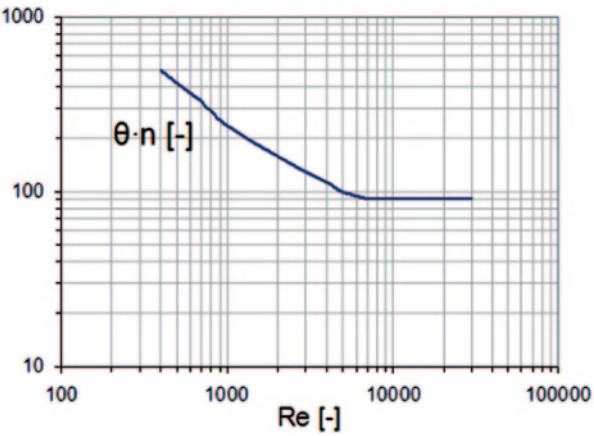

Fig. 11.2 Mixing behavior of the impeller mixer

11.5.2 Scale-Up from Small Scale with Similar Tank and Mixer Geometries

There are several approaches to scale-up or scale-down of: (1) the mixing process based on a constant specific power impact: $\frac{P}{V} = constant\ criterion$ (and therefore a comparable mechanical stress at both scales (Perry and Green 2008; Hughmark 1974), (2) constant mixing time Θ (Norwood and Metzner 1960), (3) bulk velocity (Barker and Treybal 1960), (4) bulk shear, and (5) impeller tip shear rates. In this case study, a constant P/V ratio is used as the scale-up criterion to scale-up X-mAb mixing process from a 50 to a 300 L tank with similar tank and mixer geometries (see Fig. 11.3).

11.5.2.1 Outline of Scale-Up Procedure

Once optimal mixing parameters have been identified at the pilot-scale vessel, the parameters are used to determine target operating parameters for the 300 L tank:

- From small-scale vessel information, set scale-up criterion and calculate P/V constant
- For larger scale tanks, calculate P
- Calculate "*n*" using knowledge of torque (M_d) as a function of mixer speed for each tank
- Calculate Re and obtain Θ using correlation in Fig. 11.1
- After identifying the operating parameters for the larger tanks, the values are confirmed through appropriate experiments at scale.

For any given test fluid with certain protein concentration (viscosity and density) the mixing characteristics can be estimated as shown in the example below.

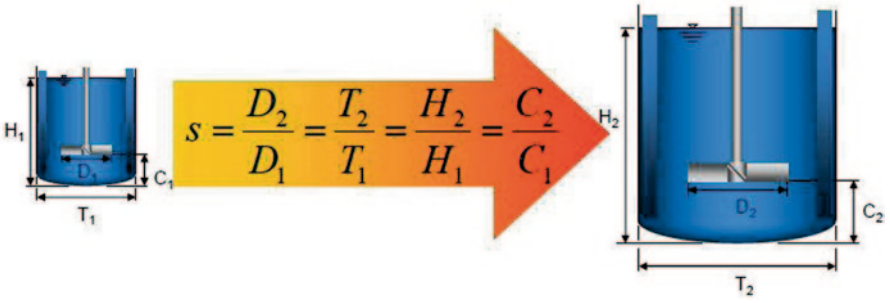

Fig. 11.3 Geometric similarity—a single scale ratio, *s*, defines the relative magnitude of all linear dimensions between the small and large scale

11.5.2.2 Step 1

Calculate the (Re) of the test fluid for a given rotational speed (e.g., RPM = 90)

$$Re = \frac{n.D_{mixer}^2}{v} = \frac{\frac{90}{60s}(0.150m)^2}{2.43.10^{-6}\frac{m^2}{s}} = 13888$$

11.5.2.3 Step 2

Determine the Ne number from the Fig. 11.1 which is 0.37

11.5.2.4 Step 3

Calculate power, P, from the following equation
We know:Ne = 0.37

$$\rho = 1030\frac{kg}{m^3}$$

$$D_{mixer} = 150.10^{-3}\ m$$

$$n = \frac{90}{60s}$$

$$Ne = \frac{P}{\rho . D_{mixer}^5 . n^3} \quad 0.37 = \frac{P}{1030\frac{kg}{m^3}.\left(150.10^{-3}m\right)^5.(90/60s)^3}$$

$$P = 0.37.1030.(150.10^{-3})^5.(90/60)^3$$

$$P = 0.137W$$

11.5.2.5 Step 4

Calculate the scale-up criterion

$$\frac{P}{V} = Constant, \frac{0.137W}{50 \times 0.001m^3} = 2.74.\frac{w}{m^3}$$

Table 11.4 Summary of mixing studies at pilot scale

Mixing conditions studied at pilot scale (50 L)	Temperature = 25 °C	Temperature = 5 °C
	Fill ht = Min batch size (15 L)	Fill ht = Max batch size (50 L)
	Mixing time = 30–100 min	Mixing time = 40–130 min
	Mixing Speed = 40–140 RPM	Mixing Speed = 90 ± 20 RPM
Objective	Identify optimal mixing speed	Identify optimal mixing time
Performance criteria for range of speed/time	No foaming, no vortexing, no product impact (no aggregate formation)	Formation of homogenous solution with no adverse impact on product
Summary of results		
Reynolds number (Re)	13888	13888
Newton number (Ne)	0.37	0.37
Power number (P)	0.137	0.137
P/V criterion	9.13 W/m^3	2.74 W/m^3
Optimal mixing speed	90 ± 10 RPM Note: foaming observed for rpm > 120	90 ± 10 RPM
Optimal mixing time	Homogeneity observed in < 10 min. No negative impact on product quality observed up to 100 min	60 ± 10 min Note: homogeneity observed in 40 min

Scale-up criterion (comparable stress), $\frac{P}{V} = 2.74.\frac{w}{m^3}$

The operating mixing parameters that were found acceptable and optimal at pilot scale (50 L) from both process performance and product quality perspectives (Table 11.4) were used below as input into the model to estimate operating mixing parameters for large scale (300 L).

Scale-Up to 300 L Tank In order to identify the appropriate mixing parameters that would create mixing conditions and produce shear stress level in a 300 L tank identical to that of 50 L scale, the mixer of required diameter for 300 L tank is determined using the knowledge of product solution/ tank parameters (Table 11.5) along with similar geometry criteria.

$$\frac{D_{mixer}}{T_{vessel}} = constant = 0.368$$

$D_{mixer} = 312.8\ mm$

Given the scale-up criterion of $\frac{P}{V} = constant = 2.74\frac{w}{m^3}$

The mixer power for the 300 L tank can be computed as follows

$$\mathrm{P} = 300 \times 0.001\ \mathrm{m}^3 \times 2.74\frac{W}{m^3} = 0.82\ \mathrm{W}$$

Table 11.5 Product solution and 300 L scale tank parameters

Mixing speed (RPM)	n=? (Output from scale-up model)
Mixing time	(Θ)=? (Output from scale-up model)
Test fluid parameters	ρ=1.03 g/ml
	η=2.5 mPas
	v=2.43•10^{-6}m^2/s
Vessel dimensions	T_{vessel}=850 mm $H_{filling\ level}$=900 mm (V_{fluid}=300 L)
Mixer dimensions	D_{mixer}=? mm (Output from scale-up model)
Temperature	5 °C

The mixing speed required to create the same shear stress level can be calculated using the power, P=0.82 W and the corresponding torque, M_d=0.343 (measured from the equipment) using the following equation

$P=2\cdot ¶ \cdot n\cdot M_d$

$$n = \frac{0.82}{0.343\times 2\times 3.14}\times 60 = 23\ \text{RPM}$$

If P/V is used as scale-up criterion longer mixing times and higher Reynolds numbers are achieved for the 300 L tank. From Fig. 11.1 is known that at Re>10000 the Ne number is almost constant (0.35). By using the formula for the Ne number

$$Ne = \frac{P}{\rho\cdot D_{mixer}^5\cdot n^3}$$

and the value for the Ne number 0.35 the rotational speed for the large 300 L vessel can be determined without knowing the corresponding torque of the mixer (here: 19 rpm).

Thus the mixing speed calculation for 300 L tank can be repeated for any small scale tank (50 L) speed that is considered optimal from a homogeneity, product quality (shear) and foaming perspective.

Once we know the mixing speed, the Re can be calculated.

$$Re = \frac{n.D_{mixer}^2}{\upsilon} = \frac{\frac{23}{60}(0.313m)^2}{2.43.10^{-6}\,\frac{m^2}{s}} = 15320$$

Knowing the "Re" the mixing time in the 300 L scale tank can be estimated using the correlation between Re and n Θ (Fig. 11.2).

Table 11.6 Summary of estimates of operating parameters for 300 L tank

Mixing conditions studied at commercial scale (300 L)	Temperature = 25 °C	Temperature = 5 °C
Vessel dimensions	T_{vessel} = 850 mm $H_{filling\ level}$ = 900 mm	T_{vessel} = 850 mm $H_{filling\ level}$ = 900 mm
d mixer	312.8 mm	312.8 mm
Reynolds number (Re)	17470	15320
Newton number (Ne)	0.37	0.37
Power number (P)	0.456 W	0.82 W
P/V criterion	9.13 W/m^3	2.74 W/m^3
θ·n (from Fig. 11.3)	75	90
Mixing speed	26 RPM	23 RPM
Mixing time (Θ)	2.8 min	4 min

Review of the correlation between Re and n Θ in Fig. 11.2 indicates that for the 300 L scale the intensity of mechanical impact on the product solution represented by nΘ is 90.

Thus the mixing time in the 300 L tank, $\theta = 235$ s

The above predictedoperating parameters were tested and verified at two worst case conditions similar to pilot scale studies using X-mAb, during the engineering runs. Samples were drawn and tested for both homogeneity and product quality attributes, the results of which are summarized in the Table 11.6

11.5.3 Condition 1

11.5.3.1 Input Parameters

- Batch size 35 kg
- Temperature: 25 °C
- Mixing speed: 26 RPM

11.5.3.2 Output Parameters

- Sampling (top, bottom, and dead spots): Test for protein concentration for homogeneity and SE-HPLC for purity/aggregates
- Visual observation: Inspect for splashing, foaming, and vortexing (Table 11.7).

Table 11.7 Maximum speed (RPM) determination through visual inspection for 35 kg batch size

Mixing speed (RPM)	Visual inspection
10	Solution in motion, no vortex, no foam generated, stratification observed
15	Stratification starts dissipating, no foam generated, no vortexing or splashing observed
25	Stratification and gradients disappeared, no foam, no vortexing or splashing observed
30	Foaming and vortexing observed

11.5.4 Condition 2

11.5.4.1 Input Parameters

- Batch Size 300 kg
- Temperature: 5 °C
- Mixing speed: 23 RPM

11.5.4.2 Output Parameters

- Sampling (top, bottom, and dead spots): Test for protein concentration for homogeneity (see Fig. 11.4) and SE-HPLC for purity/aggregates
- Visual observation: Inspect for splashing, foaming, and vortexing

The results from the scale-up data indicates good agreement between the predicted and the experimental data demonstrating that the model approach developed from the small scale vessel is appropriate for scale-up activities. The completion of mixing in comparison to the model estimates is summarized in the Table 11.8 appearing on page 229. The differences in mixing time as compared to the model ranged from 0–15%.

11.5.5 Scale-Up when Tank Geometry is not the Same

When the shape of the compounding vessel in the manufacturing plant or the type of mixer is different than the previously studied vessels, the Ne-Re- and the mixing diagram cannot be used. The characteristics of the flow pattern in the vessels are different.

11.6 Design Space

The extensive prior knowledge, initial risk assessment, coupled with the mixing characterization studies at the pilot scale that led to the identification of operating conditions and their verification at scale helped to create a design space ensuring a

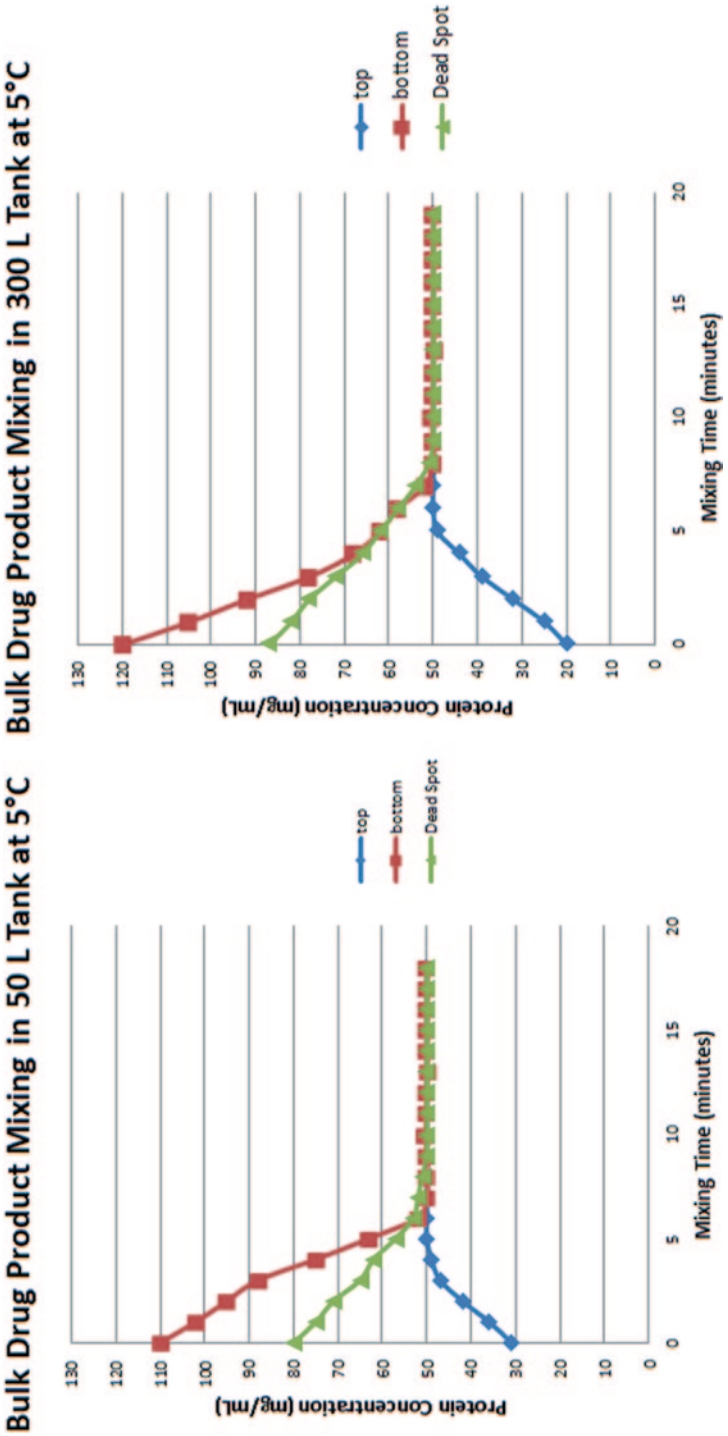

Fig.11.4 Mixing time determination through protein concentration (*Homogeneity*).

Table 11.8 Summary of differences between the experimental and predicted mixing times required for compounding vessels from 50–300 L

Temperature	Tank size = 50L	Tank size = 300 L		
	Experimental	Experimental	Predicted	% Difference from model
5°C	4	5	4	20

homogeneous product for the compounding operations (Table 11.9). The engineering run studies at scale also confirmed that the sub-unit operations in the compounding step do not impact purity (aggregation and particle formation) of X-mAb.

11.7 Control Strategy

The proposed control strategy for the filtration process has a dual purpose:

1. Ensure product quality and safety.
2. Ensure that the commercial manufacturing process is consistent and robust.

Product quality and safety are ensured by controlling all quality-linked process parameters (WC-CPP) within the limits of the design space. Process consistency is ensured by controlling key process parameters (KPPs) within established limits and by monitoring relevant process attributes.

The control strategy for the given process parameters outlined in this case study for mixing is covered below. The outcome of the studies executed in order to explore the design space allows the differentiation between critical process parameters (CPP) and key performance parameters (KPP), and general process parameters (GPP) as shown in Table 11.10.

11.8 Final Risk Assessment

A final risk assessment was undertaken to understand risk mitigation from a process parameter perspective. The final risk assessment was completed by taking the designation of CPP, WC-CPP, KPP, and GPP into account. In addition the assessment of a NOR/PAR width of parameter and control strategy was included in the assessment.

11.8.1 Final Risk Assessment Tool Description

If a NOR/PAR has a wide range, there is less inherent risk that the range can be met by a process and therefore, has a lower risk value. A narrow range is more difficult to control and translates to a higher risk value. The control strategy can be

Table 11.9 Knowledge space ranges

Process parameter	Designation	Unit ops	Quality attribute	Process ranges explored	Design space: PAR	Control space: NOR	Target	Rational for ranges
PS 80 fill volume	CPP	Excipient handling	Peroxide value	0.14–0.97 volume to headspace ratio	0.46–0.97 PS 80 volume to headspace ratio	0.50–0.95 PS 80 volume to headspace ratio	0.50–0.95 PS 80 volume to headspace ratio	Greater fill volume correlate to lower peroxide values
PS 80 headspace	CPP	Excipient handling	Peroxide value	air-nitrogen	air-nitrogen	air-nitrogen	air-nitrogen	Less peroxide formation with less air content
Mixing speed—DS dilution	CPP	DS dilution	Aggregation, oxidation protein content	5–120 RPM	5–120 RPM	5–25 RPM	10 RPM	Covers typical operations without increase in aggregate
Hold time of DS at CRT	CPP	DS dilution	Bioburden, aggregation, oxidation	0 days to 12 months	0–10 days	0–7 days	1 day	Covers typical operations without increase in aggregate, oxidation or bioburden
Hold time of bulk drug product at CRT	CPP	DS dilution	Bioburden, aggregation, oxidation	DS experiments to support	0–10 days	0–7 days	I day	Covers typical operations without increase in aggregate, oxidation or bioburden
Temperature of DS dilution	WC-CPP	DS dilution	Aggregation, oxidation protein content	5–30 °C	5–25 °C	10–20 °C	10–20 °C	Covers typical operations without increase in aggregate

Table 11.9 (continued)

Process parameter	Designation	Unit ops	Quality attribute	Process ranges explored	Design space: PAR	Control space: NOR	Target	Rational for ranges
Mixing time of DS dilution	WC-CPP	DS dilution	Aggregation, oxidation	0–600 min	3–600 min	20–40 min	30 min	Covers typical operations without increase in aggregate
Hold time of diluent at CRT	WC-CPP	Diluent preparation	Bioburden	0 days to 3 months	0–10 days	0–7 days	3 days	Covers typical operations
Diluent mixing speed	KPP	Diluent preparation-excipient dissolution	Excipient dissolution, foaming	25–150 RPM	75–150 RPM	100–150 RPM	125 RPM	Dissolution complete in less than 30 min
Diluent temperature	KPP	Diluent preparation-excipient dissolution	Excipient dissolution, foaming	5–25°C	15–25°C	17–23°C	20 °C	Dissolution complete in less than 30 min at speeds (NOR)

Table 11.10 Control strategy of mixing process parameters

Process parameter	Designation	Control strategy
Excipient weight accuracy	CPP	Batch record
Order of excipient addition	GPP	Batch record
Mixing time of dissolution	KPP	Batch record
Mixing speed of dissolution	KPP	Batch record, equipment calibration
Temperature-DS dilution	KPP	Batch record
pH meter accuracy	CPP	Preventative maintenance and calibration at time of use
Hold time of diluent	CPP	Documentation controlling cumulative exposure to cold and room temperature conditions in batch record
Drug substance weight accuracy	CPP	Batch record, equipment calibration
Hold time of drug substance	CPP	Documentation controlling cumulative exposure to cold and room temperature conditions in batch record
Mixing time of DS dilution	CPP	Batch record
Mixing Speed—DS dilution	CPP	Batch record, equipment calibration
Compounding tank size	GPP	Batch record
Impeller/mixer configuration—compounding tank	GPP	Batch record
Hold time bulk drug product	CPP	Documentation controlling cumulative exposure to cold and room temperature conditions in batch record

considered as high/tight, medium or low. A tight control of a system has less risk than one that is medium or low and therefore, has a lower risk. PP risk was ranked as CPP > KPP > GPP. Values assigned for each designation, the NOR/PAR width of parameter and control strategy level described in Table 11.11. From these values, a risk mitigation number can be calculated.

A Risk Mitigation Number of ≤ 200 was considered mitigated. Values > 200 require additional mitigation strategies. This cut-off suggests that a:

- CPP with a narrow range for NOR/PAR must be controlled well to pass 200 (be considered mitigated)

Table 11.11 Risk assessment values

Risk value	Designation	NOR/PAR width of parameter	Control strategy level
1	GPP		High
3		Wide	
5	KPP		Medium
7		Narrow	
9	CPP		Low

Risk Mitigation Number = designation * NOR/PAR width × control strategy level

- CPP with a wide range for NOR/PAR must be controlled well to medium to pass 200
- KPP with a narrow range for NOR/PAR must be controlled well to medium to pass 200 KPP with a wide range for NOR/PAR will always pass 200
- GPP with a narrow range for NOR/PAR will always pass 200
- GPP with a wide range for NOR/PAR will always pass 200

For compounding, the risk mitigation scores for the individual process parameters outlined in the initial risk assessment are calculated in Table 11.12.

Requirements for further risk mitigation for hold times of drug substance and bulk drug product were identified by the Risk Mitigation Number in the final risk assessment. An additional control strategy was included to address these hold times. A hold time log affixed to each holding tank was added to the batch record. A re-evaluation was completed for these process parameters after the increase in control strategy was implemented demonstrating risk mitigation (Table 11.13).

Based on the new control strategy and further risk mitigation reassessment, all process parameters that were previously designated as CPPs now fall below the 200 in the risk mitigation score and are designated as well controlled (WC-CPPs).

11.9 Process Demonstration and Verification

11.9.1 Hold Time of Diluent and Bulk Drug Product

The hold time studies that were performed at small scale in 1 L stainless steel tanks and the Proven Acceptable Ranges (PARS) that were identified and used to support the clinical batches were verified at scale during the engineering runs. Only the worst case scenarios were verified during engineering runs. The diluent was held for 2 weeks at room temperature (17°C–25°C) while the bulk drug product was held for 1 week at room temperature (17°C–25°C). Samples were drawn at the end of hold time using novaseptum and tested for bioburden along with the quality attributes, the results of which are summarized in the Table 11.14. The test results met all the acceptance criteria defined for diluent and bulk drug product.

11.10 Life-Cycle Management

The details outlined in the compounding section demonstrate the use of models for scale-up to allow flexibility in the manufacture of X-mAb drug product batches from 50–300 L in size. The model has been confirmed experimentally and will not require further experiments at scale.

Table 11.12 Risk mitigation scores for mixing process parameters

Process parameter	Designation CPP/KPP	Risk value	NOR/PAR width	Control strategy level	Risk mitigation (D × W × C)	Risk mitigated (Y/N) (D × W × C ≤ 200)
Excipient weight accuracy	CPP	9	3	1	27	Y
Order of excipient addition	GPP	1	7	5	35	Y
Mixing time of dissolution	KPP	9	3	2	54	Y
Mixing speed of dissolution	KPP	9	3	5	135	Y
Temperature-DS dilution	KPP	9	3	1	27	Y
pH meter accuracy	CPP	9	7	1	63	Y
Hold time of diluent	CPP	9	3	5	135	Y
Drug substance weight accuracy	CPP	9	7	1	63	Y
Hold time of drug substance	CPP	9	7	5	315	N
Mixing time of DS dilution	CPP	9	3	5	135	Y
Mixing speed of DS addition	CPP	9	7	1	63	Y
Compounding tank size	GPP	5	3	5	75	Y
Impeller/mixer configuration—compounding tank	GPP	1	3	5	15	Y
Hold time bulk drug product	CPP	9	7	5	315	N

Table 11.13 Further risk mitigation assessment

Process parameter	Designation CPP/KPP	NOR/PAR width	Control strategy level	Risk mitigation (D × W × C)	Risk mitigated (Y/N) (D × W × C ≤ 200)
Hold time of drug substance	9	7	1	63	Y
Hold time of bulk drug product	9	7	1	63	Y

Table 11.14 Diluent and bulk drug product hold time data

Diluent after hold time (10d RT)				
	Bioburden	*pH*	*Osmolality*	*Conductivity*
Acceptance Criteria	< 1 CFU/10 mL	5.0–5.5	280–350 mOSm/Kg	Report results
Lot 1	0 CFU/10 mL	5.2	310	0.8 mS/cm
Lot 2	0 CFU/10 mL	5.2	301	0.9 mS/cm
Lot 3	0 CFU/10 mL	5.3	296	0.9 mS/cm
Bulk drug product after hold time (5d RT)				
	Bioburden	*Aggregates*	*Subvisible particulates (HIAC)*	
Acceptance criteria	< 1 CFU/10 mL	< 5 %	10 µm < 6000 25 µm < 600	
Lot 1	0 CFU/10 mL	2.1	140/mL ≥ 10µm 18/mL ≥ 25µm	
Lot 2	0 CFU/10 mL	1.8	165/mL ≥ 10µm 25/mL ≥ 25µm	
Lot 3	0 CFU/10 mL	2.0	170/mL ≥ 10µm 32/mL ≥ 25µm	

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Chapter 12
Application of QbD Elements in the Development and Scale-Up of a Commercial Filtration Process

Feroz Jameel

12.1 Introduction

Aseptic processing using sterilizing grade filtration is commonly employed in biopharmaceuticals as an alternate to methods of terminal sterilization that degrade proteins. The "one filter type for all applications" approach only works to a limited extent. To ensure highly efficient production processes, both for bulk filtration as well as in-line filtration, specific sterile filters suitable for a specific product and process need to be selected. The selection of the filter should: (1) ensure that it is capable of retaining microorganisms, and generating a sterile filtrate (FDA 2004; ASTM 1988, 2005), (2) be compatible with the product and process, (3) is non-toxic, (4) does not adsorb formula components or add extractables to the process (Sundaram et al. 2001), (5) can remove bioburden, (6) is integrity testable, and (7) is sterilizable (Jornitz et al. 2001). From the process perspective, the filtration process should be scalable, feasible at the commercial site and impart no negative impact to the drug product quality attributes (Jornitz and Meltzer 2000, 2006). In order to meet the above requirements, a thorough characterization of the filter(s) and the process parameters need to be performed to understand and identify a robust filter and a filtration process. This chapter describes in detail, with illustration, how Quality by Design (QbD) elements could be applied in identifying optimal filter(s) and filtration processes that are well understood, well controlled, safe, and robust (U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Center for Veterinary Medicine (CVM) Office of Regulatory Affairs (ORA) 2006).

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12.2 Definition of the Target Product Filtration Process

The X-mAb (X-monoclonal antibody) drug substance (DS) is shipped to manufacturing sites in multiple containers and the contents of each container are pooled into a sterilized stainless steel vessel using a peristaltic pump. The pooled DS is mixed for 10–20 min in a pooling vessel that is equipped with a mixer to achieve homogeneity of the solution. Pooled bulk is then transferred through a filter under pressure into another sterilized stainless steel holding tank. The pooled bulk is then either stored at 2–8 °C for an intermediate storage in the stainless steel or is delivered immediately to a fill and finish suite for further processing. The pooled bulk solution is sterile filtered using 0.2 μm filter in a class C area into a class A suite directly into a surge vessel placed on the filling machine (in-line filtration). Sterile filtered bulk solution is then filled into sterilized and depyrogenated vials. The process flow includes a first filtration to reduce bioburden followed by in-line filtration at which point the DS is claimed sterile.

Filter and filtration process requirements include:

- The filter and the filtration process should be able to cover the batch size range of 50–150 L.
- The process should be operative in the temperature range of 4–23 °C.
- The process should be operative under low and high pressures to enable high-flux and low-contact time of the product with the membrane during bioburden reduction filtration to minimize loss of excipients, especially protein, and polysorbate.
- The filter should be nonreactive with the product and should not shed any particles.

12.3 Prior Knowledge

Given the similarity in the formulation composition (excipients), physicochemical properties and the target filtration process of mAbs, a platform approach was created and adopted. In this approach, extensive assessments and evaluations were performed to identify filter(s) and a filtration process that can be commonly used for all mAbs for early phase drug product development and manufacturing of early stage clinical batches. The assessments involved a broad range of filters with regard to their suitability for the intended mAb filtration process (see Table 12.1) based on knowledge from vendor specifications and literature for the following criteria: membrane material, capsule availability, available connections, scale-up range (membrane area), double layer option (prefilter), flow rate (WFI), size (length), housing and supporting material, sterilization method options, recommended flushing volume, hold-up volume, leachables/extractables, and operating experience.

The aforementioned attributes were assessed on a weighted scale of 10 to 1 based on their criticality to filter selection criteria with 10 being high, 5 medium, and 1

Table 12.1 Comparison of different filter membrane types used with antibody products

Attribute	PVDF	PES	PES/ composite	Nylon	Cellulose acetate
Pore size (μm)	0.2, 0.1	0.2, 0.1	0.5, 0.2	0.2	0.2, 0.1
Charge	Neutral, positive	Neutral	Neutral	Neutral, positive	Neutral
Protein binding	Low	Low	Low	High	Low
Hydrophilic	Yes	Yes	Yes	Yes	Yes
Manufacturers	Millipore, Pall, Sartorius, Meissner	Millipore, Pall, Sartorius, Meissner	Millipore	Millipore, Pall, Sartorius, Meissner	Sartorius

low. The filter was ranked 10, if all the desirable attributes for the intended filtration process were met. Examples of desirable attributes include: if it is available as a capsule (to eliminate housing cleaning and cleaning validation and integration into single-use disposable systems), it is equipped with a variety of connections to manufacturing equipment, its hold-up volume is low, various sterilization methods can be applied (e.g., autoclaving by several cycles, gamma-sterilization, or in-situ steam sterilization), or scale-up is possible, leachables and extractables are low. Filters that could fulfill only some of the desired attributes were ranked 5, and the filters that had only few suitable features were ranked 1. This process of ranking of various filters is summarized in the Table 12.2.

Only those filters that achieved an overall performance score of > 500 were qualified for further evaluation of their performance with fluid characteristics of mAbs products. Based on the above selection criteria and scoring, membranes of PVDF (Polyvinylidene fluoride) and PES (Polyethersulfone) material construction were chosen for further characterization of filters with mAbs solutions (A-mAb, B-mAb, and C-mAb). The characterization work included flow characteristics (V_{max}), product quality impact (shear stress, protein/excipient adsorption, particle shedding), and rinsing volumes prior to integrity testing (Fig. 12.1).

12.4 Characterization Studies

Filters tend to shed particles that are mostly composed of the material they are made of. Filters were flushed with known volumes of WFI and the change in the number of particles per volume fractions (in liter increments) was measured. The minimum WFI rinsing volume required to ensure zero particle shedding was determined for each filter prior to initial filter integrity testing. The V_{max} and repeated filtration and product quality impact studies were performed subsequently on filters that were rinsed with WFI, integrity tested, and autoclaved.

Table 12.2 Initial filter screening and performance assessment

Filter	Attribute/Weight scale														
	Membrane material	Capsule availability	Connections	Membrane area (m^2)	Double layer-prefilter available	Flow rate	Size/Length	Housing material	Supporting material	Sterilization method options	Recommended flush vol	Hold-up volume	E/L	Operating experience	Performance score
Weight scale	5	10	5	10	5	5	1	1	1	10	5	1	10	10	
PVDF vendor 1	10	10	5	5	1	5	10	10	10	10	5	10	10	10	620
PVDF vendor 2	10	10	5	10	10	10	10	10	10	10	10	5	10	5	710
PES vendor 1	10	10	10	5	5	10	5	10	10	10	5	5	10	1	590
PES, vendor 2	10	10	5	10	10	10	10	10	10	10	10	5	10	5	710
Cellulose vendor 1	10	5	10	5	10	5	5	10	10	10	5	5	10	1	540
Cellulose vendor 2	10	10	5	10	10	5	10	10	10	10	10	5	10	5	685

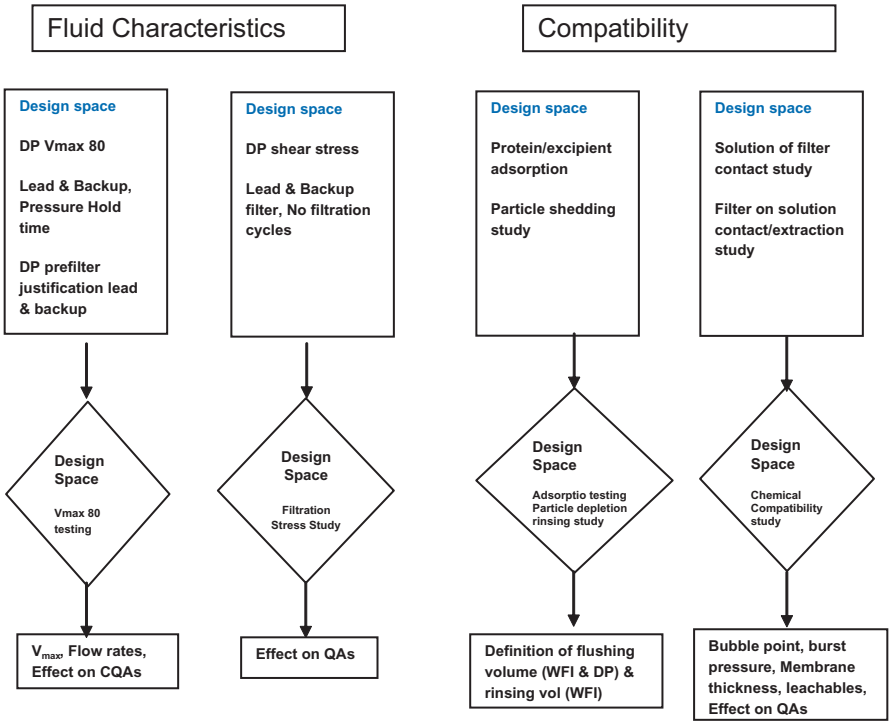

Fig. 12.1 General characterization program that was used to establish platform approach for selection of filter to be used in early process development

12.4.1 V_{max} Studies

The $V_{\max}$ method was used as one of the tools to discriminate the performance of different filter membranes, and the flow characteristics of each selected filter was studied as a function of pressure and protein concentration using various mAb products. A simple fractional factorial design, Table 12.3, was followed where the protein concentration was varied from 30 to 80 mg/mL and pressure was varied from 7 to 20 psi. In order to eliminate the filter geometry (capsule housing, pleated vs. disk filters) and system configurations effects on $V_{\max}$ studies, all the $V_{\max}$ experiments of all the selected filter membrane materials were conducted on 47 mm disk filters in stainless steel filter housing.

The outputs of this study were (1) the flow rate (2) determination of maximum volumetric throughput ($V_{\max 75}$), and (3) determination of formation of any particles or aggregates. The effect of temperature on $V_{\max}$ was not included in this study.

Table 12.3 Experimental design for V_{max} Study

Number	Pressure [psi]	Concentration [mg/mL]	Filter material type
1	7 (−)	30.0 (−)	PES
2	20 (+)	30.0 (−)	PES
3	7 (−)	80 (+)	PES
4	20.0 (+)	80 (+)	PES
5	12.0	50	PES
6	7 (−)	30.0 (−)	PVDF
7	20 (+)	30.0 (−)	PVDF
8	7 (−)	80 (+)	PVDF
9	20.0 (+)	80 (+)	PVDF
10	12.0	50	PVDF

+ (Maximum)
− (Minimum)

12.4.2 Repeated Filtration and Product Quality Impact Studies

Any sensitivity to shear impact on the protein integrity and any interactions between the formulation ingredient and the filter membrane (protein/excipient binding) or leachable/extractables during the filtration process were evaluated by subjecting the mAb products to repeated filtrations (3×) and analyzing the filtrates for changes: in the purity of the protein (aggregates), in the protein/excipients content and number of particles per volume fractions (in liter increments). This analysis of the filtrate for loss of protein/excipient and number of particle per volume fractions helped to determine if a preflush was necessary and if so, how much volume was required. Since subtle changes in the product quality are not evident at $t = 0$, samples were put on short-term stability to detect any changes in the product quality over time.

12.4.3 Determination of Minimum WFI Rinsing Volume Prior to Postuse Integrity Testing

The bubble point value for a filter wetted with WFI differs from a filter wetted with mAb product due to differences in solution properties such as surface tension, contact angle, temperature, and diffusion rates. Therefore, bubble point specifications for WFI and mAb product need to be defined separately. First, the postuse integrity test is performed on the filter as is (wetted with the product) after the completion of filtration of the product. If it fails, the test is repeated on filter wetted with WFI by rinsing off the product completely with WFI. For this reason, it is necessary to determine the minimum flushing volume of WFI required to rinse off the product completely.

Experiments were performed by soaking the filters with various mAb product solutions for a defined time. The soaked filters were then rinsed with WFI in 1 L increments and the corresponding bubble point was measured along with the analysis of the filtrate for protein/excipient content. The minimum rinsing volume required was defined when the bubble point values and the protein/excipient content reached a plateau.

The knowledge and scientific understanding gained through the filter screening/characterization work to establish platform filters (lead and back up filter) and filtration process represents the prior knowledge. Small-scale process justification studies were performed with X-mAb solution as part of the early phase process development to confirm the application of platform process and demonstrate appropriate stability of X-Mab drug product. This platform filter and filtration process was then initially used for the manufacturing of early phase supply of X-mAb for toxicology, phase 1 and 2 clinical studies. This prior knowledge in conjunction with the experience gained during clinical manufacturing was utilized in the initial risk analysis of filter and filtration process for X-mAb product.

12.5 Initial Risk Analysis

Filtration process parameters that can potentially impact the critical product quality attributes (CQAs) are listed in Table 12.4. Using a "cause and effect" matrix they were assigned criticality scores based on prior knowledge and clinical manufacturing experience. The purpose of this analysis is to identify those critical process parameters (CPPs)) that need to be reexamined/characterized in detail specifically with the X-mAb product.

A cause and effect matrix consists of columns of quality attributes, and rows of process parameters. Quantitation of risk is calculated by multiplying the ranking of each quality attribute (the number at the top of each column) by the parameter ranking (the number in each row of the appropriate column). This is an assessment of the severity of the consequences of an adverse event in combination with the relative likelihood of that event happening. The functional risk prioritization incorporates these CQA/CPP attributes to develop the failure modes and effects analysis (FMEA) during the verification process. Scores are categorized "high", "medium," and "low." High: > 250, Medium: 150–250, and Low: < 150. Items over 250 are highlighted in red, items between 150 and 250 in yellow, and items less than 150 in green as shown in Table 12.5.

Table 12.4 Initial risk assessment of filtration process

Process Step	Quality Attributes								Rationale	Score
	Protein content	Purity (Agg/ Frag)	PS 20	Visible Particles	SVP	Sterility	Clarity	Endotoxin		
Rank	10	10	10	10	10	10	7	10		
Pre flushing volume (WFI)	1	5	1	7	10	1	1	1	Effect of particles and oxidative species might influence the formation of aggregates or subvisibles	267
Filter integrity test, Pre & post	1	1	1	7	7	10	7	10	Impact on sterility and particulate matter	419
DP flush vol (pre filtration)	5	5	10	7	10	1	5	1	Adsorption of protein and/or excipient on filter membrane	335
Flow rate per membrane area	1	7	1	1	7	1	7	1	Impact shear stress and might lead to aggregation	239
N_2 Pressure	1	5	1	1	1	1	5	1	The pressure will influence flow rate that might lead to shear stress and foaming	145
Bioburden levels (pre-filtration)	1	1	1	1	1	7	1	7	Bioburden level can affect process performance	197
Filter size (membrane area)	5	1	5	1	1	7	1	1	Filter size have to be adapted to the respective batch size	217
Filter contact time	1	1	1	1	1	7	1	1	Contamination due to Microbial growth through the membrane	137
Geometry	1	5	1	1	1	1	1	1	Shear stress caused by the geometry of the filter (fluid dynamics)	117
Batch size	1	1	1	1	1	1	1	1	No impact on CQAs expected	77
Filtration Temperature	1	1	1	1	1	1	1	1	No impact on CQAs expected	77
Particle burden (pre-filtration)	1	1	1	1	1	1	1	1	Impact on filter clogging, Vmax 75% and flow rate	77

Table 12.5 Criteria used for the definition of initial risk assessment values

Criticality Score	Criticality Level	Criteria
< 150	**Low**	All quality attributes for the given process parameter are ranked a 1
150 < score < 250	**Medium**	At least one parameter is ranked a 7 for an attribute with a criticality scale of 7
> 250	**High**	At least one parameter is ranked a 10 for an attribute with a criticality scale of 10

12.6 Design Space

In this section, the process leading to the definition of design space for the sterile filtration operations is described. All predefined process parameters assigned with criticality scores were superimposed with the available prior knowledge and fed into the definition of the design space. Based on the criticality scores assigned, four sets of experiments were identified to evaluate X-mAb product-specific effects on the performance of the filtration process, flow rate, V_{max} values, and impact from

Table 12.6 Design of experiments for V_{max} study using PVDF

Number	Pressure (bar)	Temperature (°C)
1	0.5	3
2	1	12
3	2.0	23

shear stress due to the filtration process on the CQAs (purity, visible and subvisible particles) of X-mAb. Additionally, the compatibility of both filters with X-mAb solution properties, specifically, the protein/excipient adsorption study to define flush volume prior to sterile filtration, particle shedding/depletion study and rinsing volume with WFI for bubble point measurements for pre- and postintegrity tests were verified. A simple fractional factorial design of experiments was followed to execute these studies and is summarized in Table 12.6 below. The X-mAb drug product-specific effects on the flow rate and the V_{max} values were evaluated at the higher end of the protein concentration specification, 57.5 mg/mL, representing the worst case.

12.6.1 Design Space Estimations

12.6.1.1 Estimation of V_{max} by Gradual Pore Plugging Models

Badminton Model (Badmington et al. 1995) The filter capacity of a filter membrane under constant pressure can be determined by V_{max} test methodology using a gradual pore clogging model. This methodology allows accurate selection of the sizing of filter membranes for filtrates of particular nature and batch size using relatively small quantities of material. By appropriate manipulation of the general equation for flow decay:

$$\frac{\mathrm{d}^2 t}{\mathrm{d}V^2} = k\left(\frac{\mathrm{d}t}{\mathrm{d}V}\right)^n$$

and using the parameter of ½ for n under gradual pore clogging, the fact that the system is under constant pressure, and the definition $Q = \mathrm{d}V/\mathrm{d}t$, the following equation may be derived:

$$\frac{t}{V} = \left(\frac{K_s}{2}\right)t + \frac{1}{Q_0}.$$

Furthermore, by performing a simple integration on the flow decay model, and setting $\mathrm{d}V/\mathrm{d}t = 0$ when $V = V_{max}$, it is possible to demonstrate that:

$$V_{\max} = \frac{2}{K_s}.$$

Therefore:

$$\frac{t}{V} = \frac{1}{V_{\max}} t + \frac{1}{Q_i} \tag{12.1}$$

Which is a linear equation of form $Y=mX+b$ that may be plotted out using only t and V data. In this case, $V_{\max}$, the parameter of interest, may be determined as the inverse of the slope of the line generated by plotting t/V as a function of time.

To determine what portion of the data is linear, an r^2 value for the linear Eq. 12.1 is calculated at each point along the t/V versus t plot—working backward from the terminal time point. The $V_{\max}$ results are obtained when a linear equation is fit to a minimum of 6 data points resulting in a correlation coefficient of ≥ 0.99. It is advisable to select data points with the largest slope as they represent the worst-case estimation of $V_{\max}$.

Brose Model (Brose et al. 2004) Brose adsorptive-sequestration filtration model (ASM) can also be used for estimation of filter characteristics. The mechanism of plugging membranes pores is the same in both pore-plugging models. Experimental data could be plotted using Eq. 12.2.

$$\frac{t}{V(t)} = \frac{1}{J_0 A_0 P} + \frac{K_{\text{Ads}}}{A_0} t \tag{12.2}$$

where J_0 is an initial pressure-normalized liquid flux before any filtration has occurred, A_0 is the tested membrane area, P is operating pressure, and K_{Ads} is an adsorptive filter-plugging constant, which is dependent on the characteristics of the solution being filtered. J_0 and K_{Ads} are calculated from the slope and intercept of Eq. 12.2. Similar to Badminton model, r^2 is calculated at each point along the t/V versus t plot (working backwards from the terminal time point) and J_0 and K_{Ads} are selected at the point where r^2 reaches the value not less than 0.999.

In addition to an ASM, Brose also proposed a sieve retention model (SRM), which describes the process of filter plugging due to formation of layer (or "cake") on the surface of the membrane rather than inside the pores of the membrane. In this process of filter plugging a boundary layer of increasing thickness is formed as a function of volume filtered, thus increasing resistance to flow. In order to evaluate and estimate the solution and filter characteristics for SRM model experimental data can be plotted using the following equation:

$$\frac{t}{V(t)} = \frac{1}{J_0 A_0 P} + \frac{K_{\text{Sieve}}}{2 J_0 A_0^2 P} V(t) \tag{12.3}$$

where, K_{Sieve} is a filter-plugging constant that is dependent on the characteristics of the solution being filtered. J_0 and K_{Ads} are calculated from the slope and intercept of Eq. 12.3. Similar to other models described above, r^2 is calculated at each point along the t/V versus V plot (working backward from the terminal time point) and J_0 and K_{Sieve} are selected at the point where r^2 reaches the value not less than 0.999.

The obtained filtration data can be analyzed using both ASM and SRM models. Brose suggested that if the plot of $t/V(t)$ versus t is linear, then the adsorptive-sequestration model is operative. If the plot of $t/V(t)$ versus $V(t)$ is linear, then the sieve-retention model describes the filtration process more adequately. In some cases neither correlation will give a straight line and it can be assumed that both mechanisms are operative to some extent. Brose et al. 2004) suggests using a conservative approach, which consists of utilizing whichever model under-predicts flux, resulting in an over-design of the system or over-sizing of the filter area.

12.6.1.2 Estimation of Volume of Filtered Solution Using Models

For the Badmington gradual pore-plugging model, the volume of filtered solution as function of time can be estimated using Eq. 12.4:

$$V(t) = \frac{A_0}{\frac{1}{V_{max}} + \frac{1}{Q_1 t}} \tag{12.4}$$

For the Brose adsorptive-sequestration model (similar to gradual pore plugging), the volume of filtered solution as function of time can be estimated using Eq. 12.5:

$$V(t) = \frac{J_0\ A_0\ Pt}{1 + J_0\ K_{Ads} Pt} \tag{12.5}$$

In comparison to Eqs. 12.4, 12.5 has a pressure component in the equation and advantage can be taken of it to assess the effect of pressure on the filtered volume.

For a filtration process that follows sieve-retention model, the volume of the filtered solution can be estimated based on the following equation:

$$V(t) = \frac{A_0}{K_{Sieve}} \left(\sqrt{1 + 2 J_0 K_{Sieve} Pt} - 1 \right) \tag{12.6}$$

12.6.1.3 Estimation of the Flow Rate during In-line Filtration

The flow rate as a function of time can be estimated using Eq. 12.7 derived from the Brose model (Brose et al. 2004):

$$Q(t) = \frac{A_0 J_0 P}{\left(1 + J_0 K_{\text{Ads}} P t\right)^2} \tag{12.7}$$

The effect of temperature on normalized flow rate J_0 can be estimated using Eq. 12.8 :

$$J_0(\mathrm{T}) = J_0\left(20^{\circ}\mathrm{C}\right)\frac{\eta\left(20^{\circ}\mathrm{C}\right)}{\eta(\mathrm{T})} \tag{12.8}$$

where, viscosity of X-mAb as a function of temperature η(T) can be estimated from Eq. 12.9

$$\eta(\mathrm{T}) = \exp\left(-7.8843 + \frac{5413.53}{1.987\mathrm{T}}\right) \tag{12.9}$$

12.6.1.4 Estimation of Filtration Time for the Bulk Filtration

Based on Brose model, the amount of time required filtering certain batch size V_B through the filter of area A at transmembrane pressure P can be estimated using Eq. 12.10:

$$t = \frac{V_B}{J_0 P\left(A - V_B K_{\text{Ads}}\right)} \tag{12.10}$$

J_0 and K_{Ads} can be estimated using Eq. 12.2 and data from small-scale experiments.

12.6.1.5 Estimation of Filtration Area

The filter surface area required for the filtration of a given batch of size, V_B, during given period of time, t, for adsorptive-sequestration model (10) can be estimated using the following Eq. 12.11:

$$A_{\min} = V_B\left(\frac{1}{J_0 t P} + K_{\text{Ads}}\right) \tag{12.11}$$

For sieve-retention model (Daniel et al. 2004), the minimum required filter surface area can be estimated using Eq. 12.12:

$$A_{\min} = \frac{V_B K_{\text{sieve}}}{\sqrt{1 + 2 J_0 K_{\text{Sieve}} P t} - 1} \tag{12.12}$$

For gradual pore-plugging model (Badmington et al. 1995), the minimum required filter surface area, A_{min}, can be estimated using Eqs. 12.13 (V_{max} based filter sizing) and 12.14 (flow rate based filter sizing):

$$A_{min} = \frac{V_B}{V_{80}} \tag{12.13}$$

where V_{80} is the filtered volume at the moment of filtration when flow rate reaches 20 % of its initial value.

$$A_{min} = \frac{V_B}{KQ_i t_{filtration}} \tag{12.14}$$

where $t_{filtration}$ is the desired maximum filtration time (normally considered as 1 h) and K is a flow decay coefficient, with an initial estimate of $K \approx 0.9$ for most filtration processes encountered.

12.6.1.6 Surface Area Safety Factor

To account for variability arising from filtration parameters (pressure, temperature) and filter characteristics (lot-to-lot variability), a surface area safety factor is normally used during process scale-up. Some vendors are currently using V_{75} as one of the criteria to size the filters on commercial scale. V_{75} can be estimated from Eq. 12.15 (Badmington et al. 1995)

$$V_{75} = V_{max}\left(1 - \sqrt{\frac{Q}{Q_i}}\right) \text{where } \frac{Q}{Q_i} = 25\ \% \tag{12.15}$$

or, solving for V_{75}/V_{max}

$$\frac{V_{75}}{V_{max}} = 0.5 \tag{12.16}$$

At that time, 50 % of filter area considered to be plugged or otherwise nonaccessible (Badmington et al. 1995), as shown in Eq. 12.16. The minimum surface area A_{min} for a given batch volume V_B is given by Eq. 12.17:

$$A_{min} = \frac{V_B}{V_{75}} \tag{12.17}$$

Thus, the recommended volume per surface area is one-half the calculated V_{max} parameter, or, by inversion, for a given volume, it is recommended that the surface area be increased by a factor of 2.

Determine allowable % *Plugging* to avoid operating in the region of flux or time where diminishing returns are apparent. The % *Plugging* is calculated by the following equation:

$$\%Plugging = \left(1 - \frac{Q}{Q_0}\right) \times 100\% = \left(1 - \frac{J}{J_0}\right) \times 100\%$$

Where, Q and J are the flow rate and flux at any point and Q_0 and J_0 are the initial flow rate and flux. The allowable % *Plugging* of 75% ($0.5V_{max}$) is suggested for commercial and 50% plugging level ($0.29V_{max}$) for clinical manufacturing since more variability is expected in clinical manufacturing.

12.6.2 Filter Capacity Determination (V_{max}) Experimental Studies

Approximately 0.7 kg of X-mAb was pooled into a stainless steel surge vessel. The pressure vessel was supplied with pressure by a regulated house nitrogen line set to 12 psi. The outlet of the pressure vessel was capped with a 47 mm test filter housing, holding a 0.22um PVDF test filter disc. A 2 L teflon bottle was placed on a 32 kg scale and tared as a receiving vessel. Prior to the start of the test, the tubing after the filter was sealed with a hemostat. The equipment was assembled and pressure was applied and allowed to equalize. The filter was vented, and immediately thereafter the hemostat was removed and measurements began. Cumulative weight values were recorded at 20-s intervals for 31 min. The interval was expanded to 1 min up through 23 min. When flow rate was observed to have dropped to under 50% of the initial rate (14.7 g/min versus approximately 4 kg/min), measurements were ended and the remainder of the bulk transferred into the final container.

Accumulated mass versus time data was plotted according to standard methodology (time/kg versus time), and a V_{max} value was derived. Plotted data and regression analysis with residuals are shown below in Figs. 12.2 and 12.3.

Plotting of residuals versus time shows a distinct pattern of a single arc crossing the 0 line in a roughly parabolic fashion, suggesting that there are other higher-order effects potentially unaccounted for in the model. An r^2 value for the linear model of 0.9991 indicates that the linear model fits the data well enough for extrapolation. In addition, increasing residuals with respect to time indicates a downward deflection of the flow decay curve, which will in turn lead to a smaller slope, and hence a higher value for the V_{max} (1/m). The V_{max} as obtained from earlier data will therefore represent a worst-case scenario.

As shown in the Fig. 12.2, the slope of the best-fit line is 1.2474. The inverse of the slope is used to calculate the V_{max} of the 47 mm test filter and is 0.801667 kg. The total effective filter area of a 47 mm test filter, due to 2 mm of occlusion by gasket material about the circumference, is:

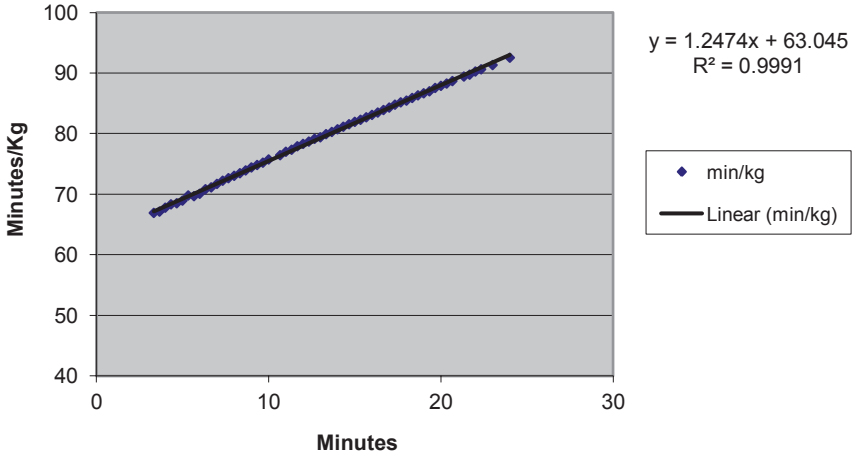

Fig. 12.2 Accumulated mass versus time

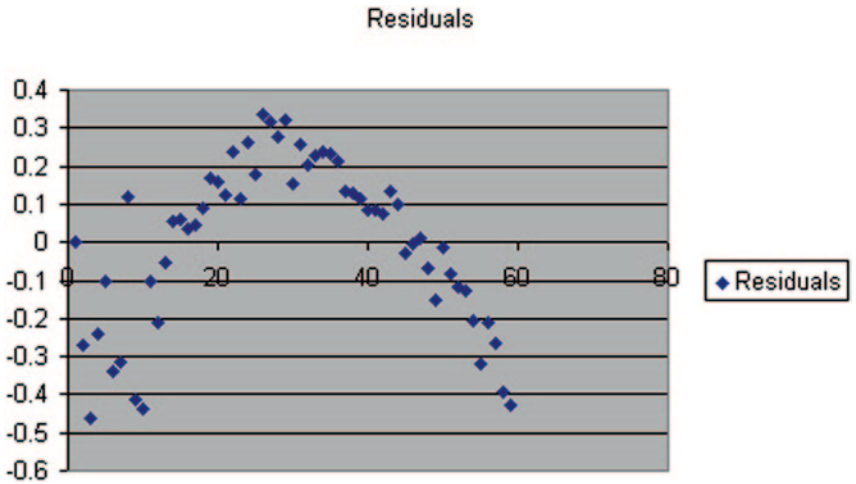

Fig. 12.3 Model residuals

$$\left(\left(\frac{0.047}{2}\right)-0.002\right)^2\times\pi = 0.00145\ m^2$$

Yielding a final V_{max} of: $\frac{0.801667\ kg}{0.00145\ m^2} = 552.87\frac{kg}{m^2}$.

Given a total membrane area of 0.18 m^2, and a total membrane area for the filter of 0.7 m^2, the V_{max} values for those filters are as follows:

$$552.87 \frac{\text{kg}}{\text{m}^2} \times 0.18 \text{ m}^2 = 99.52 \text{ kg}$$

$$552.87 \frac{\text{kg}}{\text{m}^2} \times 0.7 \text{ m}^2 = 387.00 \text{ kg}.$$

As the filter medium reduces the permeability of the filter and the flow rates drop off by the square of consumed filter capacity, it is typically not recommended to filter material in quantities more than 50 % the calculated V_{max}. At 50 % total capacity, flow rates are approximately 25 % that of initial, and quickly decrease to unacceptable levels thereafter. Therefore, for a filter of area 0.18 m^2, it was recommended to filter not more than 49.80 kg of X-mAb, and for a filter of 0.7 m^2 it was recommended to filter not more than 193.50 kg of X-mAb.

12.6.3 Filter Characteristics at Different Temperatures

As indicated in the target process both bulk and in-line filtration processes at the commercial site could be performed within the temperature range between 3 and 25 °C and pressure range between 7 and 14.5 psi. Since the flow rate during filtration is quite sensitive to the temperature, it was imperative to either perform V_{max} experiments or use scale-down validated model to estimate the flow rate and V_{max} values as a function of temperature range. Filtration experiments were performed as a function of temperature, as expected, filtration of the same amount of solution took much longer at 3 °C as compared to 12 °C and especially compared to 20 °C. For each temperature, at least three experiments were performed with fresh material. Data were plotted in the form of Eqs. 12.1 and 12.2 in order to estimate filtration coefficients. An illustration of a $t/V(t) = f(t)$ plot (gradual pore-plugging and adsorptive sequestration models) is depicted in Fig. 12.4. The filtration characteristics as a function of temperature were estimated from the slope and intercept of plot in Fig. 12.4 and are summarized in Table 12.7. Filter characteristics were chosen at the point where r^2 value was ≥ 0.999.

As it can be seen from the above table, the flow rate (J_0) decreases with the decrease in temperature, most likely due to increase in viscosity of the solution. The calculated data were compared to actual J_0 values at the temperatures and the differences were less than 5 % suggesting one can use J_0(20 °C) value along with Eqs. 12.8 and 12.9 to estimate $J_0(T)$ for any given temperature within the range from 3 to 20 °C. The experimental values of filtration characteristics summarized in the above table (Table 12.7) were used for the filter sizing at various temperatures.

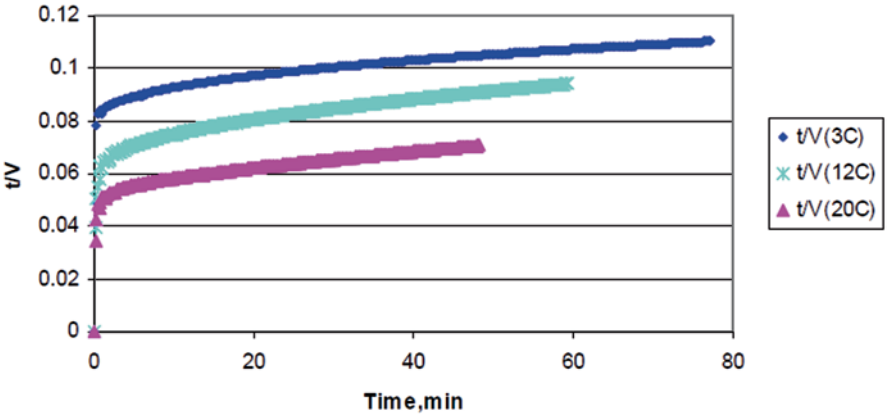

Fig. 12.4 Plot of $t/V=f(t)$ for X-mAb for various filtration temperatures. Filtration pressure was 10 psi

Table 12.7 Flow characteristics of 47-mm PVDF membrane estimated for the filtration of 57.5 mg/mL X-mAb. (10 psi, flow characteristics were estimated for the data set where $r^2 \geq 0.999$)

Temp (°C)	Adsorptive-sequestration model		Gradual pore plugging model	
	J_0 mL/min/psi/cm^2	K_{Ads} cm^2/mL	V_{max} L/m^2	Q_i L/m^2/h
20	0.1257	0.00356	2798	759
12	0.0990	0.00401	2385	589
3	0.0695	0.00289	3390	425

12.6.4 Bulk Filtration

It is likely that batch sizes of X-mAb drug product for fill and finish at commercial site could be in the range of 30–150 L The minimum recommended filter surface area (m^2) for the bulk filtration of different batch sizes of X-mAb were estimated and plotted as a function of temperature, pressure, and batch sizes using Eq. 12.11 (see Fig. 12.5).

Bulk filtration time (min) for the processing of various batch sizes of X-mAb was estimated as a function of temperature and pressure using Eq. 12.10 and flow characteristics from Table 12.1 (see Fig. 12.6). A surface area safety factor of 2 was taken into consideration during calculations. Calculations were performed for 10″ (0.69/2 m^2) Durapore filter.

The calculations indicate that the filtration time to filter 150 L of X-mAb at worst conditions (4 °C and 0.5 bar) could be as high as 95 min which is slightly higher than the normally desirable time of ≤ 1 h. If 95 min of time are not operationally feasible; option of either using two filters in parallel or increasing the filtration pressure could be explored.

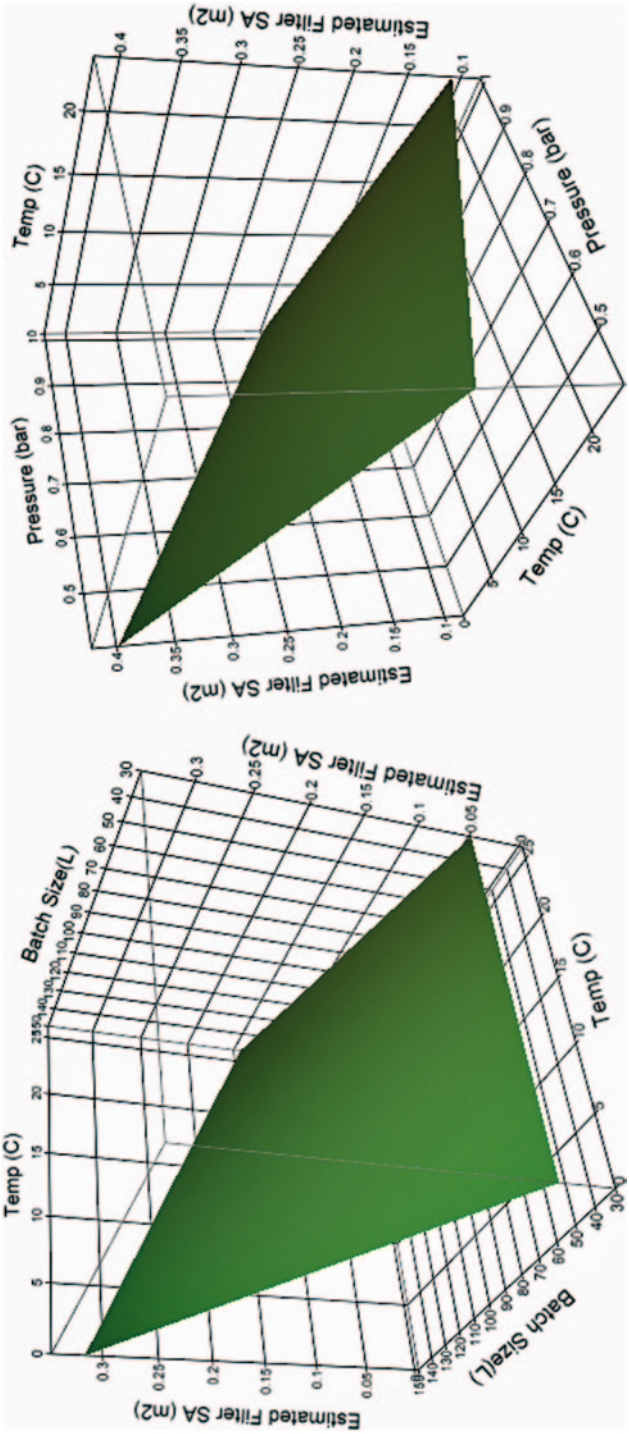

Fig. 12.5 Estimations of filter surface area as a function of batch size, temperature, and pressure

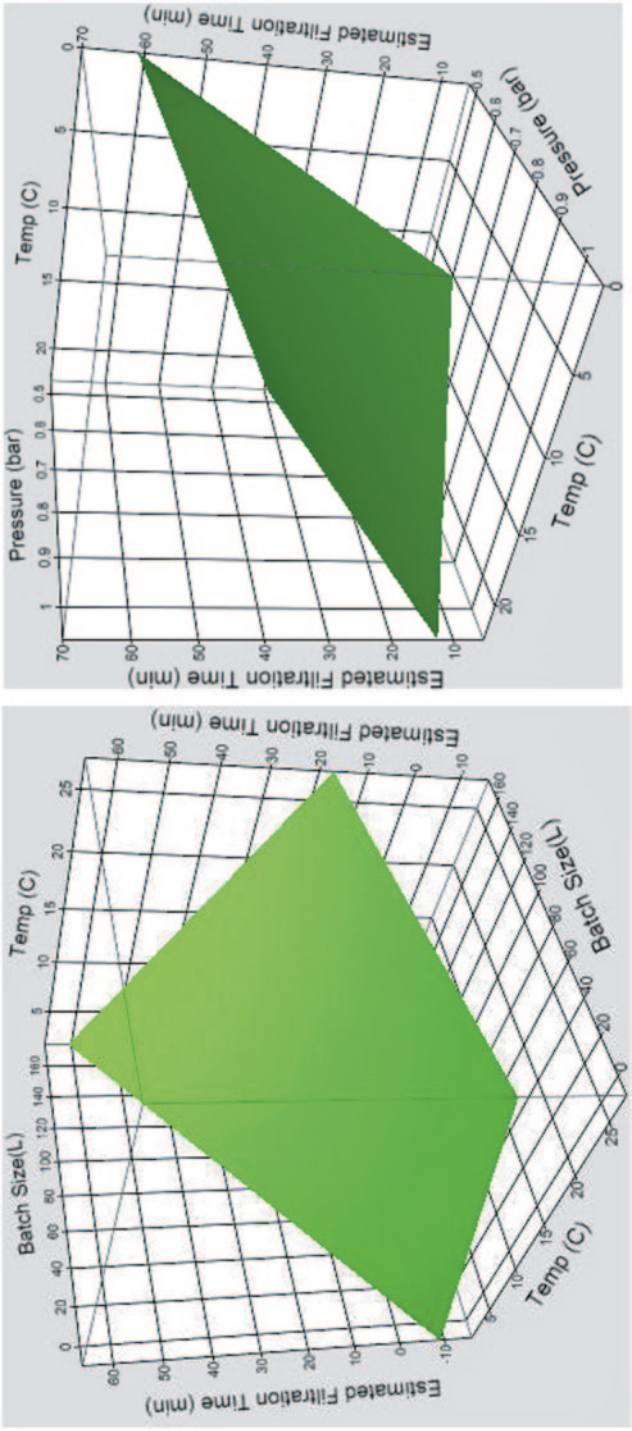

Fig. 12.6 Estimation of filtration time as a function of batch size, temperature, and pressure

12.6.5 In-Line Filtration (Sterile Filtration)

The commercial manufacturing process requires that the bulk drug substance (BDS) to be filled and finished be stored at 2–8 °C in the holding tanks. The BDS is transferred from the tank into the surge vessel of the filler through a filter at a constant pressure of 7 psi. One of the requirements of the sterile filtration process is that the filtration flow rate matches with the syringe-filling rate constantly throughout the filling process without any interruptions or delays. Based on the syringe-filling rate of 250 syringes/min/0.98 mL, a minimum filtration flow rate of 14.7 L/h was estimated. Since the flow rate constantly changes at constant pressure due to filter plugging, it is imperative that a filter of appropriate size be identified that maintains the flow rate above the minimum flow rate of 14 L/h all the times especially at the end of the filtration process. PVDF filters of various sizes were characterized and assessed for their maximum volume of DS they can filter under a safety factor of 2 at various temperatures while maintaining the flow rate above. Equations 12.5 and 12.7 , and the filter characteristics summarized in the Table 12.4 were used to determine the volume of filtered solution and the flow rate. The results are plotted and depicted in Figs. 12.7, 12.8 and 12.9.

Based on the calculations at worst conditions (4 °C and 0.5 bar) from the plotted data it was estimated that Millipak-200 filter can potentially filter a maximum of 12 L while 4″ and 5″ Durapore filters can filter 105 and 280 L, respectively, while maintaining the flow rate above the minimum required flow rate of 14.0 L/h. Depending upon the batch size any of these filters could be used. A 5″ Millipore 0.22 μm Durapore filter was recommended for in-line filtration of X-mAb for a batch size of 150 L within the temperature range of 4 to 20 °C providing some additional flexibility to go beyond 150 L.

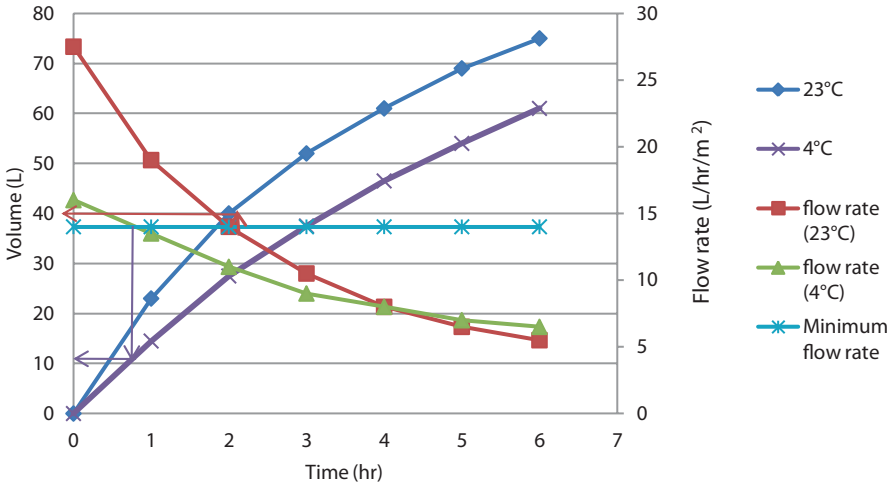

Fig. 12.7 Filtration of X-mAb through Millipak-200 filter unit (0.1 m^2) under a pressure of 7 psi and a safety factor of 2

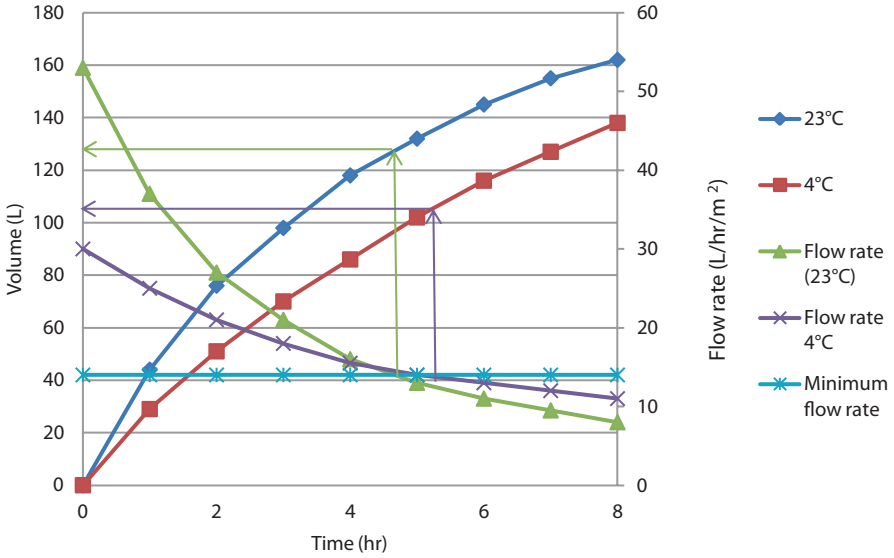

Fig. 12.8 Filtration of X-mAb through Millipore 4″ Durapore filter (0.19 m^2) under a pressure of 7 psi and a safety factor of 2

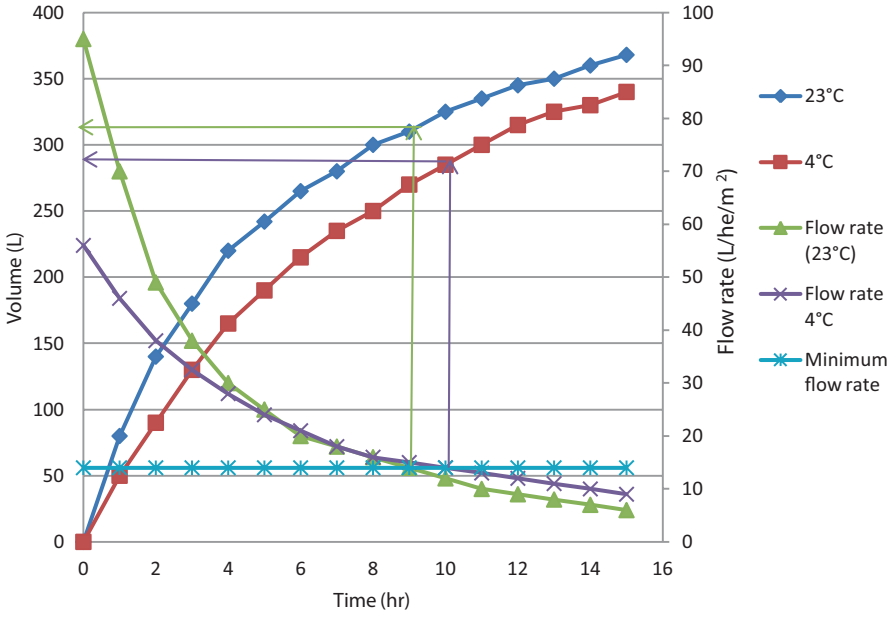

Fig. 12.9 Filtration of X-mAb through Millipore 5″ Durapore filter (0.35 m^2) under a pressure of 7 psi and a safety factor of 2

One filter configuration that conformed to the recommendation for in-line filtration was a Millipore 0.22 μm Durapore 5″ Opticap XL capsule filter.

The above results show that the PVDF filter lay within the design space covered by the platform approach (A-mAb, B-mAb, and C-mAb).

12.6.6 Product Quality Assessment: Impact of Multiple Filtrations on Product Quality

The X-mAb product was subjected to multiple filtrations (3×) replacing with new filter every time and samples were collected before and after filtration. Specific Polysorbate 80 test method and reverse phase high-performance liquid chromatography (HPLC) were employed to assess any losses due to adsorption. The stress impact from multiple filtrations on the protein integrity of X-mAb was also evaluated using stability indicating assays SE-HPLC and CEX-HPLC. The results are also summarized in Table 12.8. Comparison of test results before and after filtration indicates no loses due to adsorption and no adverse impact from multiple filtrations on the purity of X-mAb molecule.

The filtrate obtained after multiple filtrations was also analyzed for visible and subvisible particles to determine any particle shedding. HIAC was employed for determination of subvisible particles (≥10 μm and ≥25 μm). A certified inspector performed inspection for visible particles. No visible particles were observed and the subvisible particle count was well below the USP requirement.

Above test results confirm the findings of the platform work (prior knowledge) that after a flushing volume of 100 mL for PVDF filter configurations no change in formula composition or particle contamination can be observed. However, three options were identified and recommended as good manufacturing practice to manufacturing,

Table 12.8 SE-HPLC, CEX-HPLC, RP-HPLC, and PS 80 filtration testing results

Sample	SE-HPLC		CEX-HPLC			RP-HPLC	% PS 80 (w/v)
	% HMW peaks	% Main peaks	% Acidic peaks	% Main peaks	% Basic peaks	Conc. mg/ml	
Prefiltration	0.6	99.40	3.753	93.926	2.321	50.20	0.104
Post 3× filtration after 10 ml of filtration	0.70	99.30	3.812	93.749	2.438	49.06	0.101
Post 3× filtration after 20 ml of filtration	0.82	99.18	3.721	93.811	2.468	50.80	0.105
Post 3× filtration after 30 ml of filtration	0.57	99.43	3.767	93.777	2.456	51.05	0.110
Post 3× filtration after 40 ml of filtration	0.45	99.60	3.763	93.906	2.331	50.31	0.107

Option 1: Wait until the filler surge tank has been filled with X-mAb drug product to its capacity before starting the filling process to dilute/neutralize the loss effect.
Option 2: Recirculate X-mAb drug product through the filter back into the feed tank to ensure that if there is any binding, it is saturated before filling begins.
Option 3: Discard vials equivalent to 300–500 mL of the product.

12.7 Control Strategy

The control strategy for the given process parameters outlined in this case study for sterile filtration is covered below. The outcome of the studies executed in order to explore the design space allows the differentiation between CPP and key performance parameters (KPP) and general process parameters (GPP) as shown in Table 12.9. According to this designation the control strategy is defined to mitigate the criticality of the given parameters and feed in to the specification of the proven acceptable range (PAR) and the normal operating rang (NOR) for the process parameters at each unit operation listed in Table 12.10. Based on the level of control and the availability to mitigate the corresponding risk (Risk level≤200) CCPs will become WC4793 CPPs.

Table 12.9 Control strategy of process parameters for sterile filtration

No	Parameter	Designation (CPP/KPP/GPP)	Control Strategy
1	Preflushing volume of WFI	CPP	Follow specification of NOR and PAR for WFI preflush volume and document in the batch record
2	Filter integrity test (before and after filtration)	CPP	To follow the specifications and document in the batch record
3	Flushing (Prerun) volume of DP bulk solution	CPP	To follow the specifications of NOR and PAR volume of DP bulk solution and document in the batch record
4	Flow rate per membrane area	CPP	To follow filter specification and NOR and PAR for N_2 pressure and document in the batch record
5	Material of construction	CPP	Follow filter specification and document in the batch record
6	N_2 pressure	CPP	Follow specification of NOR and PAR for pressure and document in the batch record
7	Filter size (Filtration volume per unit membrane area based on Vmax 80%)	CPP	Follow specification based on batch size
8	Filtration temperature (RT)	GPP	Environmental controls

Table 12.10 Control strategy of process parameters of sterile filtration

Process parameter	Quality attribute	Process ranges studied	PAR	NOR	Target	Rationale for ranges
Preflushing volume of WFI	Vis. and SVP clarity	1–50/L	>10/L	>20/L	20/L	Based on particle shedding study
Filter integrity test (prior to filtration)	Vis. and SVP, clarity, endotoxins, sterility	Must pass minimum water-wetted bubble point as defined by vendor	Must pass minimum water-wetted bubble point as defined by vendor	Must pass minimum water-wetted bubble point as defined by vendor	Must pass minimum water-wetted bubble point as defined by vendor	In accordance with vendor specification
Batch size (filtration vol.)	No negative impact on CQAs expected	0.5–200 L	30−200 L	30–150 L	150 L	Based on V_{max75} study
N_2 pressure	Aggregation	0.5–2 bar	0.5–2 bar	0.5–1 bar	0.7 bar	X-mAb Drug product specific characterization studies
Filtration temperature	No impact on CQAs expected	4–23 °C	4–23 °C	4–23 °C	18 °C	X-mAb Drug product specific characterization studies
Filter size, bulk filtration (membrane area per filtration volume based on V_{max75} %	PS 80 content, bioburden, sterility					X-mAb Drug product specific characterization studies
Prerun flushing volume with DP bulk solution	Protein content, PS 80 content, Vis. particles SVP	0–1 L	0–1 L	0.3–0.5 L	0.3 L	X-mAb Drug product specific characterization studies
Duration filtration of bulk drug product (filter contact time)	Sterility	36 h	≤ 24 h	≤ 12 h	≤ 8 h	To eliminate bacteria growth in the membrane
Filter integrity test (postfiltration)	Sterility	Must pass minimum water-wetted bubble point as defined by vendor	Must pass minimum water-wetted bubble point as defined by vendor	Must pass minimum water-wetted bubble point as defined by vendor	Must pass minimum water-wetted bubble point as defined by vendor	Correlation pore size to bubble point by vendor

12.8 Final Risk Assessment

If a NOR/PAR has a wide range, there is less inherent risk that the range can be met by a process and therefore, has a lower risk value. A narrow range is more difficult to control and translates to a higher risk value. The control strategy can be considered as high/tight, medium, or low. A tight control of a system has less risk than one that is medium or low and therefore, has a lower risk. PP risk was ranked as CPP > KPP > GPP. Values assigned for each designation, the NOR/PAR width of parameter and control strategy level described in Table 12.11. From these values, a risk mitigation number can be calculated.

A risk mitigation number of ≤ 200 was considered mitigated. Values > 200 require additional mitigation strategies. This cut-off suggests that a:

- CPP with a narrow range for NOR/PAR must be controlled well to pass 200 (be considered mitigated)
- CPP with a wide range for NOR/PAR must be controlled well to medium to pass 200
- KPP with a narrow range for NOR/PAR must be controlled well to medium to pass 200 KPP with a wide range for NOR/PAR will always pass 200
- GPP with a narrow range for NOR/PAR will always pass 200
- GPP with a wide range for NOR/PAR will always pass 200

The final risk assessment verifies that the established control strategy is sufficient to mitigate the criticality of all process parameters and demonstrates control of the whole filtration process, see Table 12.12.

Table 12.11 Risk assessment values

Risk value	Designation	NOR/PAR width of parameter	Control strategy level
1	GPP		High
3		Wide	
5	KPP		Medium
7		Narrow	
9	CPP		Low
Risk mitigation number = designation × NOR/PAR width × control strategy level			

Table 12.12 Risk mitigation assessment for filtration process

Parameter	Designation CPP/KPP	Risk value	NOR/PAR width	Control strategy level	Risk mitigation (D × W × C)	Risk mitigated (y/n) (D × W × C ≤ 200)
Preflushing volume of WFI	CPP	9	3	1	27	Y
Filter integrity test (prior to filtration)	CPP	9	7	1	63	Y
Batch size (filtration vol.)	KPP	5	3	1	15	Y
N_2 pressure	KPP	5	7	1	35	Y
Filtration temperature	GPP	1	7	1	7	Y
Filter size (membrane area per filtration volume based on V_{max80}%)	CPP	7	7	1	49	Y
Prerun flushing volume with DP bulk solution	CPP	9	3	1	27	Y
Duration of filtration of bulk drug product (filter contact time)	KPP	5	3	1	15	Y
Filter integrity test (postfiltration)	CPP	9	7	1	63	Y

12.9 Process Demonstration/Verification

Based on the thorough characterization of the filtration operations, the high level of prior knowledge, the availability of valid scaledown models coupled with mathematical models that can be used to define filter and parameter specifications according to the desired batch size demonstrate a scientific understanding of the filtration process, which allows to obviate a formal process demonstration/verification and perform only a continuous process verification/monitoring at full scale.

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Chapter 13
Application of QbD Elements in the Development and Scale-up of Commercial Filling Process

Feroz Jameel, Cenk Undey, Paul M. Kovach and Jart Tanglertpaibul

13.1 Introduction

The dogma that says the "Process makes the Product" is true. The formulated drug substance is processed through several unit operations of manufacturing before it is made into drug product to be used by the patient or healthcare personnel. Filling process is one of the critical unit operations of manufacturing and is drawing attention and scrutiny because of its potential to induce stress to the protein molecule manifesting into formation of aggregates and/or particles (Cromwell et al. 2006; Vázquez-Rey et al. 2011; Meireles et al. 1991). Additionally, the filling process is expected to provide accurate dosing (fill weight) with no splashes and no drips.

To ensure product quality and elegance, it is imperative that one thoroughly understand the principles on which the various fillers function, their operating conditions and parameters that can potentially affect the product quality attributes and elegance of the drug product. Quality by Design (QbD) approach can be very useful in the development of a filling process for shear-sensitive protein products with wide range of viscosities and temperatures as it enables product and process understanding based on sound science and quality risk management (ICH Q8 (R2); ICH Q9; ICH Q10; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Center for Veterinary Medicine (CVM) Office of Regulatory Affairs (ORA), Pharmaceutical CGMPs 2006; Website www.ich.

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org,Quality-Guidelines-menu,under Q8, Q9 and Q10). The elements that comprise quality by design are (1) quality target product profile (QTPP), (2) prior knowledge, (3) critical quality attributes (CQAs), (3) risk assessment: linking material attributes and process parameters to drug product CQAs, (4) design space, (5) control strategy, and (6) product life cycle management and continual improvement.

In this chapter, it will be illustrated through a mock case study the application of various elements of QbD in the development and transfer of a commercial filling process of an X-mAb for prefilled syringe (PFS) presentation.

13.2 Definition of Target Filling Process

Syringe barrels and needle assemblies are received presterilized in tubs, ready to use. Syringe plungers are obtained rinsed, siliconized, and presterilized from vendor, ready to use. A target fill of 1.07 g with an alert limit of ±0.02 and an action limit of ±0.03 g will be used for the syringe fills. The equipment set-up includes the assembly and installation of the 2 cc pump, pump control vale, 14 ga nozzle, and silicone tubing. Fill speed and drawback is adjusted after product has been pumped through and air has been removed from the lines. Fill volume target will be adjusted and confirmed using a balance and will be based on fill weight. Once the equipment set-up is complete, a batch in the range of 100–150 L corresponding to ~100,000–150,000 syringes will be filled in a single mode and fill weight checks ($n=3$) will be performed after every 30 syringes.

Typically a filing speed of 25 syringes/minute/nozzle is employed at the target filling line facility and, with fillers of 10 head rotary piston pump and 16 pump heads for peristaltic pump, a batch of 150 L of X-mAb formulated drug product will be filled in 10 hours by rotary piston and approximately 6 hours by peristaltic pump filler. This processing time is within the limit of 36 hours fill duration validated by media fill challenge. During the fill, the formulation will be observed for dripping, binding, foaming, and speed limitations and any observations will be recorded. After completion of the fill, plungers will be placed in the syringe barrels using an in-line HYPAK autoclavable stopper placement unit (ASPU).

The drug product PFS will be subjected to a 100% manual inspection. Manual inspection involves visual inspection by a certified inspector. The certified inspector will inspect for a limited subset of defects such as visible particles, color and clarity of the solution. After completion of manual inspection the PFS will be transferred from the tubs to Rando trays. The syringes that pass the inspection will be labeled, packaged, and stored at 2–8 °C.

Process Requirements Since the early development work has exhibited product sensitivity to shear stresses, it is critical that the right filler and filling parameters be identified that do not impart shear stresses and prevents the formation of aggregates or particulates. Additionally, besides fill weight accuracy ($Cp_k \geq 1.4$ and fill weight with tolerances), the filler and the filling process should not cause any dripping or drying of the product inside the pump and outside the nozzle.

Business Strategies/Requirements To meet the supply/market demand, more than one manufacturing site is often qualified and utilized pre- and/or post launch. Under those situations it is very likely that different pumping mechanisms (filling technologies) may exists at those sites and the product may have to be filled using more than one filling technology. Hence, it is required or expected that the product be evaluated against various filling technologies, and processes be developed/characterized and design spaces for various filling technologies be kept ready to be implemented as needed. Historically, in the case of syringe filling, either rotary piston or time pressure filling systems are often utilized because of their suitability to meet syringe filling expectations/requirements and offer significant advantages in manufacturing precision and accuracy, increased throughput and reduction of equipment related downtime (Sethuraman et al. (2010)). However, within the time–pressure technology Innova filler could serve as a worst case compared to Bosch–Stroebel due to the absence of orifice and wider internal diameter of the nozzle than the orifice.

In time/pressure filler designs the product exits from intermediate vessel through extremely narrow orifices exposing product to higher shear rates and different air–liquid surface interactions compared to rotary piston fillers. Additionally, the time–pressure has Teflon tubing in its fluid path and product gets exposed to it while the Teflon is not a material of product contact for rotary piston fillers.

Lately, peristaltic pump have proven to be the best choice for protein products that are sensitive to shear stresses and tend to form particles (Kiese et al. 2008; Gomme et al. 2006). These fillers operate through different mechanisms/principles and they exert different nature of stresses upon the drug product. It will be misleading to consider one as a worst case over the other and vice versa. Hence, it is imperative and prudent to characterize all types of filling technologies independently/separately.

13.3 Prior Knowledge

13.3.1 Literature Review

This section reviews the various types of fillers their underlying principles, capabilities and limitations, and the process parameters that can impact product quality. Filling technologies include positive-displacement pumps and time–pressure, peristaltic pumps, and mass flow pumps. Typically, selection of the type of system to be used for filling is based on solution properties, manufacturing needs, and availability of equipment at the manufacturing site. Various filler types and their attributes are summarized in Table 13.1.

Piston Pump Fillers These pistons are air-operated, self-priming, positive displacement type pumps designed to meter precise quantities of liquids with a high degree of accuracy (+0.5%), or better, depending on product. The volume to be dispensed is preset by adjusting the micrometer volume control. On the downstroke

Table 13.1 Attributes of various filling pumps

Pump type	Product contacting surface	Mechanical function	Driving force	Control	Fill accuracy (% within target)	Potential product impact	Product's physical properties sensitive to temperature changes	Filling v olume >1 mL	Filling volume <1 mL	Need easy access to parts for CIP/SIP
Rotary piston[b]	Stainless steel 316	Rotation and stroking	Piston	Stroke length	0.5	Shearing by piston	++	++	+	−
Rolling diaphragm[b]	Stainless steel 316, rubber	Stroking	Diaphragm	Stroke length	0.5	Minimum	++	++	+	−
Peristaltic[a]	Silicon tubing	Rolling and compression	Roller	Rota-tion speed	1.0	Minimum	+	+	−	+
Time pressure[a]	Silicon and Teflon tubing	Pinch valve	Pressure	Time	0.5	Minimum	−	++	++	++

++ = very suitable to use, + = appropriate to use, − = not preferred to use

[a] Reference: Pharmaceutical filling system overview by Optima-Pharma 02/2008

[b] Based on historic knowledge.

of the liquid piston a vacuum is created in the liquid chamber which opens the inlet check valve diaphragm and holds the outlet check diaphragm closed. This operation permits the liquid chamber to fill itself (self priming). On the upstroke the liquid in the chamber is pressurized by the piston movement. This opens the outlet check valve diaphragm and holds the inlet check valve diaphragm closed. This action discharges the liquid out of the pump into a system. The advantage of this system is that it causes lower shear stress on the product but involve number of product contact parts and difficulty in cleaning and sterilizing the system.

Rotary Piston Pumps (Volumetric) It is a commonly used filling mechanism. It consists of a piston and cylinder requiring matched sets of piston and cylinder due to the tight clearances between the two. The pump cylinder consists of two ports, an inlet and an outlet port and the piston is designed to open and close the inlet and outlet ports by rotation as the pump cylinder moves vertically up and down. As the piston rotates and the cylinder moves in the downward direction, it provides an opening for liquid to enter into the cylinder through the inlet port. The piston then rotates, and as the cylinder moves in the upward direction, the liquid is dispensed out of the pump through the outlet port toward the filling needles. A control valve is integrated into the mechanism of the pump that controls the amount of liquid that flows in and out of the pump and, one of the advantages of this system is that it is capable of dispensing consistently and with precise fill accuracy extremely small fill volumes at high speeds. However, few disadvantages of this rotary pump system are that (1) the product itself serves as the lubricant for the pump action during operation. (2) As there is continuous contact between moving parts on the pump and the product, over the period of time coating materials and/or stainless steel material may shed from the pump surface and cause either heterogeneous particle-nucleated protein aggregation (Bee et al. 2009). (3) There is high potential of significant shear generated at the piston–cylinder interface due to the small clearance of 0.006 mm between them. The shear forces generated by the motion of the pump may unfold the protein resulting in adsorption onto the pump surface, eventually causing stability issues for products. For protein molecules that are shear sensitive it is highly recommended to thoroughly evaluate the impact of this filling technology before used for commercial filling.

Peristaltic Pump Systems These systems were originally designed for fluid transfer rather than high speed, accurate filling. The system is a motor-driven mechanical roller that applies pressure on flexible tubing, which performs both the suction and the compression of the liquid to be dispensed. The tube's restitution after the squeeze creates a vacuum, which draws more fluid into the tube, creating a gentle pumping action with minimal damage to the product inside the tube, particularly when compared to positive-displacement pump systems.

Mechanical setup has been made more repeatable by changing the dynamics of how the elastomeric tubing is pinched on the unit. As noted above, this type of filling offers complete disposability, with virtually no chance of product cross-contamination, plus fill weight is temperature-independent. Disadvantages include a limited product viscosity range, tubing relaxation, and a filling precision somewhat less than that of rotary pumps.

Time/Pressure Time-over-pressure (TP) filling technology utilizes the pressurized product tank and pinch valves to open and close silicone tubing between the tank and filling needles and their ability to control the product tank headspace pressure and temperature has made these systems more accurate than pump systems. In these systems the product is driven by over pressurization using filtered gas (either clean nitrogen or compressed air) over a defined period of time and a pumping mechanism is not involved. The fill accuracy is dependent on the temperature and viscosity of the product, manifold pressure, flow time, filling needle diameter, and flow orifice diameter. The holding tank and product manifold are maintained at a constant pressure and temperature. Pressure within the system is maintained in part by only permitting product to exit the manifold by way of extremely small orifices which have fluid flow controlled by servo motors or pinch valves. The orifice is connected by hoses to the filling needles. Fast-acting stepper or servo motors control the action of the pinch valves which regulate flow of product through the line for the appropriate amount of time based off the programmed fill target weight, fill range, product density, and flow rate. Fill control is regulated to reflect changes in product temperature through use of an integrated PLC.

Advantages of a TP system include lack of any mechanical moving parts in the product stream so the product does not come into contact with parts that can potentially shed foreign particles. Utilization of steppor or servo motor controls, PLCs, and algorithms allow constant adjustment of fill control based on temperature and pressure parameters that are monitored and corrected in real time. This results in higher accuracy than alternatives such as positive displacement pumps. Finally, there are fewer parts to be cleaned and sterilized. There are two major disadvantages of TP technology. It utilizes a small diameter orifice to control filling accuracy. This orifice restricts the product flow that can result in the product experiencing high shear for a brief duration of time while the product is being transferred through the orifice to the individual filling lines. This high shear may be deleterious to protein products (Sethuraman et al.). Hence, impact of shear on the product during filling using the TP system needs to be evaluated as part of the technology evaluation process. Additionally, TP systems do take more time to tune in at the start of a fill, and require control and/or compensation for product temperature as this affects product flow properties.

13.3.2 Mitigation of Shear Rates

The drug product experiences substantial shear during flow through nozzle and tubing, which is maximum at highest line speed for higher fill volume. For shear sensitive products: The shear exposure time, or duration is directly proportional to the length of tubing and fill rate. Therefore, wherever possible the smallest practicable length and largest practicable inner diameter is recommended for product supply lines to minimize shear exposure for shear sensitive products.

For shear sensitive products: To minimize the shear impact on shear sensitive drug products, it is recommended to fill with largest practicable orifice diameter (for TP filler) at slowest practicable fill speed or use highest practicable clearance between cylinder and the piston (for RP filler) at the slowest possible fill speed.

13.3.3 Additional Filling Considerations and Product Quality

Filling processes can impact product quality by introducing physical stresses such as shear, friction, and cavitation (Thomas and Geer 2011; Neumaier 2000; Van Reis and Zydney 2007). Other detrimental factors include temperature generated in the process of filling, foaming, and contact with filling system materials, including processing aids such as silicone oil. Certain pumps may shed extrinsic particles that may lead to heterogeneous nucleation-induced aggregation (Tyagi et al. 2009). These various pumping mechanisms produce different levels of mechanical stresses on the protein solutions, and each of these stresses may lead to antibody denaturation and subsequent aggregation and/or formation of particulates.

Aggregates can induce immune response impacting the efficacy of the drug product and even the endogenous version of the therapeutic protein. Aggregates could be soluble/insoluble, covalent/noncovalent, reversible/irreversible, and native/denatured. Aggregates with native like structure, are most likely to be the most potent in eliciting an immune response.

Nayak et al. (Nayak et al. 2011a, b) in their work utilized microflow imaging, size-exclusion chromatography (SEC), and turbidimetry to quantify subvisible particles, aggregation, and opalescence, respectively. The filling process was performed using several commonly used filling systems, including rotary piston pump, rolling diaphragm pump, peristaltic pump, and time–pressure filler. They found that rolling diaphragm pump, peristaltic pump, and time–pressure filler generated notably less protein subvisible particles than the rotary piston pump, although no change in aggregate content by SEC was observed by any pump.

Similar study by Tyagi et al. (Tyagi et al. 2009) investigated factors associated with vial filling with a positive displacement rotary piston pump leading to formation of protein particles in a formulation of an IgG. They hypothesized that nanoparticles shed from the pump's solution-contact surfaces nucleated protein aggregation and particle formation.

The other concern with the piston pump is that the protein solution serves as a lubricant to the piston during the operation and potential shear generated at the piston–cylinder interface due to the tight clearance of 0.006 mm between the piston and the pump cylinder. Hence, there is a potential risk of damaging the drug product while handling highly shear-sensitive protein molecules (Cromwell et al. 2006).

13.3.4 Product Knowledge

The product X-mAb is an IgG1 mAb having a molecular weight of 145 kDa (including 3 kDa of glycosylation) with an isoelectric point (pI) of 8.7. It is formulated at protein concentration of 50 mg/mL in 10 mM sodium phosphate with sucrose, pH 7.0.

Knowledge and experience with clinical manufacturing of this product indicates its sensitiveness to shear stresses during processing resulting in the formation of aggregates/particulates. Since it is a high protein concentration product formulated with sucrose, some filling difficulties relating to dripping and accuracy of fill weights were encountered that were attributed to high viscosity of this product.

13.3.5 Process Specific Knowledge

Historically, rotary piston pump and time–pressure filler have been interchangeably used in the production of mAb drug products. Both have shown success at commercial scales with some limitations but required thorough characterization and optimization. During the early stages of the product development of X-mAb, studies were conducted with rotary piston pump with single pump head simulating commercial batch sizes to evaluate the performance of the filler and the product quality attributes. Performance assessments included ability of the pump to fill commercial batch sizes without process deviations (fill weight deviations), dripping, and drying of the product. The drying of the product inside and/or outside of the pump results in pump seizures, manifested with significantly reduced deliverable volume, piston movement and particle generation in the pump from either the pump itself or from the product. In-process samples of the drug product were drawn and analyzed for visible and subvisible particles using HIAC. The effect of the magnitude of clearance between the piston and cylinder were also evaluated (2 cc pumps (for syringe fill). The 2 cc pumps test included small and large clearances of the pumps available, thus bracketing the clearance ranges). Although, normally the drug product undergoes a single pass through the pump head, few transfers were made to understand the propensity of the drug product to repeated shears. The drug product was found to be sensitive to repeated shear.

Additionally, previous experience with high-concentration mAb formulations suggests consideration of the effect of the viscosity on the filling operation. The formulations were found to drip from the nozzle of the filler resulting in inaccurate fill volumes. The ability to accurately fill the viscous solutions may be compromised if appropriate process optimization and characterization is not performed.

Previous experience also indicated that the filling of high-concentrated mAb formulations containing high percentages of sugars could potentially lead to pump blockage resulting in the stop of the filling machine if temperature, humidity, laminar flow, and filling process interruptions (downtime) are not controlled and monitored.

Historically, a fixed constant fill weight is applied/used regardless whether the protein concentration values of the drug substance to be filled are at the upper end or lower end of the protein concentration specifications. This puts into the risk of either being over or under the label claim resulting in wastage of expensive protein or failing to meet the drug product protein content specifications. In order to mitigate this risk a thorough characterization of the fill weight as a function of protein concentration and creation of a design space for filling process would be beneficial.

During the formulation design the impact of filling process/equipment on the robustness of the formulation or compatibility of the formulation with the filling equipment was assessed with a similar mAb and the results are described below.

13.3.6 Initial Screening of Filler Pumps

Particulate matters or aggregates may be generated during filling. The following case study discusses two formulated drug products filled with different filling technologies.

- Monoclonal antibody A—20 mg/mL in citrate buffer, pH 6.5
- Monoclonal antibody B—10 mg/mL in citrate buffer, pH 6.0

Concentrations of polysorbate 80 in the formulations varied from 0 to 0.01 %. Each solution was filled using rolling diaphragm, peristaltic and rotary piston pumps. Samples were analyzed for particulate matter using a HIAC particle size analyzer.

Figures 13.1, 13.2, 13.3, and 13.4 show cumulative particle count per millileter of different sizes of product A with levels of surfactant polysorbate 80 in the formulation.

These data suggest that particulate matter generation in formulated product depends on the formulation, filling technology, and intrinsic nature of the protein. The three pump types have minimal impact on particulate matters in product A when polysorbate 80 is present in the formulation; however, rolling diaphragm and rotary piston pumps generate more particulate matters compared to peristaltic filling in product B at a similar concentration of polysorbate 80 in the formulation (Figs. 13.5, 13.6, 13.7, and 13.8).

Figures 13.5, 13.6, 13.7, and 13.8 show cumulative particle count per millileter of different sizes of product B with levels of surfactant polysorbate 80 in the formulation.

Depending on particle size, further characterization of the filling operation may determine which operating parameters for a specific filling technology have the

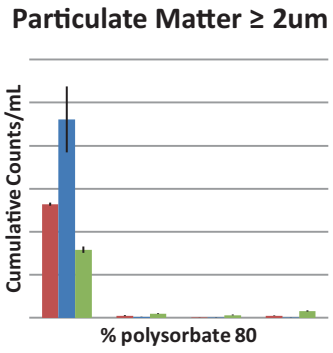

Fig. 13.1 Particulate ≥2 microns in product A

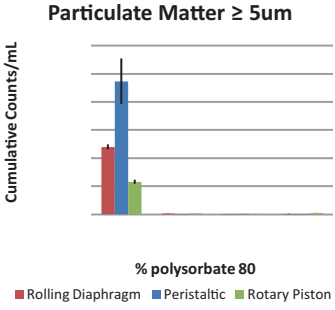

Fig. 13.2 Particulate ≥5 microns in product A

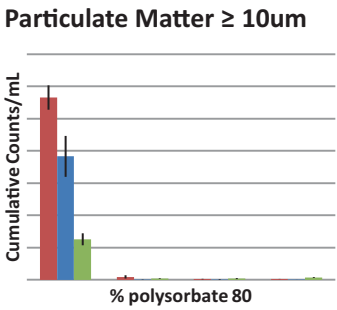

Fig. 13.3 Particulate ≥10 microns in product A

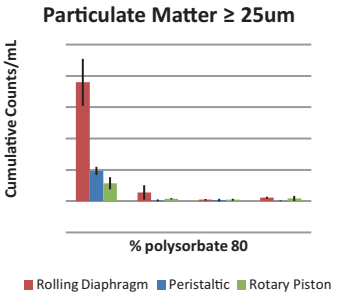

Fig. 13.4 Particulate ≥25 microns in product A

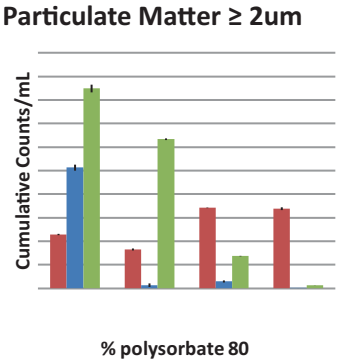

Fig. 13.5 Particulate ≥2 microns in product B

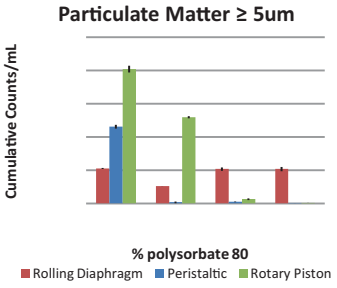

Fig. 13.6 Particulate ≥5 microns in product B

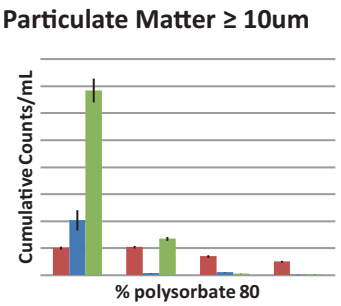

Fig. 13.7 Particulate ≥10 microns in product B

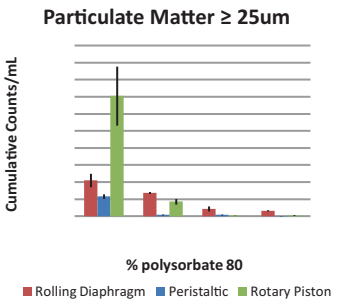

Fig. 13.8 Particulate ≥25 microns in product B

greatest impact on the particulate matters generation at a fixed concentration of polysorbate 80 that is the subject of the following sections.

13.4 Critical Quality Attributes (CQAs)

All the quality attributes of the product were listed and risk assessment was performed using cause-effect matrix. The CQAs were identified based on the risk priority number (RPN) in relation with the target product profile (TPP), for details see chapters 3 and 21. The CQAs of this product comprises color, aggregation, fragmentation, visible particulates, subvisible particles, oxidation, charge heterogeneity, solubility, and viscosity. However, only three quality attributes (aggregation, visible particles, and subvisible particles) were chosen to exemplify the construction of the design space.

13.5 Initial Risk Assessment (Ranking) and Critical Process Parameters (CPPs) Determination

To develop a filling process that is robust, reproducibly operates within the established "Design Space" and delivers product that meets CQAs, a thorough understanding of the relationship between the operating parameters, equipment design, and perfor-

mance parameters/output attributes is required. An initial list of operating parameters that could potentially impact the consistency and performance of the process leading to the impact on the product quality attributes (e.g., aggregates, particulates, etc.) was created and ranked based on a risk ranking and filtering (RRF) tool that assesses each process parameter for its main effect and potential interaction effects with other process parameters. Main effect and interaction effect impact ranks are multiplied to generate an overall severity score, and the severity score determines the type of characterization studies that need to be performed (i.e., univariate or multivariate). The risk assessment/analysis considered process capability and prior knowledge accumulated from the laboratory, nonclinical, clinical, and commercial manufacturing experience relating to impact of fill parameters on similar mAb product quality attributes.

For illustration, the following Tables 13.4 lists all the filling process parameters that can potentially impact the drug product quality attributes along with the RPNs. Process parameters that scored severity ≥ 8 or RPN > 72 were categorized as CPPs and the parameters that scored RPN < 72 were categorized as key process parameters (KPPs).

The results from this initial risk assessment were then used to guide the process development and characterization studies.

The scoring criteria are summarized in Table 13.2. Definitions for the relative impact descriptions and ranking are provided in Table 13.3.

Effect is considered for variation of parameter across a proposed design space range.

The resulting list of recommended process characterization studies based on this type of risk assessment and the ranges of various CPPs studied in a design

Table 13.2 Scoring criteria for risk ranking

Severity score	Experimental strategy
≥ 32	Multivariate study
8–16	Multivariate, or univariate with justification
4	Univariate acceptable
≤ 2	No additional study required

Table 13.3 Definition of main effect impact and scoring

Impact description	Definition	Score
No impact	Effect causes variation in process output which is not expected to be detectable (e.g., no effect or within assay variability)	2
Minor impact	Effect causes variation in process output which is expected to be within acceptable range	4
Major impact	Effect causes variation in process output which is expected to be outside acceptable range (can be near edge of failure)	8

Table 13.4 Risk ranking study for the piston pump and peristaltic pump filler process parameters

Process parameter	Proposed design space range		Main effect score	Rationale for (*M*)	Interaction score	Rationale for (*I*)	Severity score	Potential interaction parameters	Recommended characterization studies
	Low	High	(*M*)	Main effect	(*I*)	Interaction effect	(*M*×*I*)		
Filtered bulk hold time	24 h	1 week	*4*	Microbial contamination, high bioburden, Proteolytic bulk degradation	*2*				Univariate study with temperature
Pump speed/ head (spm)	10	40	8	Shear effects and foaming due to air interaction may cause aggregation	4	Other parameters may exacerbate foaming and dripping effects	*32*	Temperature, fill volume, nozzle position	Multivariate study with fill temperature, nozzle diameter, and nozzle position
Fill temperature (°C)	2	23	8	X-mAb has good stability even at RT but low temp could impact filling process due to high viscosity	4	May have additive effect, dripping and drying effects at the nozzle and inside the pump	32	Pump speed	See pump speed study
Nozzle diameter (mm)	1	3	4	Diameter affects jetting of solution leaving nozzle	4	May have additive effect	16	Pump speed	See pump speed study
Nozzle position (mm)	0.5	2.5	4	Height affects amount of air interaction	4	May have additive effect	16	Pump speed, nozzle diameter	See pump speed study
Fill weight settings (target fill weight)	1.04	1.1		Under/overdose					Will be performed as part of engineering run at scale

Table 13.4 (continued)

Process parameter	Proposed design space range		Main effect score	Rationale for (*M*)	Interaction score	Rationale for (*I*)	Severity score	Potential interaction parameters	Recommended characterization studies
Batch size (L)	40	200	8	Volume affects number of pump strokes. Product in between piston and wall may be over stressed leading to aggregation	4	May have additive effect	*32*	Pump speed	Multivariate study with pump speed and number of strokes per pump head
Pump speed/ head (RPM)	50	250	8	Shear effects and foaming/splashing. Air interface interaction may cause aggregation	4	Other parameters may exacerbate foaming and dripping effects	*32*	Acceleration, reverse and delay time	Multivariate study with acceleration, reverse and delay time
Acceleration	50	250	8	Shear effects that could cause stress leading to aggregation	4	Other parameters may exacerbate foaming and dripping effects	*32*	Pump speed reverse and delay time	Multivariate study with pump speed, reverse and delay time
Delay time between the fills (s)	1	4	8		4	May have additive effect	8	Pump speed	See pump speed study
Reverse (back suction)	0	5	4	Dripping	4	May have additive effect	16	No relation to other parameters	Univariate study
Tubing/needle diameter (ID in mm)	0.6	1.6	8	Fill weight accuracy	4	May have additive effect	32	Pump speed, acceleration, reverse	Multivariate study with pump speed, reverse and acceleration

Table 13.4 (continued)

Process parameter	Proposed design space range		Main effect score	Rationale for (*M*)	Interaction score	Rationale for (*I*)	Severity score	Potential interaction parameters	Recommended characterization studies
Batch size (L)	40	200	4	Continuous use of tubing can cause tubing relaxation resulting in fill wt inaccuracy	4	May not have additive effect	*16*	Pump speed	Univariate study with pump speed, reverse, needle size and acceleration
Product temperature (°C)	2	23	8	Fill weight accuracy, dripping and drying	4	May not have additive effect with needle size, speed and reverse	*16*	with needle size, speed and reverse	Univariate study with pump speed, reverse, needle size and acceleration

of experiments (DOE) are summarized for piston pump and peristaltic pump as examples in the Table 13.4.

Since the extent of knowledge may vary depending upon the stage of development of the product, this assessment was reevaluated throughout the development as more safety and efficacy information was gained.

Once experience and understanding/knowledge gained after manufacturing of few X-mAb commercial batches at full scale, the risk assessment will be reevaluated to finalize the control strategy.

13.6 Design Space (Process Characterization)

The ultimate goal of these studies was to build a knowledge space for the filling unit operation, hence, to define an acceptable design space and a control space. Outcome from these studies will demonstrate understanding of the filling process; help address typical process optimization needs in the future and address nonconformances during commercial manufacturing (Table 13.5).

Table 13.5 Modular process characterization study

Development activities	Study objective	Processing parameters
Pumping study	To evaluate effects of the piston pumping mechanism by single pump head, simulating commercial batch sizes to evaluate the performance of the filler and the product quality attributes	Pump speed
		Number of piston strokes
Filling study	To evaluate the effect of filling parameters by varying pumping speed along with its nozzle ID and position using sterile, filtered X-mAb	Pump speed
		Nozzle ID
		Nozzle position
Environment conditions study	To evaluate the effect of filling room/isolator climate conditions on the performance of the filler	Temperature
		Relative humidity
		Air flow rate
		Duration of interruptions
Engineering runs	To confirm the results of filling X-mAb under various full scale operating conditions within the given limits	Pump speed
		Fill weight accuracy
		Line interruptions/drying time
		Temperature
		Relative humidity
		Air flow rate

13.7 Strategy and Study Design (DOE)

The results of theoretical risk analysis performed in the earlier section identified CPPs and KPPs, and recommended that a multivariate filling study be performed on CPP to better understand not only the main effects impact on CQAs but also two way interactions. In order to confirm experimentally the theoretical assessments, fill process conditions or operating parameters that were used in the following experimental study were selected based on the knowledge of the filler dimensions existing at the commercial manufacturing sites and potential variability in the operating conditions.

The DOE for multivariate study often results in 15–18 set of studies depending upon type of filler and would require enormous resources and time. Hence, a strategy could be designed wherein a theoretical model [See Appendix] could be developed and utilized to perform simulation studies. As shown in the appendix the shear rates as a function of process conditions and filler dimensions such as speed, nozzle dimensions, etc., can be estimated and their impact on the drug product quality attributes can be evaluated using scale-down models. Scale-down models can be constructed based on capillary fluid flow and the desired shear rates and exposure time can be created.

Based on the results of simulation studies a design space can be constructed. The validity or authenticity of the model prediction can be verified experimentally by performing filling experiments at two extreme conditions or worst cases at the pilot scale involving short-term stability assessment of the product. This strategy would enable reduction of number of experiments required in the construction of the design space.

Since a single pump head is considered as a scale-down model and the process is scaled up by increasing the number of pump heads it was thought it was not necessary to verify the results of both pump study and filling characterization studies under full scale operating conditions at the commercial manufacturing site. However, the optimization of the KPPs that impacts the consistency and yield of the process was performed at a full scale during the engineering runs at the commercial filling site.

13.8 Piston Pump Study—Effect of Solution Properties on the Performance of the Pump

Methods In addition to assess the impact of filling conditions on the drug product quality attributes of X-mAb, the impact of solution characteristics/properties on the performance of the filler was evaluated. The evaluation included pumping study where 2 L of the drug product was pumped three times simulating the clinical batch sizes of 40 L filled by six pump heads. The rationale for such recirculation was twofold (1) to exaggerate the stresses and understand the sensitivity of the product to

Table 13.6 Summary of pump study results

Assays	Number of pump passes			
	Control	1	2	3
SE-HPLC (Monomer) %	99.3	98.8	98.8	98.6
SE-HPLC (HMW) %	0.7	1.2	1.2	1.4
Visible particles	0	0	0	0
Subvisible >10 μm (MFI)	5	8	16	25
Subvisible >25 μm (MFI)	1	1	1	1

filling related shear stresses although in reality the drug product goes through only single pass (2) assess capability of the pump to pump clinical batch size of 40 L without seizure and leakage.

Additionally, the filling process was interrupted for various periods of time mimicking the commercial filling interruptions to observe whether the pause has caused any drying or crystal growth of the product either at the nozzle or inside the pump that can negatively impact smooth filling process or pump seizure. The effect of variations of starting temperatures of the drug substance on the performance of the filler was also as evaluated.

Results No adverse impact from repeated passes of the drug substance was observed on the performance of the pump; no seizure or leakage/dripping of the solution was noticed. Results of purity and particle analysis that are summarized in Table 13.6 indicate no significant increase in the formation of higher order aggregates on SE-HPLC; however, some subvisible particles were noticed.

13.9 Filling Study—Effect of Filling Conditions on the Drug Product Quality Attributes

Theoretical (Mathematical Model) Shear rates that the product would experience at the above conditions were theoretically estimated using mathematical model. Formulated X-mAb drug product was exposed to high shear rates of approximately 2179 s^{-1} using a lab-scale model consisting of a capillary fluid flow apparatus of known internal diameter, length, and controlled flow rate. These shear parameters have been previously calculated to be representative of the shear that the product would experience in the piston pump filler based on the filler dimensions, design, and future operating conditions.

The X-mAb drug product was filtered through 0.2 μm PVDF membrane and stored at 2 °C–8 °C prior to use. Syringe barrel/needle RNS Hypak, 1 mL long with 27GX 1/2 in., 5 Bevel and plungers Hypak TSCF from BD were used. Cozzoli piston pump was employed. Syringes in a 10 × 10 tubs in a nested arrangement were used to fill and stopper using vacuum assisted plunger placement unit. All product contact containers and filler parts were cleaned prior to use and between each prod-

uct run. The cleaned filler parts were assembled and line was purged with water for injection (WFI) and with 50 mL of X-mAb drug product to remove WFI from the lines. Approximately 2050 units were filled using piston pump. In addition, 100 syringes were hand filled to serve as controls. All filled syringes were analyzed for visible and subvisible particles in addition to stability indicating assays prior to set down for accelerated stability studies. The filling conditions followed the DOE listed in the Table 13.7.

A single head piston pump was employed to fill the drug product at various filling conditions. Since the temperature has significant impact on the viscosity of concentrated drug product which in turn will have impact on the flow properties/filling, the effect of drug product temperature on the filling process was studied by either filtering the solution immediately after taking it out of 2 °C–8 °C storage followed by immediate filling into syringes or equilibrating at room temperature (23 °C) prior to execution of filling operation. To assess the filling nozzle effects, the position and the size of the nozzles were also varied. The nozzle position was defined as the insertion depth of the nozzle tip into the syringe during filling. The nozzle's internal diameter was measured at the opening canal.

Table 13.7 Filling study design of experiments

Number	Pattern	Temperature (°C)	Nozzle ID size (mm)	Nozzle position (mm)	Pump speed (Unit/min)
1	++−−	23	2	0.5	10
2	+−0−	23	1	1.5	10
3	++−0	23	2	0.5	25
4	−−−−	4	1	0.5	10
5	++00	23	2	1.5	25
6	−+−0	4	2	0.5	25
7	−−00	4	1	1.5	25
8	−−++	4	1	2.0	35
9	−+−+	4	2	0.5	35
10	−++−	4	2	2.0	1
11	+−+0	23	1	2.0	25
12	++++	23	2	2.0	35
13	−+0−	4	2	1.5	10
14	−++0	4	2	2.0	25
15	+−−+	23	1	0.5	35
16	++0+	23	2	1.5	35
17	−+0+	4	2	1.5	35
18	+++−	23	2	2.0	10

+ represents the higher limit within a specific range
− represents the lower limit within a specific range
0 represents the midpoint within a specific range

The results of filling study clearly indicated that among all the process parameters tested, the filling speed and the size of the nozzle or orifice significantly impacted the quality of the drug product, specifically the purity/integrity of the X-mAb, and were considered as the CPPs. The temperature of the drug product at the time of filling influenced the viscosity of the product which in turn influenced the performance of the filling process and was considered as the KPP.

This is not surprising, based on the relationship between shear rate (γ) and Hagen–Poiseuille equation described below

$$\gamma = \frac{8u}{d_p}$$

Where u is local velocity which can be determined according to the Hagen–Poiseuille model (Macosko and Larson 1994; Darby 2001):

$$u = \frac{{d_p}^2}{32\mu}\left(\frac{\mathrm{d}P}{\mathrm{d}x}\right)$$

As the liquid flows through the nozzle, higher the filling speed (flow rate) and smaller the constriction or the internal diameter (ID) of the nozzle/orifice greater will be the shear that the protein will be exposed to.

Based on the results of the filling study a knowledge space was constructed which is illustrated in the Fig. 13.9. The size of the black spheres corresponds to the level of high molecular weight species (HMWS); the larger the size of the sphere, the greater will be the measured level of HMWS/aggregation. Within the knowledge space, an operational space represented by light green box and a control space represented by pink box are defined where formation of higher order molecular weight species (HMW) is monitored and controlled while providing adequate scope for manufacturing optimization and flexibility.

The proposed design space is as follows: Temperature between 5 °C and 23 °C, Pump speed between 10 and 30 syringes per minute per head, nozzle ID between 1 and 2 mm, and nozzle position between 0.5 and 2.0 mm.

Knowledge and understanding (design space) gained from this DOE study may be used as a prior knowledge and leverage upon for similar mAb products with similar composition of the formulation. Instead of repeating the whole study, the applicability of the design space may be verified/tested by performing experiments under the worst case conditions (++++) of the design space (i.e., 23 °C, 35 spm, nozzle diameter of 2 mm and nozzle position at 2 mm). If no adverse impact on the drug product quality attributes is observed under these conditions, then one may apply the design space illustrated above to the new product.

13.9.1 Peristaltic Pump

A full factorial study matrix was created as listed in Table 13.8 and a total of 15 runs were performed with center point conditions acceleration (175), pump speed (200),

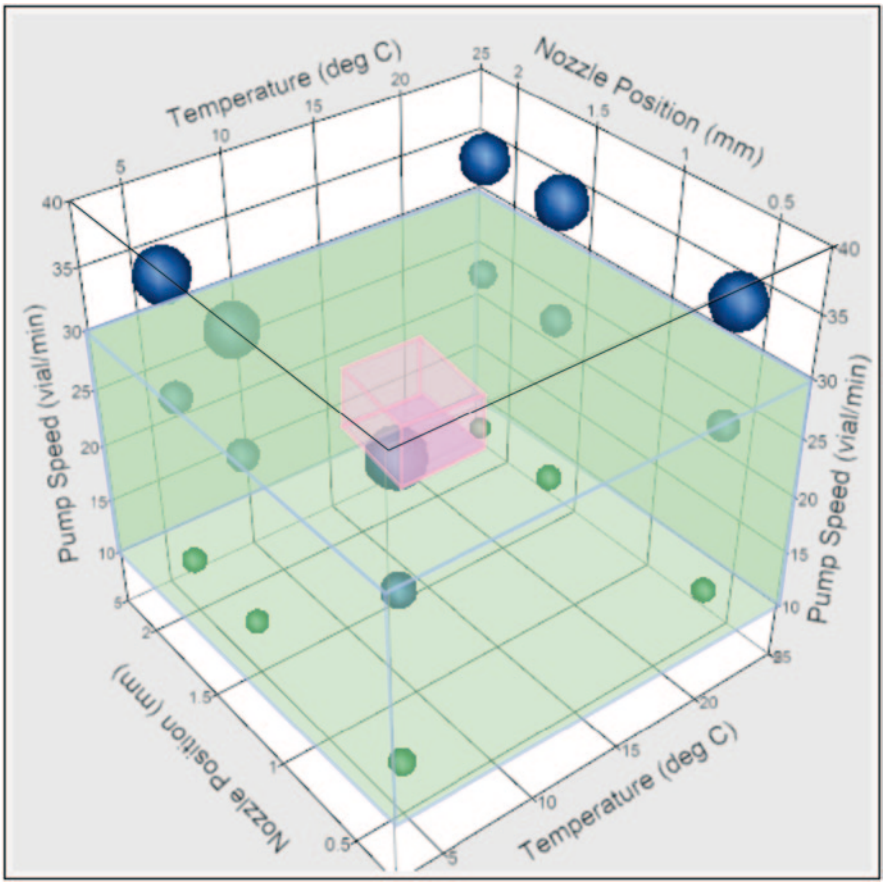

Fig. 13.9 Knowledge space matrix from X-mAb filling study

and delay (2 s) and reverse (2). The syringes were filled to the target fill weight of 1.04 g with the product. A total of 300 syringes were filled for every run and the fill weight check was performed every 10 syringes.

The impact of variation of filling process parameters were evaluated against the fill characteristics (dripping, foaming, splashing, and fill weight accuracy) and product quality attributes specifically subvisible and visible particles (see ANOVA in Fig. 13.10). No HMW were found and particle count was quite minimal compared with the piston pump suggesting protein does not get sheared with peristaltic pump filling technology. One way analysis of variance (ANOVA) as shown in Fig. 13.10, indicates that the nonspherical subvisible particle count is significantly higher when piston pump was used in comparison to when peristaltic pump case.

Dripping, splashing, and foaming were monitored and evaluated through visual inspections during the filling while the fill weight accuracy was determined through Cp_k values. Microscope focal imaging (MFI) was employed to measure subvisible particles. The above studies determined that the maximum pump speed to operate without creating foaming, splashing, and dripping was 200 RPM with an

Table 13.8 Full factorial DOE study matrix

Run order	Pattern	Acceleration	Pump speed (RPM)	Delay (sec)	Reverse
1	++−−	200	250	1	1
2	Center point	175	200	2	2
3	−−−−	150	150	1	1
4	++++	200	250	3	3
5	−−−+	150	150	1	3
6	−−+−	150	150	3	1
7	+++−	200	250	3	1
8	Center point	175	200	2	2
9	−+−+	150	250	1	3
10	−−++	150	150	3	3
11	++−+	200	250	1	3
12	−+−−	150	250	1	1
13	−++−	150	250	3	1
14	−+++	150	250	3	3
15	Center point	175	200	2	2

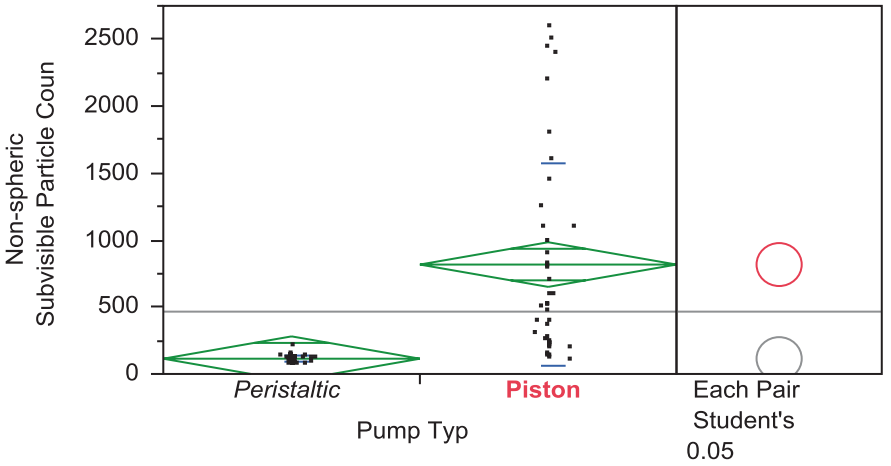

Fig. 13.10 One way analysis of nonspherical subvisible particle count by pump type

acceleration set at 200. Operating at pump speeds lower than 100 RPM resulted in the formation of droplets. The process capability index (Cp_k) values for T1 limits were greater than 1 in those run in which the pump speed was 200 RPM.

Tubing relaxation or breakage resulting in dosing inaccuracies is a common phenomenon observed with peristaltic pump filling. In order to evaluate the tubing durability the filling process was continuously run for 3–4 h without changing the

tubing. No breakage of tubing was observed, however, the dosing decreased over the time. All the fill weight values were well within T1 limits. This was further evaluated during the engineering run at full scale to understand whether the batch size needs to be reduced or frequent calibration is required to keep the fill weights within the T1 limits.

13.9.2 Impact of Temperature on Pump Performance

The temperature of the drug substance affects the pump's ability to deliver accurately at the target fill weight at colder temperatures especially with high protein concentration formulations due to viscosity related issues. This study mimicked the manufacturing procedures and temperature exposure conditions prior to filling. The fill weight and temperature were monitored every 30 fills. As expected the fill weights varied slightly initially and the fill weights were lower than the target value, but stabilized over time as the temperature of drug product equilibrated to room temperature. However, all the data points were within T1 limits and the Cp_k value of >1 for T1 limits demonstrated that the temperature effect does not impact the pump/process capability to deliver accurate fill weights.

13.9.3 Piston Pump Environmental Conditions Study

Depending upon the nature of the problem, the fill-line and/or the filling process is often interrupted for various lengths of time and these interruptions can cause drying of the product leading to pump blockage. The drying of the product that occur either inside the pump between the cylinder and the piston or at the tip of the nozzle is caused by the environmental conditions. The environmental conditions that contribute to the drying of the product during the interruptions of the filling process are temperature, relative humidity, interruption time, and air flow. Since these conditions can have significant impact on the performance of the filler and the filling process, it is important to study these variables and draw optimal/operational conditions that assure no pump blockage. The DOE is listed in the Table 13.9 where the temperature was varied from 15 °C to 30 °C, the percentage relative humidity was varied from 34 to 75, and time of interruptions from 15 to 60 min keeping the laminar air flow rate 0.45 m/s constant. The study was performed using 2 mm tubing and 1.6 mm needle with eight piston pumps.

This will provide a wide characterization space that supports the expected manufacturing process at various sites and allows for significant room for any future process changes.

The results of the environmental characterization studies which are displayed in the Figs. 13.11, 13.12, and 13.13 indicate that elevated temperatures, low-percentage relative humidity at constant laminar air flow coupled with process interruptions result in pump blockage. No pump blockages were observed when the tem-

Table 13.9 DOE for evaluation of effect of environmental conditions on the performance of rotary piston pump

Number	Pattern	Temperature	% RH	Interruption time
1	313	30	34	60
2	331	30	75	15
3	321	30	50	15
4	112	15	34	40
5	111	15	34	15
6	131	15	75	15
7	231	20	75	15
8	311	30	34	15
9	223	20	50	60
10	332	30	75	40
11	312	30	34	40
12	221	20	50	15
13	121	15	50	15
14	212	20	34	40
15	313	30	34	60
16	233	20	75	60
17	322	30	50	40
18	232	20	75	40
19	222	20	50	40
20	122	15	50	40
21	211	20	34	15
22	323	30	50	60
23	123	15	50	60
24	113	15	34	60
25	133	15	75	60
26	213	20	34	60
27	333	30	75	60

perature was maintained between 17 °C and 25 °C, the percentage relative humidity was within 35–65 and the duration of interruptions was ≤ 30 min even with constant laminar air flowing in the direction of filler. Pump blockages were observed when the duration of interruptions exceeded 30 min with temperatures reaching above 25 °C and relative humidity dropping below 35 % with laminar air flow on, however, no blockages were observed under the same conditions if the laminar air flow was off suggesting air flow parameter is critical to the drying of the product inside the pump.

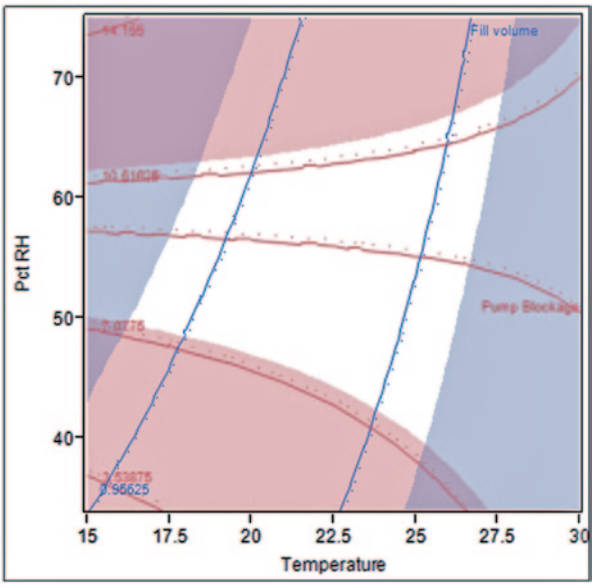

Fig. 13.11 Percent relative humidity (PctRH) vs. temperature in relation to fill volume and pump blockage rate

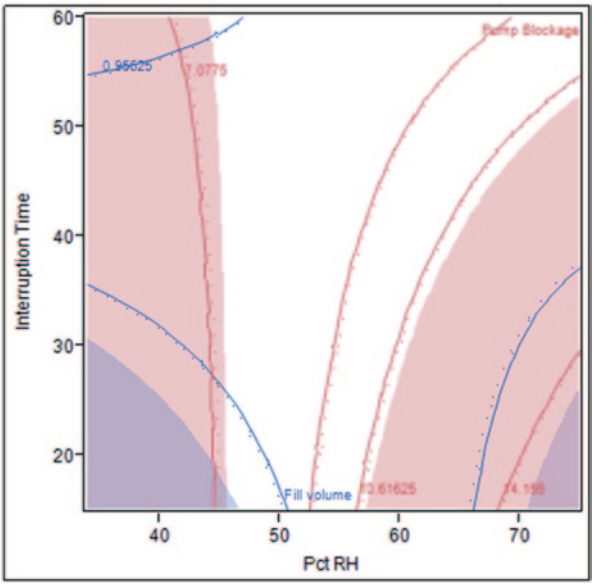

Fig. 13.12 Percent relative humidity (PctRH) vs. machine interruption time in relation to fill volume and pump blockage rate

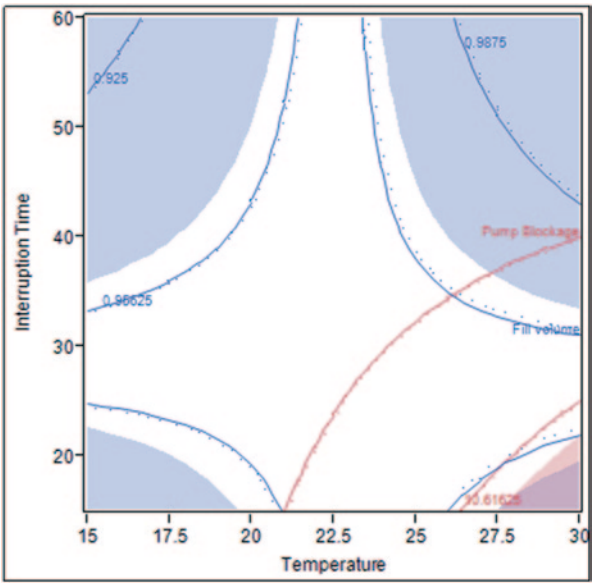

Fig. 13.13 Temperature vs. machine interruption time in relation to fill volume and pump blockage rate

13.10 Scale-Up and Engineering Runs

13.10.1 Identification of Site Specific CPPs

A risk assessment using FMEA was performed during the technology transfer leveraging on the design space and the knowledge of the manufacturing site to identify site specific gaps/parameters of high risks that need to be examined in detail (CPPs).

Table 13.10 lists the process parameter risk analysis for the filling process.

13.10.2 Fill Weight Strategy

The challenges to meet protein content (mg/syringe) specification limits for drug products are compounded due to limits associated with fill weights (g) and drug substance protein concentrations (mg/mL). The drug substance protein specification allows a variability of ± 10 % in protein concentration. In order to ensure the delivery of 50 mg ± 5 % of protein per syringe a variable fill weight strategy was adopted where the target fill weight was calculated based on the protein concentration of the drug substance to be filled. The fill weight, and drug substance and drug product protein concentration variabilities were assumed to be 0.007 g, 0.7 mg/mL, and 0.4 mg/syringe, respectively. The Fig. 13.14 illustrates that as drug substance protein concentration and/or fill weight approximate their lower or upper limits, the probability of an out-of-specification (OOS) result in drug product protein content increases significantly. Thus, the understanding of the drug product protein content

Table 13.10 FMEA risk analysis of commercial site

Process parameter	Input/output	Target value/ normal operating range	Proven acceptable range	Failure	Impact	RPN	Parameter classification	Justification/remarks/ actions
Fill weight control	Output	Target±0.034 g range (Target=1.04 g	Target: 1.04 g±0.04 g range	Protein content too high or too low	Dosage per syringe too high or too low	75	Key	If a fill weight is out of PAR the syringes are discarded, the impact on quality is low. A variable fill weight approach is implemented depending on the protein concentration of the pooled and 0.2 µm filtered DS, the corresponding target fill weight will be used
Duration of filling	Output	–	According to media fill	Filling takes too long	Exceeding of the aseptic filling time	72	Key	The aseptic filling time is documented in the batch record. The aseptic filling time is covered by the current media fill validation
Filling speed	Input	Max (~225 syringes/min)	Max (~225 syringes/min)	Filling speed too high	Cosmetic defect leading to high product reject rate	515	Noncritical/ WC-CPP	Effect of variation in fill speed on product quality studied and defined acceptable operating range from design space Speed of each pump is documented during each fill accuracy check The parameter is easy to adjust

Table 13.10 (continued)

Process parameter	Input/output	Target value/ normal operating range	Proven acceptable range	Failure	Impact	RPN	Parameter classification	Justification/remarks/ actions
Consistency of aseptic filling and batch uniformity	Output	–	X-mAb specifications	Filling and filtration affects product quality attributes during the process	Impact on API quality	300	Critical	Samples will be taken according to the sampling plan at the beginning, middle, and end of filling

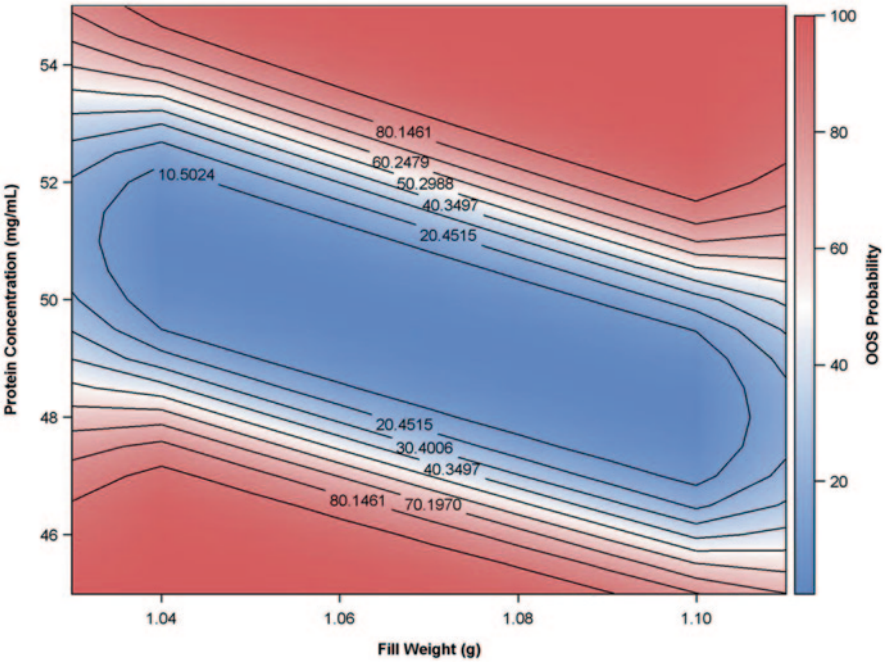

Fig. 13.14 Simulated drug product protein content (mg/syringe) risk space

risk space was critical to properly optimize in-process limits for drug substance protein concentrations and fill weights.

Based on the filler capability on precision and accuracy a target fill weight of 1.07 g with the tolerances of ± 0.02 g (T1) and ± 0.03 g (T2) have been defined for drug product to ensure a target of 50 mg/syringe of protein.

In addition to verification of the design space generated at pilot scale, the intent of the engineering runs was to assess the overall machinability of the filling line that includes the container closure integrity of the primary packaging, the fill weight accuracy/precision, the performance of the piston pump, and the behavior of the X-mAb drug product solution under full production operation.

The engineering runs were performed at full scale operating conditions spanning the extreme conditions as well as a targeted set point within the defined control space using piston filler; a Cozzoli 10 pump heads filler with a 2 cc piston pump. Results from these runs allowed setting of the specific key parameters and critical parameters within the design space that provided a high level of confidence of its successful performance during the process validation/process performance qualification runs. Table 13.11 outlines the critical parameter that was studied for the three engineering runs. Samples were drawn from the beginning, middle, and the end of the filling process for each run in order to test the impact of filling conditions on the product quality attributes. The filling units has the capability of performing automatic weight check and adjust the fill weight as needed. To test the fill weight precision at the production scale, the fill weight data was analyzed using Cp_k.

Table 13.11 Processing parameters outline of engineering runs

Engineering run number	Conditions	Pumping speed (unit/min)
1	Worst	35
2	Best	20
3	Target	25

Three batches of X-mAb bulks were filled at the 100 L scale at the conditions summarized in Table 13.11. The product quality and process performance results from these engineering runs were compared with the pilot scale results and were found to be in agreement and consistent both in terms of processing capability and product compatibility, demonstrating successful scale-up and transfer of the filling process. Samples were drawn at various stages of the filling process and tested for integrity/purity of X-mAb using SEC-HPLC. The percentage of HMW as a function of time for the three engineering runs is plotted in Fig. 13.15. No discernable differences in the % HMW were observed between the samples and the results were well below the acceptance criteria, demonstrating the proposed design space is applicable at full scale.

Life Cycle Management As indicated earlier, the marketing demands requires expansion of foot prints and transfer of filling operation from one site to another, different pumping mechanisms may be introduced. For prefilled syringes, piston pump, peristaltic pump, and time pressure filling pumps are the most commonly used filling systems. Filling studies with piston pump and peristaltic pump are described here, however, if new manufacturing site require use of time pressure filling system, filling studies would be conducted for X-mAb with the new pump system leveraging on the experience, knowledge, and approaches gained through the above studies.

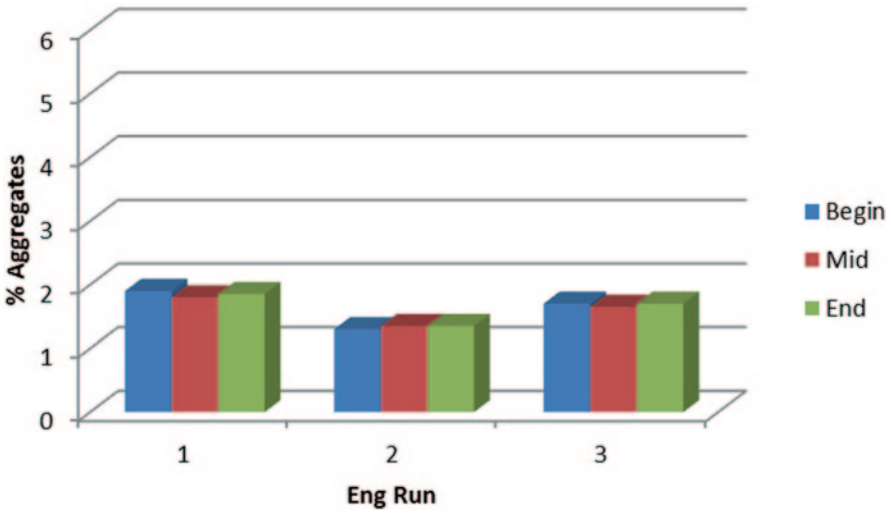

Fig. 13.15 Engineering run result summary for X-mAb

Control Strategy Classification of each in-process control (IPC) parameters of filling process as critical, key, and nonkey followed the general guidelines discussed in the earlier chapters. However, the establishment of the control and action limits of these parameters were based on the process performance, commercial site filler characterization, and aligned with the filler performance qualification acceptance criteria. Fill weight, filling yield, and reconciliation are the IPCs of the filling unit operation that are monitored during the commercial production.

13.10.3 Fill Weight

This is monitored as an IPC to ensure that the filler delivers the target product volume into the syringes during the filling process. The low fill weight is classified as CPP to ensure that the syringes meet the label claim amount of protein per unit, any amount lower than the label claim will impact the therapeutic efficacy of the product. The high fill weight is classified as KPP not only because of it reflects inconsistency in the process but also concerns on waste of drug substance (COGM). It has no impact on the patient as the material is injected from the graduated syringes.

The minimum and maximum filling lot sizes for the X-mAb are presented in the Table 13.12 with the in-process sample rate and total number of syringes checked for fill weight during manufacturing process. During the set-up, syringes are checked for fill-weight to ensure the process is in steady-state and ready to start production.

The in-process fill weight check is performed at a regular interval that is specific to the filling machine. For X-mAb the piston pump filler was operated at the speed of 250 syringes per minute and sampled 1 syringe every 30 s, so 1 syringe out of approximately 125 syringes is checked for fill weight. Additionally, a confirmatory test is performed at the release by the deliverable volume method for each batch where in 5 units are tested.

The machine is capable of performing a 100% IPC (check weight of each syringe before, during, and after filling). The 100% IPC is normally performed during set-up or after extended machine downtimes. During production mode IPC, the position where the weighing starts is random but continues weighing consecutively (i.e., if IPC

Table 13.12 Number of syringes sampled for in-process fill weight check

Product, X-mAb (run length)	Label claim (1 mL)	Batch size (kg)	Filling yield (%)	Target fill weight (g)	No of syringes filled	Sample rate (% syringes)
X-mAb (min)	1.0	50	80	1.07	39,000	2
X-mAb (max)	1.0	100	99	1.07	79,000	2

The action and control limits for the fill weight IPC were derived using the fill weight data collected during the engineering runs, validation lots, and also leveraged on some data from clinical manufacturing

starts at needle 3 it will end in needle 2 and continue this IPC pattern during the process.

- Target fill weight (gm) = 1.07
- Action limit (gm) = 1.04–1.1
- Control limit (gm) = TBD

13.10.4 Filling Yield and Reconciliation

Upon filling, the overall performance of the sterile filtration and filling operations is monitored by percent of theoretical yield and reconciliation. The percentage of theoretical yield is monitored at this stage as a critical performance parameter. This yield is defined as the ratio between the total number of units for inspection including samples and the theoretical number of units expected.

Reconciliation is monitored at this stage as a key parameter to ensure process accountability and process output consistency. This parameter is calculated as the ratio of the total quantity of units produced (including waste, QA samples, and others) and the theoretical quantity of units expected.

Action limits for filling yield and reconciliation were established based on statistical evaluation of a significant number of historical lots and reflect the expected results for the specific equipment and procedures in that manufacturing area. The historical data used to calculate these limits were grouped according to batch size since that factor can significantly impact the values for both parameters.

Action limits for filling yield and reconciliation were recommended to be 95–101 % and 98–102 %, respectively.

13.10.5 Proposed Control Strategy

The proposed control strategy for the filling process has a dual purpose:

1. Ensure product quality and safety
2. Ensure that the commercial manufacturing process is consistent and robust

Product quality and safety are ensured by controlling all quality-linked process parameters (Well controlled—CPP) within the limits of the design space. Process consistency is ensured by controlling KPPs within established limits and by monitoring relevant process attributes.

The control strategy for the given process parameters outlined in this case study for the filling process is covered below. The outcome of the studies executed in order to explore the design space allows the differentiation between CPPs and key performance parameters (KPPs) and general process parameters (GPP) as shown in Table 13.13. According to this designation the control strategy is defined to mitigate the criticality of the given parameters and feed in to the specification of the proven acceptable range (PAR) and the normal operating rang (NOR) for the process parameters at each unit

Table 13.13 Control strategy of process parameters for filling process

No	Parameter	Designation (CPP/KPP)	Control strategy	Final designation
1	Filtered bulk hold temperature	CPP	Follow specification of NOR and PAR for bulk temperature and document in/review the batch record	GPP
2	Filler speed	CPP	Speed of each pump is documented during each fill accuracy check. Follow specification of NOR and PAR for filler speed	WC-CPP
3	Fill weight settings	KPP	In-process fill weight check. Follow action and control limits based on the fill weight data from clinical manufacturing, the engineering runs and validation lots If a fill weight is out of PAR the syringes are discarded	KPP
4	Nozzle position	CPP	Train operators Follow SOPs that specify filler set-up and train operators Document nozzle position during set-up Follow NOR and PAR from design space for nozzle position	WC-CPP

operation listed in Table 13.13. Based on the level of control and the availability to mitigate the corresponding risk (risk level≤200) CCPs will become WC-CPPs.

13.10.6 Final Risk Assessment

The final risk assessment verifies that the established control strategy is sufficient to mitigate the criticality of all process parameters and demonstrates control of the whole process.

Tool Description If a NOR/PAR has a wide range, there is less inherent risk that the range can be met by a process and therefore, has a lower risk value. A narrow range is more difficult to control and translates to a higher risk value. The control strategy can be considered as high/tight, medium, or low. A tight control of a system has less risk than one that is medium or low and therefore, has a lower risk. PP risk was ranked as CPP>KPP>GPP. Values assigned for each designation, the NOR/PAR width of parameter and control strategy level described in Table 13.14. From these values, a risk mitigation number can be calculated and is detailed in Table 13.15.

Table 13.14 Risk assessment values

Risk value	Designation	NOR/PAR width of parameter	Control strategy level
1	GPP		High
3		Wide	
5	KPP		Medium
7		Narrow	
9	CPP		Low

Risk mitigation number umbertion ateg× NOR/PAR width × control strategy level

Table 13.15 Risk mitigation assessment for filtration process

Parameter	Designation CPP/KPP	Risk value	NOR/PAR width	Control strategy level	Risk mitigation (D × W × C)	Risk mitigated (y/n) (D × W × C ≤ 200)
Filtered bulk hold temperature	CPP	9	3	1	27	Y
Filler speed	CPP	9	7	1	63	Y
Fill weight settings	CPP	7	7	1	49	Y
Nozzle position	CPP	9	3	1	27	Y

A risk mitigation number of ≤200 was considered mitigated. Values >200 require additional mitigation strategies. This cut-off suggests that a:

- CPP with a narrow range for NOR/PAR must be controlled well to pass 200 (be considered mitigated)
- CPP with a wide range for NOR/PAR must be controlled well to medium to pass 200
- KPP with a narrow range for NOR/PAR must be controlled well to medium to pass 200 KPP with a wide range for NOR/PAR will always pass 200
- GPP with a narrow range for NOR/PAR will always pass 200
- GPP with a wide range for NOR/PAR will always pass 200

The risk mitigation and assessment results demonstrate that the filling process is well under control as defined.

Acknowledgment I would like to thank Amgen colleague Ozzie Diaz for his technical discussions and assistance with the simulation of drug product protein content risk space.

Appendix

Shear Rate Estimations and Evaluation of their Impacts On In the case of time pressure filler, shear rate experienced by drug products while flowing through the manifold orifice is one to two orders of magnitude greater than the shear rate experienced while flowing through the inlet and outlet tubing, and the filling needles. In the case of products filled using the rotary piston filler, the highest shear is experienced in the clearance between piston and cylinder for maximum of few seconds.

These shear rates can be estimated from the standard shear rate Eq. (13.1) described below.

$$\gamma = \frac{4Q}{\pi R^3} \qquad \text{(Eq. 13.1)}$$

whereγ is the shear rate, Q is average flow rate, and R is the radius of tubing or orifice.

Shear rate in the clearance between cylinder and the piston (RP technology) is estimated from the first principle. During each filling cycle, the cylinder moves up and down while the piston makes 360° rotation. In the clearance between cylinder and piston, fluid experiences both vertical and radial motion. Vertical motion is associated with the upward and downward stroke movement of the cylinder while the radial motion is associated with rotational and horizontal motion of the piston. For the purpose of shear estimation, both motions are evaluated separately and root mean square value is estimated. This simplification will be an approximation, but the resulting shear values will cover the worst case conditions.

The shear rate calculations for X-mAb for a fill volume of 1.04 mL along with Reynolds number and transition length are summarized below. The estimates of Reynolds number and transition length are necessary to demonstrate a fully developed velocity profile as well as a laminar flow condition, the Reynolds number should be between 2100 and 10,000 for laminar.

Calculations for Shear Estimation for rotary piston filling technology.

Assumptions

- The fluid is incompressible and Newtonian
- The flow is in steady state and in laminar regime with no flow separation (might not be possible at all locations)
- The flow profile is fully developed (might not be possible at all locations)
- No slip condition is assumed as boundary condition

Shear Estimation Through the Nozzle The Cozzoli filler that will be used at commercial manufacturing will have 10 heads/nozzles. Depending upon the filling speed chosen, the time required to fill single syringe can be calculated as follows.

To fill each syringe, the filling nozzle arm completes a full elliptical cycle, half of the cycle time is used to fill the syringes and the other half is taken for the half of the arm to return back to its original position. For a filling speed of 250 syringe/min

with 10 nozzles, time required to fill a single syringe would be 25 syringes/nozzle/min = 50 half cycles/nozzle/min = 1.2 s/syringe.

Flow rate can be calculated by dividing the target fill volume by the filling time. For a fill volume of 1.04 mL.

The flow rate would be (Q) = 1.04/1.2 = 0.86 Ml/s

For a 1.59 mm internal diameter (D) nozzle, discharge velocity V = Discharge flow rate (Q)/Area.

$$V = \frac{Q}{\pi r^2}$$

Discharge velocity = 0.86/(× 0.159)$^{2/4}$

Discharge velocity (v) = 452.6 mm/s

For a given flow rate and the nozzle dimension of internal diameter, 1.59 mm, the shear rate can be calculated using the Eq. 13.1

$$\gamma = \frac{4x0.86}{\pi\ (0.795)^3} \times 1000 = 2{,}179\ \text{1/s}$$

The Reynolds number (Re) through nozzle can be determined based on discharge velocity and physical properties of fluid. Density (ρ) of X-mAb = 1.03 gm/cc and viscosity (μ) 2.7.

$$\text{Re} = \frac{D \cdot v \cdot \rho}{\mu},$$

Where, Re is Reynolds number (should be < 2100 for laminar flow in pipe), D is internal diameter of pipe, v is average flow velocity of fluid, ρ is density of fluid, and μ is viscosity of fluid.

$$\text{Re} = \frac{1.59 \times 10^{-3} \times 0.4526 \times 1003}{0.0027} = 267$$

The exposure time is estimated based on the length of nozzle. For a 138.5 mm nozzle

Exposure time = length of Nozzle/discharge velocity

Exposure time = 138.5/452

Exposure time = 0.30 s

Time/Pressure Filler—Shear Estimation for Flow Through Orifice Same procedure as above is used to estimate shear rates experienced by fluid while flowing through the orifice. For various orifice diameters and orifice length, the discharge velocity, Reynolds number, transition length, exposure time, and the wall shear rate are calculated. As an example, for a given orifice diameter of 0.7 mm and orifice length of 2.34 the following are tabulated.

Discharge velocity	6591 mm/s
Reynolds number	1762
Transition length	61.66 mm
Exposure time	0.0004 s
Wall shear rate	75328 s^{-1}

Evaluation of Impacts of Shear Rates on the Product Quality Attributes As shown above the shear rates as a function of process conditions and filler dimensions such as speed, nozzle dimensions, etc., can be estimated and their impact on the drug product quality attributes can be evaluated using scale-down models. Scale-down models can be constructed based on capillary fluid flow and the desired shear rates and exposure time can be created.

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Chapter 14
Lyophilization Process Design and Development Using QbD Principles

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14.1 Introduction

The target freeze-drying process must deliver dry product that is safe, stable, and efficacious. That is, the process must ensure acceptable product quality attributes, such as low-residual water content, short reconstitution time, and retention of potency, as well as exhibit pharmaceutical elegance. Since freeze drying is a costly and time intensive process, from an operational point of view, the process should be short, reproducible, and robust. An optimized process that operates within the constraints of the equipment, plant utilities, and appropriate safety margins should be the goal. The freeze-drying process essentially consists of three distinct phases: (1) freezing of the solution, (2) primary drying or ice sublimation, and (3) secondary drying (removal of unfrozen water).

The initial freezing process is of critical importance since it may influence subsequent drying phases. During freezing, stability problems may arise due to pH changes resulting from crystallization of certain buffer components (Gomez et al. 2001), cryoconcentration, ice-liquid interface (Bhatnagar et al. 2007, 2008), phase separation (Izutsu et al. 2005; Padilla and Pikal 2010), and cold denaturation (Tang and Pikal 2005). Formulations used for freeze-drying exhibit super-cooling tendencies and thermal events such as eutectic or glass transitions (Pikal 1990). The

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eutectic transition (Teu) is generally exhibited by solutes which crystallize in the interstitial phase whereas the glass transition (Tg') or collapse temperature (Tc) is generally exhibited by the excipients that stay amorphous. For partially dried or fully dried product, there exist two other transitions—collapse temperature (Tc) and glass transition (Tg). The Tc defines the maximum allowable product temperature during primary drying; above the Tc, the solute phase possesses sufficient mobility to lose cake structure, resulting in partial or complete collapse. Typically, product temperature is maintained at 2–3 °C below the maximum allowable temperature during primary drying. The Tg of the solid "dry" mass depends upon the moisture content of the solid; the higher the moisture content, the lower the Tg and vice versa. During secondary drying, the product temperature should be maintained below Tg.

Well-designed cooling cycle (ramp and hold times) must be used in order to obtain an appropriate structure of the frozen mass. The ice nucleation temperature during the freezing step affects the size of ice crystals (Konstantinidis et al. 2011; Patel et al. 2009; Rambhatla et al. 2004; Searles et al. 2001a). A higher degree of supercooling (that is, a lower ice nucleation temperature) results in smaller ice crystals, higher product resistance and, hence, a longer primary drying time. Annealing (a succession of cooling and re-warming regimens while maintaining the frozen structure) is typically performed during the freezing step to minimize ice nucleation heterogeneity and, thereby, achieve a uniform primary drying rate (Searles et al. 2001b, Lu and Pikal 2004). Annealing is also performed to induce crystallization of excipients (such as mannitol and glycine) that tend to crystallize upon freezing (Chongprasert et al. 2001; Pyne and Suryanarayanan 2001; Li and Nail 2005; Yu et al. 1999; Sundaramurthi and Suryanarayanan 2010; Al-Hussein and Gieseler 2012; Cao et al. 2013).

The second phase in the freeze-drying process is the primary drying or ice sublimation phase, which is conducted under low chamber pressure (for example, 50–250 mTorr) and at subzero product temperatures. Under these conditions, sublimation of ice takes place as dictated by the ice/water-vapor equilibrium line of the phase diagram of water. The sublimed water vapor from the frozen matrix traverses out of the cake, into the headspace of the vial, through the vents of the closure, into the chamber, and eventually onto the cold condenser coils, where it is condensed again as ice. Thus, frozen water from the vial is vaporized by sublimation and collected on the cold plates of condenser by condensation. The sublimation of ice is a phase change that requires energy; the energy is supplied as heat from temperature controlled shelves. Freeze-drying is a combined heat and mass transfer process in which both the transfer phenomena must be carefully balanced so that sustained drying rate (mass transfer) prevails without collapsing or melting of the frozen mass due to accumulation of heat from the heated shelf (heat transfer). During the entire sublimation phase, the product temperature should always be several degrees below Tg'/Tc or Teu in order to obtain a dry product with acceptable appearance. The factors influencing the rate of heat and mass transfer are discussed in a later section.

The last phase in freeze-drying cycle is secondary drying, which consists of removal of the unfrozen water, largely by desorption, at the highest possible shelf temperature compatible with product stability (for example, 20–45 °C) and at low chamber pressure.

The typical freeze-drying process involves: (1) sterilizing the bulk solution by passing it through a bacteria-retentive filter; (2) filling into individual sterile containers with semistoppered closures; (3) freezing the solution by placing the open vials on cooled shelves in a freeze-drying chamber; (4) applying vacuum to the chamber and heating the shelves in order to sublime the ice; (5) removing unfrozen water by further heating the shelves; (6) breaking the vacuum at the end of drying using sterile air or nitrogen; (7) fully stoppering the containers within the dryer; (8) unloading the vials; (9) sealing the vials with aluminum seals.

14.2 Risk Assessment of Lyophilization Process Parameters

There are several variables in the lyophilization process that could impact the process performance as well as the final product quality. As per ICH Q9, a risk assessment is performed to gauge the impact of the lyophilization process parameters on process performance and product quality attributes. The risk assessment leverages the knowledge gained during development, first principles understanding of the freeze-drying process, and experiences gained from processing similar types of products in the past. The risk assessment procedure consists of assessing each parameter of the lyophilization process for severity (which includes product safety, PK/PD, immunogenicity, activity, or efficacy), occurrence, and detectability. The process attributes relevant to lyophilization are the completion of the freeze-drying cycle with process parameters within acceptable range without affecting any product quality attributes.

The lyophilization process involves transformation of the physical state of the product formulation to yield a more stable drug product; hence, a number of product quality attributes are relevant to this operation. These product quality attributes include residual moisture, cake appearance, reconstitution time, impact to protein potency, purity, stability, and container-closure integrity (for sterility and to prevent moisture ingress). Table 14.1 summarizes the process parameters that need to be considered. The process parameters that could impact process performance or product quality attributes along with the risk assessment score are summarized in Table 14.1. The scoring system used to define each impact and uncertainty categories that are listed in Tables 14.2 and 14.3. The dwell time for stoppering is not included as a variable in the risk ranking, since it is usually fixed as a part of development activities on the full-scale lyophilizer once appropriate stoppering forces have been identified for sealing the vial with stoppers.

Any parameter with an overall score[1] of >235 is identified as a potential critical process parameter (Table 14.1). As expected, the potential critical process parameters are:

[1] Overall score is determined as sum of product of quality attribute and process parameter score.

Table 14.1 Risk assessment of lyophilization process parameters

Process parameter	Quality attributes				Process performance	Overall score	Rationale for assigned score	Recommended characterization studies
	Stability/ potency	Residual water	Recon time	Cake appearance	Process efficiency			
Score →	10	7	7	7	7			
Loading temp	10	1	1	1	1	128	Current controls ensure that the product would not be lyophilized unless loading temperature was reached. Stability data available to support the loading temp and duration	No study (data available from development stability studies to define the acceptable temp range)
Loading time	10	1	1	1	10	191	Stability data available to support the loading temp and duration	No study (data available from development stability studies to define the acceptable hold time)
Freezing rate	5	1	5	7	10	211	Varying ramp rate could alter ice crystal morphology and change drying characteristics leading to collapse or high moisture. Denaturation of protein is also possible with larger ice-liquid interface. Smaller ice crystals could also lead to smaller pores in final freeze-dried cakes and longer reconstitution times. With annealing step the effect is expected to be moderate to none	Perform study to identify if freezing rate has any affect at t = 0 (attributes like stability, reconstitution time, residual water)
Final freezing temp	1	1	1	1	1	38	The final freezing temperature should be below Tg' to ensure complete freezing. Progress into primary drying before complete freezing can result in loss of cake structure which could further affect product stability	No study (supporting data is generally readily available from development studies)
Hold time for freezing	1	1	1	1	10	101	No impact expected as the typical hold time is much longer than minimum time needed for complete freezing	No study (supporting data is generally readily available from development studies)

Table 14.1 (continued)

Process parameter	Quality attributes				Process performance	Overall score	Rationale for assigned score	Recommended characterization studies
	Stability/ potency	Residual water	Recon time	Cake appearance	Process efficiency			
Score →	10	7	7	7	7			
Ramp rate for annealing	5	1	1	1	10	141	No effect expected as annealing will remove any heterogeneity introduced due to ramp rate	No study needed. Limited by equipment capabilities
Annealing temp	5	5	5	7	10	239	Should be above Tg' to ensure Ostwald ripening but not too high to result in melting	Perform study to identify optimal annealing temperature to minimize drying time and achieve batch uniformity in drying rate
Annealing time	5	5	5	7	10	239	Should be long enough to achieve ice crystal growth. Too short may result in no added advantage of annealing step	Perform study to identify optimal annealing time to minimize drying time and achieve batch uniformity in drying rate
Ramp rate to primary drying shelf temp	7	7	7	10	10	308	Effect of higher ramp rates is to cause rapid ice sublimation leading to overload on condenser and loss of pressure control. Thus any effect would be seen in a pressure deviation	Perform study to identify optimal ramp rate for primary drying that would not affect product quality (specifically cake appearance)
Ts for primary drying	7	7	7	10	10	308	Primary drying temperature affects cake appearance and completeness of drying e.g., low temperature results in long drying time while high temperature can cause product melt back	Perform study along with use of mathematical model to define the shelf temperature set point to achieve the target product temperature profile

Table 14.1 (continued)

Process parameter	Quality attributes				Process performance	Overall score	Rationale for assigned score	Recommended characterization studies
	Stability/ potency	Residual water	Recon time	Cake appearance	Process efficiency			
Score →	10	7	7	7	7			
Pc for primary drying	7	7	7	10	10	308	High pressure could lead to product melt back; low pressure could be difficult to control on manufacturing scale	Perform study along with use of mathematical model to define the chamber pressure set point to achieve the target product temperature profile
Primary drying time	7	7	7	10	10	308	Insufficient drying could cause collapse and/or higher residual moisture which could impact stability. However, PAT tools ensure that the primary drying is complete before progressing into secondary drying	Perform study along with use of PAT tools to mark the end of primary drying
Secondary drying ramp rate	7	7	7	10	10	308	Higher ramp rates results in collapse via a glass transition	Perform study to identify optimal ramp rate for secondary drying that would not affect product quality (specifically cake appearance)
Ts for secondary drying	7	7	5	10	10	294	Too low Ts results in long secondary drying to get down to the desired residual water level. Too high Ts may result in product degradation	Perform study to identify optimal Ts

Table 14.1 (continued)

Process parameter	Quality attributes				Process performance	Overall score	Rationale for assigned score	Recommended characterization studies
	Stability/ potency	Residual water	Recon time	Cake appearance	Process efficiency			
Score →	10	7	7	7	7			
Pc for secondary drying	1	1	1	1	1	38	Over the narrow operational range, no impact on process and product attributes is expected. During secondary drying, the product temperature is close to the shelf temperature and the partial pressure of water is much lower than the chamber pressure. As a result, the effect of chamber pressure is inconsequential	Use the same chamber pressure as during primary drying
Secondary drying time	7	10	7	10	10	329	Insufficient drying could lead to high final moisture levels and hence unacceptable stability. Too long time at high temperature could also affect stability	Perform study along with use of PAT tools to mark the end of secondary drying
Final storage temp	1	1	5	1	1	66	To be stored at intended storage temperature (typically 2–8 C) and supported by development stability data	Supporting data is generally readily available from development studies
Chamber pressure before vial stoppering	7	7	5	7	1	210	Pre-aeration pressure can have an effect on reconstitution time, and also ability to withdraw solution	Perform study to test effect of pre-aeration pressure on reconstitution time and solution withdrawal characteristics at typical limits

Table 14.1 (continued)

Process parameter	Quality attributes				Process performance	Overall score	Rationale for assigned score	Recommended characterization studies
	Stability/ potency	Residual water	Recon time	Cake appearance	Process efficiency			
Score →	10	7	7	7	7			
Hydraulic pressure for stoppering	7	7	5	7	1	210	Enough force is needed (kg/vial) to stopper fully and ensure sterility. Excessive force can cause vial breakage	Small-scale studies to identify force (kg/vial) needed to stopper and also identify a higher limit which does not cause breakage. Perform confirmatory test on full scale (stoppering force PQ) to verify forces

Table 14.2 Product quality attributes ranking

Description	Score
Established impact on product safety and/or efficacy	10
Expect impact on product safety and/or efficacy	7
Do not know for sure the impact on product safety and/or efficacy	5
No impact on product safety and/or efficacy	1

Table 14.3 Process parameter ranking based on ability to impact product quality attributes

Description	Score
Strong relationship	10
Expect a relationship	7
Do not know for sure	5
No relationship	1

1. Freezing: Annealing time and temperature
2. Primary drying: ramp rate, shelf temperature, chamber pressure, primary drying time
3. Secondary drying: ramp rate, shelf temperature, and secondary drying time

As mentioned earlier, formulation thermal characteristics along with equipment and container-closure characterization are critical to design and develop a robust freeze-drying process. The rest of the chapter presents a *mock case study* demonstrating the application of Quality by design (QbD) principles (PAT and Design Space) to freeze-drying process design, development and scale-up.

14.3 Characterization of Formulation, Lyophilizer, and the Container-Closure System

The formulation for mAb X (25 mg/mL mAb X, 4% mannitol, 2.5% sucrose, 0.01% Polysorbate 20, pH 6.0) has a Tg' of −21 °C as measured by DSC and, hence, a freezing temp of −40 °C with 2 h of hold time was sufficient to ensure that the solution was frozen completely when filled at 5.5 mL in 20 mL glass vials. The formulation has a Tc of −18 °C as measured by freeze-dry microscopy. Accordingly, the target product temperature during primary drying was set to −21 °C (3 °C below the Tc) to provide a margin of safety against any excursions in temperature and/or pressure.

First, operational limits of the freeze-dryers were established to identify the range wherein the process would operate without losing process control using OQ/IQ testing as described in (Rambhatla et al. 2006; Patel et al. 2010a). Briefly, water filled bottomless trays were loaded on the shelves and minimum achievable chamber pressure was determined for a given sublimation rate (that is, shelf temperature

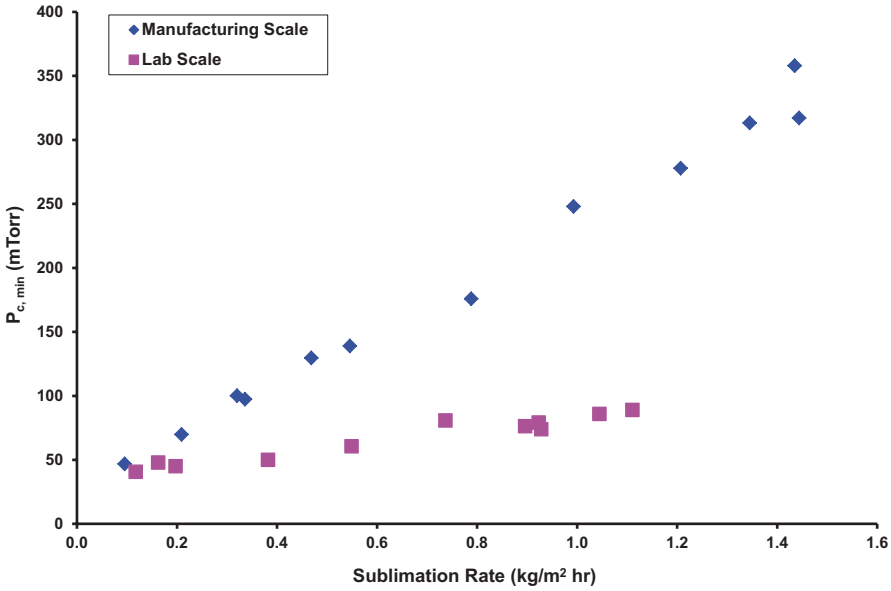

Fig. 14.1 Minimum achievable chamber pressure versus sublimation rate

and chamber pressure). Clearly, at low sublimation rates (<0.2 kg/m^2 h) the minimum achievable chamber pressure was similar for the lab and production scale dryer; however at high sublimation rates (≥0.2 kg/m^2 h), the difference was significant (Fig. 14.1). The minimum achievable chamber pressure was approximately 100 mTorr for a sublimation rate of approximately 0.4 g/h/cm^2 with the current formulation. Hence, the chamber pressure set point was >100 mTorr to ensure process control. Also, as widely published in the literature, there was no added advantage to have the chamber pressure >250 mTorr during the drying step. With these limits, the operational space for chamber pressure was between 100 and 250 mTorr (Fig. 14.2).

The vial heat transfer coefficient for 10 cc, 20 mm vials was determined gravimetrically on both the lab and production scale dryers over a pressure range from 60 to 250 mTorr (Tables 14.4 and 14.2).

Clearly, the vial heat transfer coefficient for edge vials is at least 20% higher than that for center vials (Rambhatla and Pikal 2003). Also, the vial heat transfer coefficient on a lab scale is relatively higher than that for a pilot scale (Fig. 14.3). Thus, for a given combination of shelf temperature and chamber pressure, product temperature would be lower on the production scale freeze-dryer compared to the lab scale. The vial heat transfer coefficient can be expressed mathematically for each dryer as follows (Pikal et al. 1984; Tang and Pikal 2004):

$$K_v = KC + \frac{KP \cdot P_c}{1 + KD \cdot P_c} \tag{14.1}$$

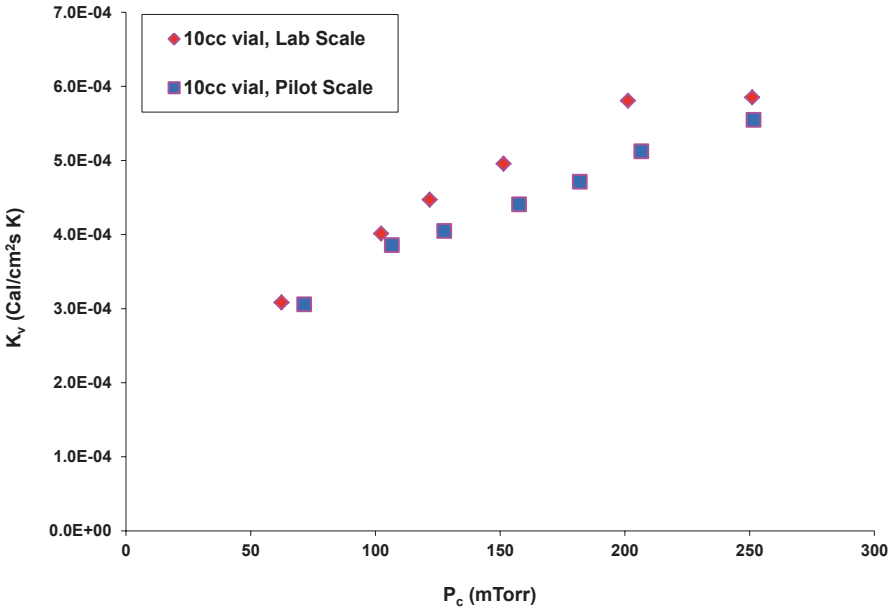

Fig. 14.2 Vial heat transfer coefficient versus chamber pressure

Table 14.4 Comparison of vial heat transfer coefficient for 10 cc, 20 mm vials on lab and production scale dryer at Pc = 150 mTorr

Vial location	Vial heat transfer coefficient, K_v ($\times 10^4$ cal/cm^2 s K)	
	Lab scale dryer	Production scale dryer
Edge	3.24 ± 0.08	2.85 ± 0.04
Center	2.15 ± 0.03	1.69 ± 0.03

where KC is the sum of heat transfer by contact conduction (K_c) and the radiation (K_r), *KP* and *KD* are constants (see Tang and Pikal 2004 for more details) and P_c is the chamber pressure.

Additionally, the shelf surface temperature mapping was performed to identify any hot or cold spot and the maximum and minimum in the heating and cooling rate that could be achieved on the lab and production scale dryers (Rambhatla et al. 2006). Both dryers were able to achieve a heating and cooling rate of up to 1.5 °C/min; however, the temperature gradient across the shelves was minimal for a ramp rate of ≤ 1 °C/min. Hence, a ramp rate of ≤ 1 °C/min defined the operational limit of the freeze-dryers during the freezing and drying step.

Lastly, the product resistance was determined on the lab scale freeze-dryer using MTM (manometeric temperature measurement). During MTM, the product chamber was isolated briefly (about 25 s) and pressure rise was recorded as a function of time (Tang et al. 2006). The MTM equation was then fitted to pressure rise data that yields product temperature, product resistance and vial heat transfer coefficient. In

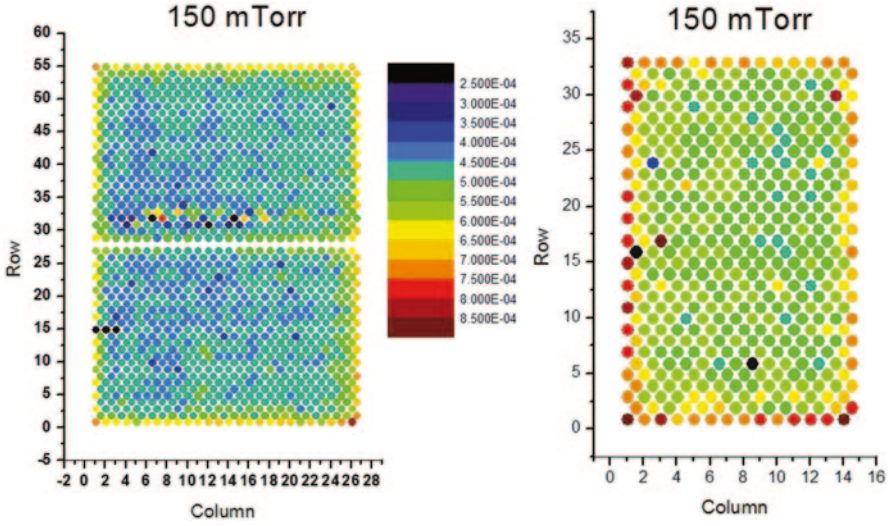

Fig. 14.3 Vial heat transfer coefficient mapping on production (*left*) and lab (*right*) scale freeze-dryer

the lab, ice typically nucleated at about −13 °C. However, in the production scale freeze-dryer, ice nucleated at about −20 °C based on historical data from previous batches. An annealing step during freezing was introduced to minimize drying heterogeneity due to differences in ice nucleation temperature. The specific surface area as measured by BET for product from lab and production freeze-dryer was comparable (that is, 0.5 m^2/g) suggesting that annealing was effective in minimizing freezing differences between the lab and production scale freeze-dryers. The product resistance was expressed by Eq. 14.2 (Pikal et al. 2005) and the average product resistance over the ice thickness was about 3 cm^2 Torr h/g.

$$\hat{R}_p = R_0 + \frac{A_1 \cdot l}{1 + A_2 \cdot l} \tag{14.2}$$

where R_0 (=2.2), A1 (=3), A2 (=2) are constants and is the dry layer thickness.

During the freezing step, the ice nucleation temperature is an important scale-up issue due to differences in the particulate matter between the production (class 100) and lab environment. The ice nucleation temperature governs the pore structure formed in the dried cake. Therefore, a higher degree of supercooling resulted in a higher product resistance and, hence, a longer drying time. Annealing during the freezing step (as discussed earlier) was performed to remove the heterogeneity in the ice nucleation temperature. Annealing time and temperature was optimized to achieve a uniform drying rate within the batch and also between different dryers. Additionally, with annealing the overall drying time was reduced by at least 15 %. An annealing temperature of −12 °C, which is approximately 9 °C above Tg', with

Table 14.5 Effect of freezing rate

Sample	Defect rate (%)	Sublimation rate (mg/vial/h)[a]	Moisture (%)[b]	Reconstitution time (m:ss)[c]
Slow freeze, 0.1 °C/min	0	0.38	0.3	1:14
Fast freeze, 1 °C/min	0	0.41	0.8	1:00

[a] Based on linear regression fit for average weight loss/vial after 1, 2, 4 and 6 h of primary drying (weight loss of 3 vials averaged per time point)
[b] Averages of 8 vials
[c] Averages of 5 vials

a 2 h hold time was selected based on the freeze-dry microscopy screening studies and previous experience with mAbs Y and Z. Mannitol crystallinity in the lyophilized cake was confirmed by polarized light microscope and XRPD. The final freezing temperature was selected to be −40 °C, which is 20 °C lower than the Tg' of the formulation, with a hold time (typically ≥ 2 h) sufficient to allow complete freezing of the solution. A freezing rate of 0.5 °C/min was within the capabilities of the freeze-dryer with minimal temperature gradient across the shelf on both lab and production scale dryers. Also, the vials were loaded on the shelves at room temperature since there were no stability concerns with this molecule at room temperature up to 72 h.

In-house data exist on freezing step optimization with similar molecules. Variations in freeze ramp rate, without the annealing step, may have potential for product impact since the freezing rate can affect the morphology of the ice formed during the freezing step. Impacts to ice morphology can potentially affect sublimation rate, final moisture content, cake appearance, and reconstitution time. Experiments were conducted at two different freezing rates, 0.1 and 1 °C/min, with the annealing step as described above. No significant differences were observed between vials produced using the two different freezing rates in terms of sublimation rate, product temperature, drying time, cake appearance, and reconstitution time (Table 14.5). In both cases, the moisture content was well within the product release specification of ≤ 3 %. Thus, the study demonstrated that, within the desired operating range, the freezing rate has minimal effect on the process and product attributes. Based on this outcome, further characterization of the freezing step in combination with primary and secondary drying parameters was deemed unnecessary.

Although separate characterization studies were not carried out for the final freezing temperature, it is well understood that the shelf temperature for the final freezing temperature should be well below the glass transition temperature of the formulation. Accordingly, for the mAb X formulation, −30 °C was chosen as the upper limit for the shelf temperature, as −30 °C was well below the glass transition temperature of the formulation (−21 °C). Thus, for mAb X, the freezing steps were as described in Table 14.6.

14.4 Mathematical Model Based on Heat and Mass Transfer

A mathematical model was developed to predict product temperature and drying time during primary drying based on heat and mass transfer characterization of the equipment, container-closure system, and the formulation. The primary drying step of the lyophilization process was modeled using the first principles of heat and mass transfer as described in (Pikal et al. 1984). The sublimation rate, dm/dt, can be described as:

$$\frac{dm}{dt} = \frac{A_{\mathrm{p}}\left(P_0 - P_{\mathrm{c}}\right)}{\hat{R}_{ps}} \tag{14.3}$$

where, A_{p} is the vial internal cross-sectional area of the vial, $\hat{R}_{ps}$ is the total area normalized product and stopper resistance and P_0 is the vapor pressure of ice, which can be calculated from Eq. 14.4,

$$\ln P_0 = -\frac{6144.96}{T} + 24.0185 \tag{14.4}$$

where T is the temperature at the sublimation interface.

The heat transfer from the vial to the product is calculated from Eq. 14.5,

$$\frac{dQ}{dt} = A_v K_v \left(T_s - T_{ice} - \Delta T\right) \tag{14.5}$$

where dQ/dt is heat transfer rate (cal/hour per vial); A_{v} is the vial external cross-sectional area (cm^2); T_{s} is the shelf surface temperature (K), T_{ice} is the temperature of the ice at the sublimation interface (K), T is the temperature difference across the frozen layer; and K_{v} is the heat transfer coefficient of the vials.

The heat and mass transfer is coupled by Eq. 14.6,

$$\frac{dQ}{dt} = \Delta H_s \frac{dm}{dt} \tag{14.6}$$

where ΔH_s is the heat of sublimation.

Based on the heat and mass transfer equations described above, the input parameters for the model are:

1. Shelf temperature
2. Chamber pressure

Table 14.6 Freezing steps for mAb X

T_s (°C)	Rate (°C/min)	Time (min)
23	NA	NA
5	0.5	36
5	NA	15
−5	0.5	20
−5	NA	15
−40	0.5	70
−40	NA	120
−12	0.5	56
−12	NA	120
−40	0.5	56
−40	NA	120
Total freezing time (min)		644

NA not applicable

3. Parameters describing correlation of vial heat transfer coefficient with chamber pressure
4. Parameters describing correlation of product resistance with dry layer thickness
5. Internal and external cross-sectional area of the vial
6. Fill volume
7. Total solids

The outputs from the model are the sublimation rate, product temperature, and drying time for the primary drying step of the lyophilization process. This model was validated on several in-house molecules to ascertain that the model was robust against varying formulation and process conditions to predict the drying differences between the dryers.

14.5 On-line Process Monitoring (PAT) and Control Strategy

14.5.1 Primary Drying

Product temperature is a critical process parameter for the freeze-drying process. During primary drying the product temperature should be below the maximum allowable temperature (that is, Tg' or Tc for an amorphous system or Teu for a crystalline system). Drying above the maximally allowable temperature results in product collapse or melt back, which could further affect product stability. Historically, product temperature has been monitored using thermocouples. However, vials containing thermocouples freeze-dry differently than most of the vials in the freeze-dryer (that is, vials without thermocouples). They nucleate at a higher temperature,

resulting in lower product resistance and, hence, faster primary drying. Further, in production dryers, thermocouples are placed in edge vials to ensure product sterility and, as shown earlier, an edge vial sublimes faster (approximately 15 %) than the center vials. Thus a thermocouple is a destructive, single-vial (that is, not representative of the entire batch) technique.

An emerging technique to monitor the freeze-drying process is tunable diode laser spectroscopy (TDLAS) (tuneable diode laser absorption spectroscopy). The technique is now commercially available on lab as well as production scale freeze-dryers. A TDLAS unit is typically mounted in the duct connecting the chamber and the condenser (Gieseler et al. 2007). A beam of laser is transmitted across the duct and output wavelength of the laser is tuned across the molecular absorption feature of the gas present in the duct. The area under the absorption line shapes is integrated and related to the concentration of water vapor. TDLAS also measures gas-flow velocity based on Doppler-shifted water vapor absorption spectrum. The sublimation rate is calculated using Eq. 14.7 based on the gas velocity (u), gas density (ρ), and the cross-sectional area of the duct (A).

$$\frac{dm}{dt} = \rho \cdot A \cdot u. \tag{14.7}$$

In this case study, monitoring of both the product temperature and the drying time, which are critical process parameters, was accomplished by TDLAS. The product temperature was monitored in real time (Fig. 14.4) based on vial heat transfer coefficient and the sublimation rate measurement by TDLAS (Schneid et al. 2009).

The end of primary drying was defined during the time at which the water vapor concentration in the duct drops, indicating that the residual water in the vials is low enough to progress into secondary drying (Patel et al. 2010b). With the current formulation, the water vapor concentration of $\leq 5 \times 10^{14}$ molecules/cc results in residual water content of ≤ 16 % on a lab scale dryer (Fig. 14.5). Thus, the end of primary drying was monitored and controlled in real time rather than relying on a fixed time measurement.

Primary drying time is scale and batch size dependent (Patel et al. 2010c), but since the drying time was determined in real time, the product was dry enough before progressing into secondary drying. Additionally, a Pirani gauge was used as an orthogonal method to monitor the end of primary and secondary drying on lab as well as production scale freeze-dryers (Patel et al. 2010b). The ramp to secondary drying was initiated when the Pirani pressure was within 10 mTorr of the chamber pressure as measured using capacitance manometer. Although not quantitative, the Pirani gauge is relatively cheap batch technique to monitor the end of primary drying.

14.5.1.1 Effect of Fill Volume

The fill volume (5.5 mL in this case study) is a potential critical fill-finish parameter that could impact freeze-drying process performance (primarily primary drying

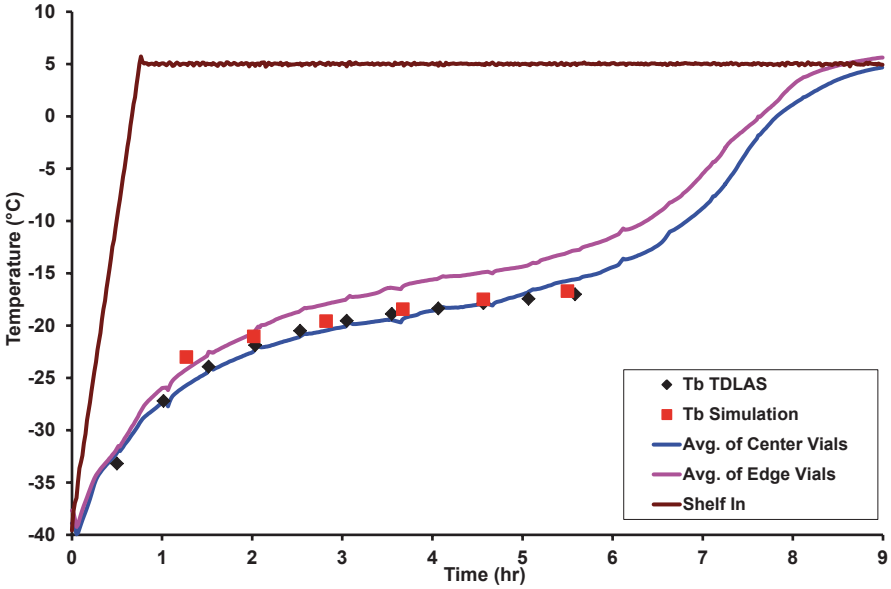

Fig. 14.4 Theoretical and experimental product temperature profile

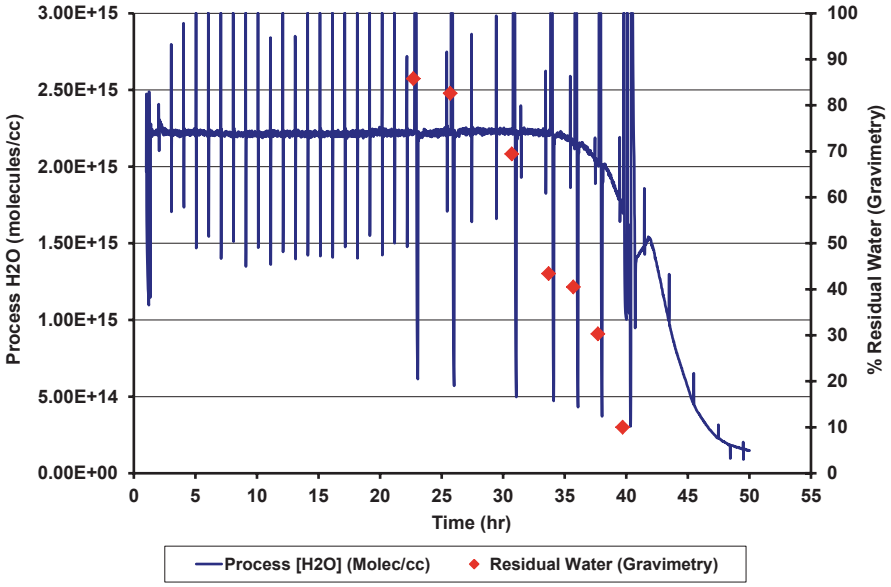

Fig. 14.5 TDLAS water vapor concentration profile along with residual water during primary drying. The *spikes* are due to MTM measurement. Reprinted from Ref. Patel et al. 2010b with permission from AAPS

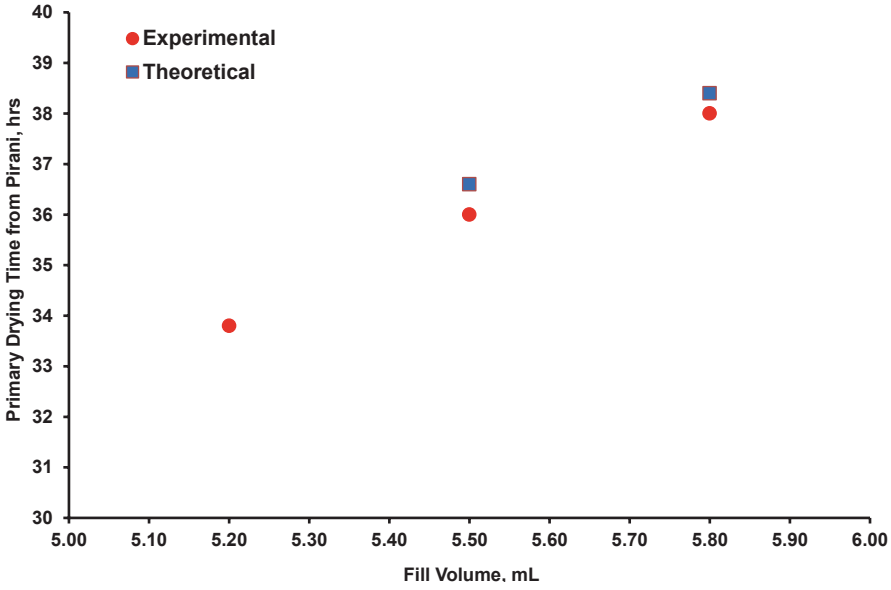

Fig. 14.6 Effect of fill volume on primary drying time

time). Generally, higher fill volume will result in higher cake height and hence longer drying time. However, for the typical fill volume variability (±3–5%) during fill finish, the impact is minimal for all practical purposes as shown in Fig. 14.6. Also, the mathematical model was able to accurately predict the effect of fill volume on primary drying time. Additionally, during early development, the drug product presentation requirement changes frequently since the dose is not decided for the clinical trial until key milestone is achieved. The likely dose range is often supported by varying the fill volume, in which case the effect of fill volume on process performance becomes even more critical. Mathematical modeling is very helpful in such scenarios to evaluate the potential impact of changing the fill volume on primary drying time as demonstrated in Fig. 14.6. With this approach, the time and material requirements are significantly reduced when supporting broad dose range by bracketing the fill volume.

14.5.2 Secondary Drying

TDLAS was used for *in situ* monitoring of residual water in real time during secondary drying (Schneid et al. 2011). The sublimation rate measured by TDLAS during primary drying was accurate within 5% of the gravimetrically measured sublimation rate and, hence, a simple integration of sublimation rate during primary drying cannot be used to determine the amount of water left at the end of primary drying. Thus, a series of calibration curves were generated on a laboratory scale

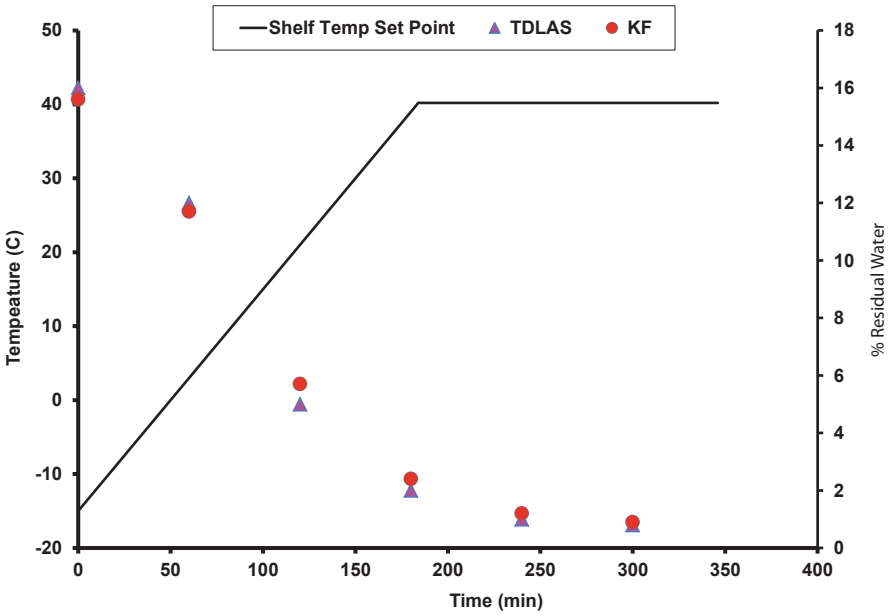

Fig. 14.7 Residual water comparison between TDLAS and KF during secondary drying. Temperature is represented by solid *black line*, whereas the residual water is represented by *circle* (KF) and *triangle* (TDLAS)

dryer relating drying rate with residual water content and temperature. To generate the calibration curves on the lab scale dryer, product was sampled from the chamber, using the sampling thief assembly, to determine residual water (by Karl Fischer) at the end of primary drying, which was also the initial residual water content for the start of secondary drying. Additionally, product was periodically sampled during secondary drying to determine residual water, which was correlated with drying rate (g/s), as determined by TDLAS. During the engineering runs, the residual water at the end of primary drying was determined from the TDLAS drying rate and the laboratory calibration curves. Given the water content at the start of secondary drying, the water content at any point in time was calculated from starting water content and the integrated secondary drying rate determined by TDLAS. There was good agreement between TDLAS and Karl Fischer residual water at the lab and production scale dryers (Fig. 14.7). For the current formulation, the end of secondary drying was defined during the time at which residual water was $\leq 0.5\,\%$. Thus, instead of using a fixed cycle time, TDLAS was used to monitor and control not only primary drying but also secondary drying.

Currently TDLAS is more commonly used as a process monitoring tool and there is no feedback loop to control the process. However, simple modifications can be easily made to the freeze-dryer software to integrate TDLAS output in order to control the process. TDLAS is a versatile tool in developing a robust freeze-drying process with minimal time and material.

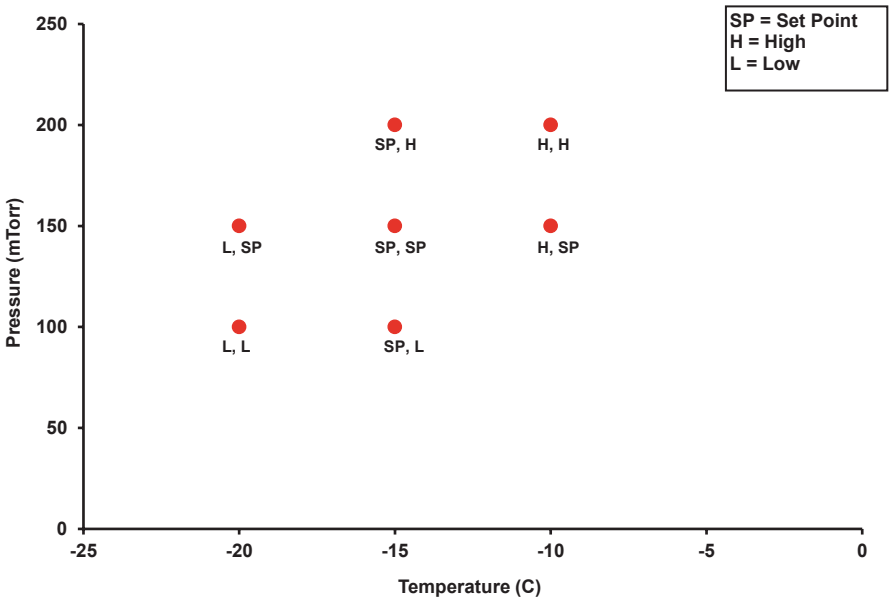

Fig. 14.8 Freeze-drying process robustness

14.6 Process Robustness

The lyophilization process can be well modeled based on the fundamental understanding of heat and mass transfer. First, the limitations and capabilities of the freeze-dryer were identified to define the operational space. Practical limits were applied based on literature and in-house data within this operational space to identify the knowledge space. Further, the heat and mass transfer principles were used to model the primary drying phase of the lyophilization process (as described above) to define the design space.

The chamber pressure, ramp rate, shelf temperature, and drying time are the parameters that define the primary drying step of the lyophilization process. The chamber pressure was set to 150 mTorr which is about 30% of the vapor pressure of ice at −21 °C and is approximately 50 mTorr higher than the minimum achievable chamber pressure of 100 mTorr at a sublimation rate of 0.4 kg/m^2 h. A ramp rate of up to 0.5 °C/min resulted in elegant cake appearance and hence a ramp rate of 0.2 °C/min (that is, 125 min ramp time) was used to advance to the primary drying step. This ramp rate offered an appropriate safety margin to account for any differences in ramp rate between different dryers. Based on the mathematical model, a shelf temperature of −15 °C and a chamber pressure of 150 mTorr resulted in product temperature of about −21 °C. Drying time was monitored in real time by TDLAS and the Pirani gauge as described above. Typically, a total of seven experiments would have been conducted to ensure process robustness as described in Fig. 14.8.

Table 14.7 Theoretical and experimental product temperature and drying time during primary drying phase of the lyophilization process on lab scale freeze-dryer at 50% of full load

Ts	Pc	Tp (theoretical, experimental)	Drying time (theoretical, experimental)	Comments
−10 (high)	200 (high)	−19.6, −19.2	32, 33	Highest product temp and shortest drying time
−15 (set point)	200 (high)	−20.6, −20.5	36, 36.5	Higher product temperature and shorter drying time
−10 (high)	150 (set point)	−20, −20.1	34, 34.2	
−15 (set point)	150 (set point)	−21, −21.4	38, 37	Set point conditions
−20 (low)	150 (set point)	−22, −22.5	42, 43	Lower product temperature and longer drying time
−15 (set point)	100 (low)	−21.5, −21.6	39.5, 40	
−20 (low)	100 (low)	−22.4, −22.6	43, 43.5	Lowest product temp and longest drying time

However, with the mathematical model approach (Patel et al. 2010d), only three experiments were performed: at the set point, high and low shelf temperature and chamber pressure conditions. Table 14.7 describes theoretical and experimental results for product temperature and drying time.

For the condition where the shelf temperature and chamber pressure is higher than the set point, the product temperature may exceed the maximum allowable temperature during primary drying and may result in loss of cake appearance. On the other hand, for the condition where the shelf temperature and chamber pressure is lower than the set point, drying may not be complete before advancing into secondary drying, which could also result in collapse or melt back (that is, loss of cake appearance). Hence, the drying time should be long enough to ensure completion of the primary drying, but not so long to result in an unnecessarily long drying cycle. Similarly, shelf temperature should be low enough to maintain product temperature below the maximum allowable temperature and high enough to result in the shortest possible cycle time with an appropriate safety margin. Under all conditions evaluated, product temperature was still below the formulation Tc (−18 °C) and no change was observed in residual water, reconstitution time, cake appearance, and stability profile post lyophilization. Since the radiation heat transfer is relatively less on a production scale dryer, a slightly higher shelf temperature on production freeze-dryer will result in the same product temperature profile and drying time as on a lab scale freeze-dryer. Engineering runs were performed on a production scale dryer at 100% of full load with a drying time of 46 h as indicated by TDLAS and the Pirani gauge; therefore, the primary drying time was fixed at 50 h (adding about

Table 14.8 Parameters for primary drying step

T_s (°C)	Rate (°C/min)	Pc (mTorr)	Time (min)
−15	0.2	150	125
−15	N/A	150	3000

10% as safety margin to address freezer-dryer scale difference) as a contingency option in the event when the Pirani and TDLAS failed to monitor and control the process. Primary drying process parameters are summarized in Table 14.8.

14.7 Secondary Drying

Similar to the primary drying step, ramp rate, shelf temperature, chamber pressure, and drying time are the parameters needed to define the secondary drying step of the lyophilization process. From previous experience with a similar formulation and drug product presentation, a ramp rate of 0.5 °C/min resulted in minor cake defects. Accordingly, a ramp rate of 0.3 °C/min (183 min ramp time) was selected, which offered an appropriate safety margin for secondary drying ramp rate. The chamber pressure was kept the same as that during primary drying (150 mTorr) since there is no added advantage of changing the chamber pressure for secondary drying (Pikal and Shah 1997) based on in-house data with similar formulation and drug product presentation.

Small-scale studies were performed to evaluate the effect of shelf temperature (25, 35, and 45 °C) during secondary drying on residual water. Vials were pulled at regular intervals during secondary drying using a sampling thief to determine residual water by Karl Fischer. As expected, at a 40 °C shelf temperature, the residual water dropped faster when compared to 25 °C. Also, post lyophilization, there was no change in cake structure and product purity (Tang and Pikal 2004). Hence, shelf temperature was set to 40 °C and the end of secondary drying was marked when the residual water content dropped to < 1% as indicated by TDLAS. On a production scale dryer with a full load, a drying time of about 240 min resulted in residual moisture of < 1%. However, at 25% of full load, residual moisture dropped to < 1% in 200 min. Overall, there was not much effect of load on secondary drying and, hence, drying time was fixed at 300 min as a contingency option in the event that Pirani and TDLAS failed to monitor and control the process. Secondary drying process conditions are summarized in Table 14.9.

Table 14.9 Parameters for secondary drying step

T_s (°C)	Rate (°C/min)	Pc (mTorr)	Time (min)
40	0.3	150	183
40	not applicable	150	300

Table 14.10 Effect of headspace pressure on reconstitution time

Headspace pressure (Torr)	Reconstitution time (m:ss)
550	1:07
650	1:13
750	1:14

The results are averages of five samples rounded to the nearest second

The final shelf temperature for storage was set to 5 °C and the vials were stoppered under partial vacuum at 650 Torr. Systematic studies were conducted to evaluate the effect of headspace pressure as described below.

14.8 Effect of Headspace Pressure

Lyophilized vials are frequently stoppered under vacuum to aid reconstitution. Based on initial development studies, the target headspace pressure for mAb X was selected to be 650 Torr. To evaluate the impact of vial headspace pressure on reconstitution behavior, a study was performed at the upper and lower extreme of the headspace pressure. As shown in Table 14.10, the headspace pressure had no significant impact on the reconstitution time.

14.9 Acceptable Range for the Freeze-drying Parameters

Based on the process knowledge, risk assessment and data from the process characterization studies, acceptable ranges for the various lyophilization process parameters were established (Table 14.11).

Table 14.11 Acceptable ranges for the lyophilization process parameters

Process parameter	Acceptable pange	Set point
Freeze ramp rate,°C/min	0.1-1	0.5
Freeze hold temperature,°C	≤−30	−40
Freeze hold duration, h	≥2	2
Primary drying ramp rate,°C/min	≤0.5	0.2
Primary drying shelf temperature,°C	−20–10	−15
Chamber pressure, mTorr	100–200	150
Primary drying duration, h	≥50	Real time monitoring and control (Pirani and TDLAS)
Secondary drying ramp rate,°C/min	<0.5	0.3
Secondary drying shelf temperature,°C	35–45	40
Secondary drying duration, h	4–6	Real time monitoring and control (TDLAS)
Head-space pressure, Torr	550–750	650

14.10 Lyophilization Process Design Space

14.10.1 Freezing

The freezing step for mAb X as shown in Table 14.6 would result in complete freezing of the solution and having the annealing step will minimize freezing heterogeneity within the batch. The acceptable range for freezing step is shown in Table 14.9.

14.10.2 Primary Drying

Optimization of primary drying step is critical to minimize the total freeze-drying cycle time. The heat and mass transfer principles along with characterization of formulation, dryer, and container closure system can help develop the design space for the primary drying step. As mentioned earlier, the lower limit of pressure control for a sublimation of 0.4 kg/m^2 h was 100 mTorr, and there is no process gain for chamber pressure >250 mTorr. Thus, the operational limit for chamber pressure for current formulation and presentation is between 100 and 250 mTorr. The choked flow or condenser overload limit further imposes a limit on the sublimation rate supported by lab and production scale dryer. Therefore, any process conditions resulting in sublimation rates in the choked flow regime (pink shaded region in Fig. 14.9) would result in "run-away" process (that is, the chamber pressure would be out of control).

Further, the maximum allowable product temperature (black dashed line Tp = −21 °C in Fig. 14.9) imposes the upper limit on product temperature above which the product would lose the cake structure. However, running a process that would result in product temperature much below the maximum allowable temperature would unnecessarily cost additional time and resources. Hence, a lower limit for product temperature is imposed (black dashed line Tp = −24 °C in Fig. 14.9) to avoid an undesirably long processing time. However, one does not control product temperature directly; rather it is the shelf temperature and chamber pressure which indirectly control product temperature. Hence, the shelf temperature isotherms (circle and diamonds) further define the chamber pressure and shelf temperature combinations that could potentially be used to achieve a product temperature between −21 and −24 °C. The region shaded in green is defined as the Design Space whereas the region shaded in blue is defined as the Control Space, which is a subset within the Design Space (Fig. 14.9). Thus, the primary drying process parameter set point (red dot in Fig. 14.9) should be selected within the Control Space and an appropriate safety margin should be added to define the Design Space. Within the Design Space, the process is always under control and is also optimized for product temperature and drying time.

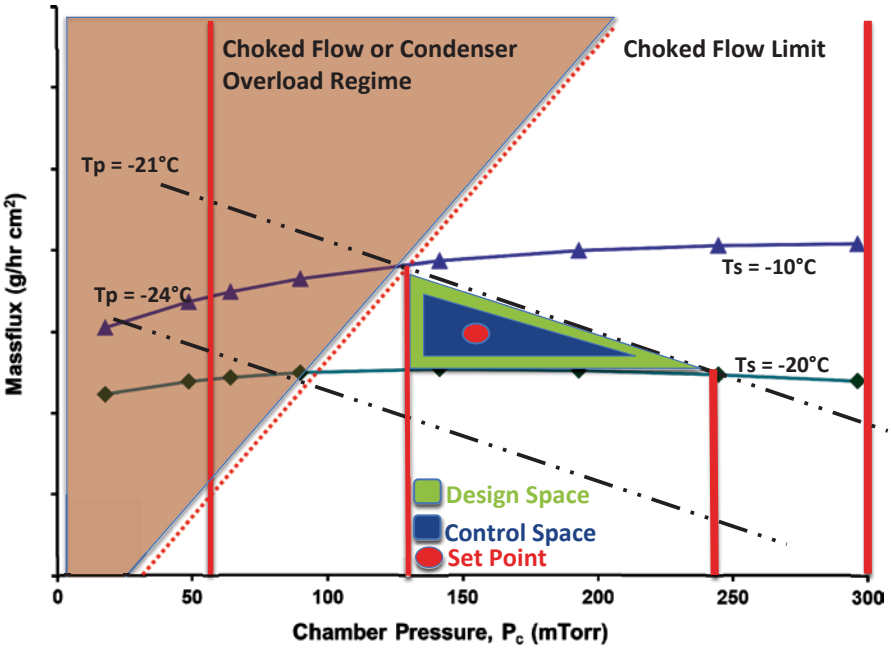

Fig. 14.9 Design and control space for the primary drying step of the freeze-drying process. Reproduced from Ref. Patel et al. 2013), with permission from J. Wiley & Sons, Inc

14.10.3 Secondary Drying

During secondary drying, the ramp rate, shelf temperature, and the hold time are the critical process parameters to ensure that product meets the quality attributes at the end of the freeze-drying process. The acceptable range for these parameters is listed in Table 14.9.

14.11 Summary

This *mock case study* illustrates the application of QbD principles for freeze-drying process design and development. Several tools are now available to monitor and control the freeze-drying process in real time so that the quality can be built within the process rather than monitoring offline at the end of the process. The Design and Control Space can be developed for the process with the help of PAT, mathematical modeling, and complete characterization of formulation, equipment, and container-closure system. Formulation characterization governs the process development, whereas processing conditions along with formulation governs the product quality. Therefore, formulation and process understanding is critical to develop a

robust lyophilization process that is scale and site independent. A process Design Space can only be claimed when systematic studies are performed demonstrating the understanding of the impact of process parameters on product quality attributes. Once a Design Space is claimed, the process parameters can be changed within this space without requiring any further regulatory filing. Additionally, the ability to assess and predict the impact of process parameters on product quality will help with tech-transfer activities when changing sites, scales, or batch size and also addressing process deviations (in temperature and pressure) due to equipment failure.

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Chapter 15
Visible and Subvisible Protein Particle Inspection Within a QbD-Based Strategy

Erwin Freund and Shawn Cao

15.1 Introduction

The expectation is using early product design as basis for the desired in vivo performance of new protein therapeutics by defining it with the Quality Target Product Profile (QTPP). This integration is the basis of a proactive science based process development. This leads to a drug product with improved attribute controls. For example, an essential part of the safety and efficacy evaluation for a drug product is achieved through monitoring of clinical trials and drug product attribute testing per defined appearance specification parameters of the therapeutic product. Clinical trial material must be tested for meeting relevant specifications and must pass the criteria for all Critical Quality Attributes (CQA) of the drug product. In case of recombinant proteins, these CQA's cover several categories, including safety (e.g., pyrogenicity, sterility, residual DNA); potency (e.g., protein concentration and biological activity); dose (e.g., injectable volume); identity (e.g., correct isoform distributions if applicable); purity (e.g., by setting maximum levels on impurity levels of host cell proteins, % oxidation, and % high molecular weight/aggregates); and appearance (e.g., color, clarity or turbidity, particles, pharmaceutical elegance, and functionality). The focus of this chapter is on inspection with respect to both visible and subvisible particulate matter as it presents a risk to drug product quality (Langille 2013).

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15.1.1 Types of Particles

Particles can be classified in different ways; and one of them is by their origin. In a parenteral drug product, particles are either composed of foreign matter or derived or related to the drug product active ingredient as in this case, the recombinant protein.

Foreign matter can be separated into three categories: extrinsic, intrinsic, and inherent. Intrinsic matter particles are capable of change and associated with the drug product container/closure (including delivery systems such as prefilled syringes with silicone oil), excipients and process or assembly process, but are insufficiently removed by the washing process. Extrinsic or extraneous particles are defined per USP <788>"as mobile undissolved particles, other than gas bubbles, unintentionally present in solutions." Extrinsic particles are additive, foreign, and are not involved in the formulation, packaging, or assembly process. Examples of extrinsic material include fibers, cellulosic matter, vegetative matter, corrosion products, paint/coatings, and building materials such as gypsum, concrete, metal, and plastic. Extrinsic particles generally do not change over the life of the product, unless by fragmentation, swelling (hydration), or degradation. Fragments of rubber, plastic, metal, and glass are examples of extrinsic particulate matter deposited in the product during assembly or not removed in the container/closure preparation process. However, if these typically extrinsic types have come from the specific container/closure and/or process in a more consistent or chronic manner, then one may consider their presence to be an intrinsic variety, with a similar level of concern.

Nano-particles are not routinely monitored at the present time and the FDA has not specified a preferred method (Susan 2014) This is out of scope for this chapter.

In 2014 the USP published chapter <787> covering subvisible particulate matter in injections as alternative to the general USP chapter <788> and specifically addressed therapeutic protein injections and analysis permitting use of smaller test volumes. "Particulate matter in therapeutic protein injections consists of mobile undissolved substances that may originate from various sources. The particles may be (a) truly foreign, or "extrinsic," e.g., unexpected foreign material, such as cellulose; (b) "intrinsic" resulting from addition or by insufficient cleaning during manufacturing, such as tank metals or gaskets, lubricants, filling hardware, or resulting from instability, e.g., changes over time, such as insoluble drug salt forms or package degradation; and (c) "inherent", such as particles of the protein or formulation components."

Inherent particulate matter applies to particles related with the active pharmaceutical ingredient (API); and in the case of therapeutic protein products, it refers to particles formed as a result of protein aggregation, which can be captured and leveraged in the form of the (WIN or 'what is normal') report that covers the typical appearance of protein aggregation and provides a minimum qualitative data as a function of time, since, the substance may be present not evident until particles form over time, even long after lot release.

Particles composed of protein aggregates can also be classified, based on their sizes, into oligomers (sometimes called soluble aggregates, and often analyzed by size exclusion chromatography), submicron or nanometer aggregates, micron aggregates (often called sub-visible particles, in the 2–100 micron-meter size range), and visible aggregates or particles (100 micron or above, Narhi 2012). Morphology, chemical modifications and dissociation/reversibility are other characteristics that are important in classifying and understanding root cause and risk especially for the SbVP (subvisible particles).

In contrast, the presence of extrinsic particles is a reflection of lack of adequate control of the manufacturing environment, in contrast to the presence of intrinsic particles which reflects the cleanliness/stability of containers and effectiveness of filtration steps. The presence of inherent protein particles reflects the stability of the formulated drug API. In this chapter the focus is on protein particles, though intrinsic and extrinsic particles are also included in some of the discussions as needed and when appropriate.

15.1.2 Purpose of Manual or Automated Inspection for Particles

15.1.2.1 Visible particles.

The 100% visual inspection process is among the final units of operation (nondestructive) and executed on the entire lot to remove visible defects that span a range of cosmetic and particulate flaws and color and clarity. The inspection process is not a sorting process but a continuous verification of the proper control of the incoming components, environmental infrastructure, and manufacturing procedures. The need for the appearance test is to ensure that the current Good Manufacturing Practices (cGMP) are effective and that the products produced are safe and effective by meeting specific requirements for identity, strength, quality, and purity. The inspection process outcome reduces process and product variation. Current practices based on the QbD approach require a detailed process and product understanding. The attributes of the visual inspection are not captured by the typical biochemical or biophysical tests on individual samples and test for the soluble entities that are homogeneously distributed among the lot. Appearance covers cosmetic defects that range from minor defects such as container surface scratches to major or critical defects such as cracked containers or deformed container closures. Particle or aggregate control in biological drug products represents one of the ongoing manufacturing and long-term stability challenges (Das 2012). Specifically, concerns are related to safety (immune toxicity and cytotoxicity), efficacy (aggregates can be sub- or super-potent) and pharmacokinetic (PK) behavior where sub-visible aggregates can slow release rates. The objective is achieving optimal manufacturing efficiency demonstrated by low false rejects and capture of all true defects coupled with the ability to do root cause analysis and trend defects by category. Information regarding the causes of rejection is critical to enable continuous improvement of manufacturing.

15.1.2.2 Subvisible Particles

The purpose is to measure and control this attribute, though rather than call it an inspection it is referred to as analysis because the tests are destructive and executed on a limited number of samples for release and without the benefit of automation.. Methods are described in Sect. 2.3 and in the USP <787> chapter.

15.1.3 Particle Analysis

Due to the wide range of particle size, from nanometer to microns to millimeters, no single analytical technology currently exists that can cover the whole size range. Instead, particle characterization in terms of sizing and counting (Cao et al. 2009; Doessegger et al. 2012) is generally done by dividing this size range into manageable subranges as described above (i.e., oligomers, nano-size range, subvisible range, and visible range), and applying different technologies (Wang et al. 2013) that are appropriate for each size range. For examples nano-size particle characterization, light scattering based technologies have been traditionally used, and some recently developed new technologies such as nanopore-based sensing (IZON, www.izon.com/media/publications/), nanoparticle tracking analysis (nano-sight by Malvern instruments), and resonant mass measurement (Archimedes by Malvern instruments), are showing promises. For subvisible particles, light obscuration has been the technology originally developed and deployed for this purpose, and recently dynamic imaging analysis (DIA, Oma et al. 2010) based techniques such as the micro flow imaging (www.proteinsimple.com) and FlowCam (www.fluidimaging.com) are showing great promise and providing additional particle morphology information. Note that all the aforementioned tests are destructive assays in contrast to measurements in the visible size range using either manual visual inspection or machine based inspection. Particle isolation (Wen et al. 2013; Ripple et al. 2012) by filtration and then characterization by microscope and spectroscopy is often done to complement these routine analyses, particularly when there is a need to identify the type or identity and origin of the particle or nature of the aggregate, (Narhi 2009; Susan 2014).

Several gaps exist in the area of visual inspection. As of today, nondestructive automated quantitative instruments are not yet available. Instead, the industry is limited to a binary characterization defined as accept or reject. Challenges remain on issues such as what size is defined as visible; establishing a correlation of particle size, number, and aggregation chemistry with clinical safety/efficacy outcome (Doessegger et al. 2012) and the trigger for an antidrug antibody response. In the area of analytical instrumentation, orthogonal methods require appropriate reference standards (i.e., protein-like particle standards) and sampling for a quantitative comparability assessment. These protein-like standards are not currently available (Ripple et al. 2011). In addition to these complications there are no universal guidelines that apply to all regulatory jurisdictions. As a result, inspection of containers filled with therapeutic proteins remains one of the last analytical frontiers that still challenge routine 100 % nondestructive inspection.

15.2 Regulatory Expectations, Impact, and Considerations

For particles, it is an expectation that they are addressed first by preventive action and secondly by corrective action (resulting in inspection rejects), with a focus on addressing the upstream sources of particles which necessitates establishing the identity. A second driver is the expectation by the regulatory agencies to that the particulate in a product be not only reduced, but controlled, including those in the subvisible range as part of QbD expectations (Martin-Moe et al. 2011). Demonstration of control by merely passing three consecutive runs as part of traditional validation was replaced in the USA as of 2011 by a new concept referred to as continuous process verification (CPV, Guidance for the Industry Process Validation 2011) that includes monitoring, statistical tracking, and evaluation of CQAs such as particle loads to ensure quality, as driven by ICH Q8, 9, 10, 11, (Korakiaiti et al. 2011) and the FDA guidance on Process Lifecycle after Performance Qualification. This process in turn enables ongoing continuous improvement, which is a part of the product life cycle process and provides for possible flexibility through adjustment of process controls within a predetermined design space. For appearance, the goal is zero defects, which are however constrained by practical considerations including protein aggregation (Cordoba 2008; Mahler et al. 2008) due to the inherent self-association nature of proteins. The preventive aspect of protein aggregation involves optimal molecule engineering of the primary amino acid sequence, formulation conditions, choice of primary container contact surfaces, and manufacturing/handling/storage condition selection. These factors cannot be optimized by modification of one component at a time and instead require the application of design of experimentation (DOE) to define the optimal configuration through the use of multivariate experimentation as expected by the QbD principles. Complementary to the CPV is continuous quality verification (CQV (American National Standards Institute ASTM E2537 2008) or continuous quality assurance (CQA) by the FDA).

A surveillance of the top reasons for observations by the agencies over the past decade reveals a significant number related to multiple t of particle-related concerns ranging from inadequate inspector training, investigations, sampling, and tracking to the source, insufficient monitoring, and preventive action. The manufacturer must demonstrate a level of control by not merely relying on final inspection as a method to achieve quality. This includes an understanding of protein aggregate formation, root cause analysis, reversibility and number/size distribution of both visible and SbVP, and kinetics of particle formation. It is specifically in the area of aggregation surveillance that biotech companies realized that the compendial methods were problematic when applied to t protein particles. These protein particles create safety concerns that are different from foreign matter so proteins require not only modified characterization methods but also a different control strategy. Based on the results of particle analysis findings of protein aggregation, a risk analysis by the industry sponsor is desirable as basis for the appropriate control strategy. The risk assessment (Carpenter et al. 2010; Rosenberg et al. 2012) evaluates the likelihood that an event will occur having an impact on safety or efficacy, and must include the ability

to detect either the event or its impact. Severe or uncertain biological consequences, even if the likelihood of occurrence is rare, make the event high risk. Gaps in the ability to detect the event or clinical consequences increase the risk. The risk assessment must be conducted by including representatives of multiple disciplines including quality, manufacturing, medical safety, clinical, toxicology and immunogenicity (Guidance (draft) for Industry 2013; Bee et al. 2012). QbD as a science-based approach is very suitable as a process development strategy to pro-actively characterize, understand, and control protein aggregation by integrating the understanding of the molecule, process parameters, and quality attributes into a holistic design space with defined boundaries of the drug product presentation that must guarantee performance suitable to meet the quality target product profile (QTPP).

CQA does typically have a quantitative value that reflects not only the ability to measure those defects but also the ability to monitor and put specifications in place. The application of validated assays in a way "allows" the presence of degradants as long as these are a part of limit specifications that enable product release and determination of expiry dating. Lack of quantitative data on a specific CQA such as visible protein particles presents a challenge with respect to control. The outcome of the clinical trials seeks to establish safety and efficacy in an environment where there is variability in both patient and different drug product lots used in the trials. The range of CQA values that cover the range of characteristics in clinical trial material serve to define release specifications for future commercial material whose quality should match or exceed clinical trial material quality.

Particles are atypical in the case of small molecule API unless they are designed as part of a controlled release strategy such as crystalline or suspensions. In contrast, recombinant protein therapeutics has sometimes insoluble protein particles. Protein molecules exhibit inherent self-association properties which are exacerbated at high protein concentrations. This self-association can result in an insignificantly small fraction of soluble protein forming a larger aggregate, which eventually appears as insoluble or visible particles as part of the complex and dynamic protein behavior and unfolding (Sharma and Kalonia 2010; Joubert 2011). Clinical testing of artificially high levels of protein aggregates is not recommended for ethical reasons. The use of clinical product with somewhat higher aggregate levels can sometimes occur for early stage clinical material and will enable "qualification" of the material in the clinic (Parenky et al. 2014).

In spite of best efforts and extensive studies, protein self-association is an inherent property of proteins and aggregation and particulation can occur, with the amount of this seen depending on the specific product. This occurrence often is time-dependent and not expressed homogeneously across all filled containers unlike color or chemical degradation, which are similarly distributed in the population of a single manufactured drug product lot. Depending on the degree of reversible association and kinetics, this aggregation can result in irreversible insoluble particles of various sizes, i.e., both visible and subvisible. This unique attribute is the foundation of the concept of protein particle probability, which can only be ascertained through statistical sampling. Protein particulation characterization is an important feature of developing design space with a preference for quantitative data,

which today are primarily based on destructive techniques such as light obscuration or DIA for subvisible particles and visual inspection for visible particles. Since the probability of detection is related to the number and size of particles present, and there are more SbVP, the approach, number of vials to test, and whether this is giving you a better fix on variability, sampling plan must be statistically justified.

The particle profile that is obtained should be evaluated through risk management in that a mitigation plan should be in place after a formal risk assessment according to a well-known risk management and tool such as Failure Mode Effect Analysis (FMEA, or Fault Tree Analysis (FTA)), or other methods as described in the ICH Q9 Quality risk management guidance. Clinical considerations which inform the risk assessment include the dosing frequency, route of administration, clearance, patient immune status, and relationship of the therapeutic protein to any endogenous protein (Bennett et al. 2004). Additional risk components are patient impact (PK, potency, biodistribution, toxicity, and immunogenicity) and detectability.

15.2.1 Particle Specification Setting Per Regulatory Expectations

The regulatory expectation for visible foreign particle matter is "practically free from particles." The expectations on inherent protein particles have been evolving over the past decade and will continue to do so based on improved inspection instrumentation and enhanced clinical understanding of immunogenicity. Any protein aggregation is undesired unless it was designed in as a requirement of the QTPP. In reality it is sometimes unavoidable and it is up to the manufacturer to deliver the evidence that the drug product is safe and effective and that the specific aggregation has no adverse impact and is under control.

- Monitoring of subvisible particles ≥10 and ≥25 micron has been a regulatory requirement and tied to release criteria (USP <788>) per compendial methods. The FDA expects that release specifications will be periodically reviewed and updated to stay current with industry practice (Susan 2014). USP <790> applies to visible particles in injections and provides guidance on the manual inspection execution for the AQL sampling as a part of batch release and action on particle complaints for products in distribution. The batch is considered essentially free of visible particles if 20 units (at least) are inspected from the reserve samples and found to be free of visible particles.
- In addition the FDA is now also requesting that subvisible particles below 10 micron to be studied and monitored as part of the Post Marketing Commitment (PMC) per 21 CFR 601.70 for biologics. At this time some quantitative methods exist and the preferred method is based on the traditional lot release method of light obscuration (Cao et al. 2010); the most recent USP <787> which supplements USP <788> addresses this. The informational USP <1787> chapter covers general information on measurement techniques. Expected are findings that include morphology, identity, method development and selection, and size distribution (e.g., ≥2, ≥5, ≥10, and ≥25 um). Permitting sufficient data and method

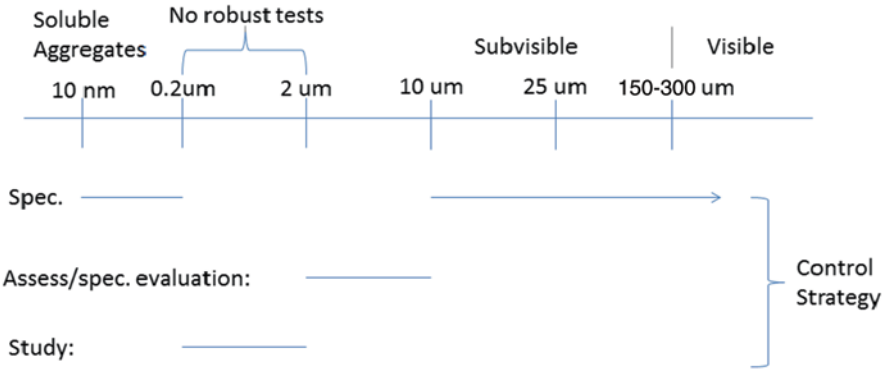

Fig. 15.1 This illustrates current capability of obtaining particle characterization as a function of particle size. The control strategy will be limited by both instrumentation capabilities and statistical power on obtaining data on very variable and dynamic phenomena like particles representative of an entire lot and not a single drug product container. The indicated size ranges are an approximation.

robustness, it is expected that this in the future is used for assessment, which over time could translate into setting specifications (for batch release, comparability, annual GMP stability lots) or action limits providing the testing technology matures. Just as stated for the oligomer aggregate case, the method validation is expected to include a challenge with stressed samples and an independent confirmation of degradants separation using an orthogonal method.

- The control strategy on protein particles in drug product is a function of the particle size. It includes studies using investigational/orthogonal methods, generating data "for information only" assessment and finally for setting accept reject criteria on release which is shown in Fig. 15.1.

As stated earlier, the FDA has no preestablished limit for sub-visible particles below 10 micron. Whenever subvisible particles are an unintended impurity, the lower their levels the better it is for the product quality (ref. 25).Susan 2014). The main driving force for setting limits is the risk of a CQA to safety and efficacy. This requires prior product knowledge, process understanding, manufacturing experience, and clinical experience. The inclusion of many lots of drug substance and drug product in clinical trials can provide a rich set of data as the basis for setting specifications. Sampling merely a single or few containers from a lot is insufficient to truly measure the particle load in that entire lot, if the particle load is not homogeneous from container to container. In case of legacy commercial products where those data are not available archived samples can be used, though due to heterogeneity of particles within a lot, it may be unlikely to provide a rich set of data. Tracking 2–10 micron data until enough has been collected to set statistically relevant specifications is another approach , though it is hampered by validation and statistical challenges with the current state of technology. As an alternative one could attempt to gather a possible correlation between aggregation data of different size ranges (e.g., oligomer vs. submicron particles) for setting limits. For the sub-

micron particles, due to the lack of protein-like particle standards and availability of robust (mature and validatable) analytical methods, which are furthermore labor intensive, this area will be one of considerable challenge in the future. Instead the use of qualitative methods is recommended, which again should include the use of stressed samples as described previously. Based on the results of these studies the sponsor should perform risk assessments and propose the control strategy that is used for technical transfer or a comparability study and not for action limits or batch release specifications until both robust tests are available and a rich data base exists. The generation of the "WIN" report in case of drug products with visible aggregation is a useful tool for setting appearance specifications.

A description in the specific monograph is one avenue to document aggregation leading to visual observation (USP <790>. From a safety perspective, the correlation between aggregate sizes, amount, and immunogenicity, is still to be determined (Joubert 2011). Based on the immunogenicity pathway and cell uptake mechanisms protein particles below 10 μm are raising most concerns (Carpenter et al. 2009; Singh et al. 2010). This aggregate size range is currently the focus of immunogenicity research (Joubert et al. 2012).

15.3 Challenges of Visual Inspection and Quantitative Analysis of Protein Particles

In general, proteinaceous particles are amorphous in shape, semitranslucent, neutrally buoyant, and mostly made up of the bulk liquid in contrast to the typically acid or heat induced dense protein visible precipitates. These properties make them hard to detect and quantitate accurately. Other confounding factors include container to container in-homogeneity, time-dependent formation or kinetics, which does not progress consistently across the entire lot. The particle makeup varies from a continuous covalent structure or one made up of loosely associated sub-aggregates that can disassemble as a function of agitation or swirling during inspection and testing (Ripple et al. 2011).

15.3.1 Interpretation of What Is Visible to a Human Inspector

For visual inspection (Melchore 2011), some background is required to interpret the practical definition of ~100 micron detection as visible in the case of spherical particles. The ability exists for humans to detect or perceive a high contrast slit of ~30 micron (20/20 visual acuity) at 10 cm distance, though some humans can detect particles that are smaller while others can only detect particles that are considerably larger; this variability in inherent human capability necessitates training and strict definitions of "what is visible." Note that the true resolving power is expressed as angular size which is about 0.5 arc minute for a perfect eye (http://arapaho.nsuok.edu/~salmonto/vs2_lectures/Lecture21.pdf). Realistically, the threshold for detecting lower contrast

and moving objects is more in the range of 50 micron as long as the object has a different refractive index from the medium. The credit for introducing the probability factor of detection and reject zone efficiency (RZE, reflecting efficiency of inspection by need to reject to worst 30% of representative defects) as a concept in inspection goes to Julius Knapp and Harold Kushner (Knapp et al. 1980; Knapp et al. 1990, 1996) who enabled a strategy to compare manual inspection to automated inspection through the use of a similar defect set. This is then followed by validation using the actual drug product. In that case the defect set contains seeded NIST-traceable standards as well as specific foreign matter, with examples of visible protein aggregates if this is normal for the product being assayed. The defect sets for automated inspection are determined on a product to product basis since components have unique defects associated with the manufacturing process for the raw materials (vial, stopper, and seal) and material processing surfaces.

The detection of particles in the visible range is based on probability (Knapp et al. 1990) and increases with increasing particle size. The probability of detecting the presence of a single round high contrast object of about 30 micron in a container is less than 1%, defined as the visibility threshold for 20/20 vision, which as a definition of what is visible has no practical application.

Instead, applying a probability of detection of ~70% is more practical (Knapp et al. 1982, 1990). Studies performed at various sites using certified inspectors demonstrate an approximate 40% ability to detect a single ideal high contrast particle of 100 micron in a translucent container when viewed under optimal conditions by the 20/20 corrected human eye (unaided), which increases to ~95% when inspecting a 200 micron particle (Smulders et al. 2012). The size range for a ~70% probability varies with conditions and covers the 100–200 micron range (Smulders et al. 2012).

The detection of fibers with a rod like shape is acknowledged to be more difficult. Stephen Langille (FDA) presented at the ECA Particles in Parenterals (September 24, 2014) meeting a distinction between visible particles and fibers, as 150–250 micron and 500-2000 micron, respectively. Even though the goal is 100% detection, the detection goal is >70% and >80% for minor and major defects, respectively.

15.3.2 Factors that Impact Particle Detection Effectiveness

Additional discussion on these visible size range values is somewhat academic as many variables impacting inspection effectiveness exists:

1. Optical differentiation: Manual or camera-based vision perceives an object by its contrast defined as the ability to distinguish its features versus the background or adjacent features. Contrast is defined as the difference in light intensity between the object image and the adjacent background relative to the overall background light intensity.
2. Particle properties: reflective vs. light scattering vs. absorption, density, spatial variation in refractive index, birefringence, shape (spheres vs rod), and fluorescence. The second factor relates to the propensity of the particle to be freely

suspended which is a function of buoyancy, adherence to inner container surface or crevices, size, number, color, contrast, shape/orientation, etc. Micro air bubbles are examples of real particles from an optical perspective but acceptable from the safety perspective.

3. Container: shape, magnification effects by the glass cylinder curvature, materials of construction, scratches, and fingerprints, all impact detection effectiveness. Uniformity of the container walls plays a big role as for instance blow moulded glass vials or interior curvature of plastic containers plays havoc with light refraction.
4. Ergonomics: sight distance, time/pacing, hand-eye coordination during agitation, repeatability, scanning strategy, and container holding, also contribute to inspection results.
5. Container content liquid properties: the density, viscosity, color, refractive index, geometric shape, opaqueness, surface glare, light polarization and surface tension, also play a role. Fill volume and presence of head space is another important parameter as they impact the agitation-induced particle dislodgement properties after inversion and spinning actions. In case of solids such as lyophilized products, the cake is typically white and does not allow access to visual inspection unless preceded by reconstitution. Recent low energy x-ray inspection may to some extend provide some though still limited inspection opportunities to foreign particles but not to aggregation.
6. Illumination: Objects interact with light that produces changes in brightness, color due to the light angles, wavelength of the incident light, light source (flicker-free), coherent/diffuse, polarization, and intensity. Inspection in areas with low ambient light is preferred except of course in the inspection booth. Excess illumination increases glare and increased eye fatigue.
7. Background media: matt black lowering retro-reflectivity and nonglare white to detect both white and dark colored particles.
8. Human inspector visual acuity (20/20 vision and contrast sensitivity), duration of inspection, eye movements, focus, fixation, number of inspection attributes applied simultaneously, use of a magnifier, and prior knowledge or increased sensitivity resulting in enhancing perception. Dark adaptation is aided by low light ambient intensity.
9. Mobility: Object detection is easier when the object moves. The approximate 70% detection of 100–200 micron particles (sphere like) stated earlier assumes the particle is in motion relative to the container. If the particle gets stuck 50% of the time it may suffer from a correspondent decrease in detection.

The prevalence of an attribute influences perception and causes an individual bias as a function of the attribute. Human ability to detect decreases with rarity of detection and this will increase false accepts. As a corollary, the ability of human inspection to detect false rejects increases with a high prevalence of a target attribute. Other confounding factors for manual inspection are the state of mind, eye fatigue, preset expectations, experience, vigilance, and ergonomic comfort. The use of mechanical tools or grippers to pick up multiple containers simultaneously to increase

throughput challenges the inspector though permits rapid comparison and improves detection of outliers such as fill volume defects. Sufficient rest periods are desired to counter fatigue.

Prior knowledge plays a big role and is a function of training, experience and the use of teaching aids, ideally videos or still images, rather than verbal descriptions. Since the visual stimuli are presented randomly, the inspector cannot anticipate when it will be seen. Here prior knowledge plays a large role because vision as a human sensory tool is a psychophysical phenomenon.

15.3.3 QbD Principles for the Inspection Process

15.3.3.1 Manual Visual Inspection (MVI)

The factors influencing effectiveness of visual detection are too numerous to study in a holistic manner as illustrated above. The current approach is to conform to standardized inspection booth parameters and inspector certification use of appropriate man-made standards and line defects, and minimize variability by standardizing ergonomic factors, container handling (inversion/swirling) and aiding inspection conditions with training material including images or videos. Special attention must be paid to recognize extrinsic particles and all foreign matter. Knapp studies must be performed to establish defect detection rates as a function of the criticality of the defect, which in turn is linked to risk assessment. The documented detection capabilities are the basis for setting process requirements for both semiautomated and fully automated inspection, which must meet or exceed manual inspection. Semiautomated inspection refers to the automated container handling within the setting of human inspection. This adds to the requirements to verify that the observer is still capable to be as effective as with total manual inspection. There is no need to repeat Knapp studies with every possible SKU as long as there is a justification of the inspection conditions which can bracket a range of parameters based on optical and geometric properties. The certification program includes the use of well-characterized defect kits (Melchore et al. 2012) reflecting possible intrinsic particles (e.g., protein aggregates) and actual container line defects. Likely, there will be a gap in the defect kit as not all possible real defects are available as standards such as unique container/closure manufacturing defects.

15.3.3.2 Automated Visual Inspection (AVI)

Machine-based inspection, while not subject to all the variability's of human inspection, lacks the data processing capability of the human inspection and the rapid adjustment to changes in brightness. This is why the acceptance quality limit (AQL) is performed using MVI on a small but statistically relevant sample size of the entire lot. The inspection machine must be verified to remain in the calibrated state both before initiation and the termination of the inspection process on a lot. AVI

requires the use of multiple cameras where for each attribute the camera speed and resolution must be optimized to effectively detect defects as a function of predetermined detection efficiencies at levels equal to or exceeding human capability. This requires a well-understood synergy of cameras, lenses, lights, container handling and vision algorithms to provide optimal detection at the throughput rates which can be as high as 600 units per minute. The disadvantage of high speed AVI is the use of lower resolution cameras leading to lower detection that may or not detract from the expected performance. Qualification of AVI systems requires defect sets to be made of each SKU spiked with single particle defects encompassing the range of possible intrinsic particles. The AVI is expected to deliver certain effectiveness for each defect category with values approaching ideally 100 % for critical defects. The AVI machine needs the capabilities to choose appropriate performance parameters which include settings on sensitivity, spin speed, postspin braking and overall throughput as well as software algorithm settings on vision programs. It is expected that the AVI vendor through DOE factorial-design experimentation determines the key critical variables and defines the design space in which to operate the AVI. This design space is to a great extent a function of the container and content characteristics as shown above. Viscosity associated with high protein concentrations is a significant obstacle in resuspending heavy or high density foreign particles into the field of view.

Reject Analysis The segregation of different defects by categories allows the trending of specific defect classes. Based on historical data triggers can be defined to commence investigations if a specific defect category exceeds set criteria, further providing an opportunity to minimize variability and address trends from a CAPA perspective.

Sampling The rationale must be documented with written justification for the statistical sampling across a lot with the emphasis to capture data during the beginning, middle and end of the fill. This excludes a random approach. If partial inspection takes place during the filling process, the real time information provides immediate feedback on the process enabling intervention.

Design space defined for particle inspection encompasses deep understanding of all critical inspection attributes that challenge both manual and automated inspection, performed during the 100 % and AQL inspection. In contrast to AVI, the MVI process is not as robust, providing the basis for pursuing AVI where possible. However, due to current limitations in both machine vision and image analysis, MVI continues to play an important role. In addition, there is a second tier analysis in case of eject inspection, which is defined as performing MVI on those units rejected by AVI that have certain attributes not within the AVI capability such as automated recognition and acceptance of micro air bubbles that are true though often transient particles but do not present a safety issue. Justification for second tier inspection of ejects must be in place.

For discussion and simplicity purpose, visible particle range is defined as 100–200 um (greater for fibers as a function of axial ratios) in this chapter. To ensure the effectiveness of the 100 % inspection, a verification test is applied. As the inspection technologies to date are not 100 % effective, the typical appearance specification

on particles defines the expectations as "essentially free of particles" in the US or "practically free of particles" in the EU.

"Essentially free of particles" is not defined as an attribute for each container but applies to an entire filled lot. This means that appearance testing must proceed for all containers as opposed to for instance oxidation as a release assay is performed on a single container. The actual release per appearance test is only formalized by an AQL as defined in the USP <790> test on a limited sample set that validates that the 100% inspection met certain criteria for effectiveness. The sampling at batch release following 100% manufacturing inspection is based on ANSI/ASQ Z1.4 or ISO 2859-1. Per General Inspection level II, execute single sampling plans for normal inspection with an AQL of 0.65%.

In either case the language reflects the inherent lack of robust inspection methodologies. It is here that the regulatory agencies are moving toward two distinct approaches: the AQL approach for foreign particles and the QbD approach for protein particles.

For extrinsic matter or where protein aggregation is not an inherent property, the definition of essentially or practically free is moving toward a statistical definition where the AQL and related sampling plan provide a statistical assurance that takes into account the practical limitations of a heterogeneous property. In contrast to a homogeneous attribute such as color or % oxidation, extrinsic particles are not distributed evenly across the containers that represent the lot population. This unique property accounts for the fact that release of a lot based on particles is not based on assessing the appearance with respect to particles by subjecting one to three containers to inspection.

For protein particles, QbD offers an alternative approach. QbD principles state that a control strategy must be in place to not only prevent presence of foreign matter but control of the type, size, and number of protein aggregates and particles to levels that do not impact safety and efficacy as established during clinical studies using characterized materials. This cannot be achieved alone by "inspecting-in" quality but requires both process monitoring and science-based release specifications that ideally have at a minimum a semiquantitative basis.

In the case of appearance inspection for visible particles, the current nondestructive automated inspection methods used on 100% of all filled containers are limited to a binary approach (pass vs. fail). This does not accommodate the specific protein stability characteristic in terms of it being quantitative. The inspection process when executed by human inspectors requires rejection of any container that has a visible particle regardless if that particle is in the visible size range or not. If anything is observed by the inspector, the unit is expected to be rejected. Another category called ejects refers to containers "rejected" due to for example transient air bubbles. These are not false rejects because air bubbles are "real," but as air bubbles pose no safety hazard they are acceptable, and can be accomplished by reinspection of these ejects by MVI. Under certain circumstances, proteinaceous aggregates are accepted on the condition that they are inherent to the formulation and are similar in appearance as observed and documented during development and in clinical trial lots.

15.3.3.3 Sub-visible particle analysis

For the analysis of particles below the visible size range, i.e., subvisible particles, technologies exist and offer the opportunity to quantitatively characterize particles in the micron size range, and qualitatively or semiquantitatively analyze particles in the submicron size range. For subvisible particles instead of micron particles, light obscuration testing and its associated limits have been traditionally applied to produce quantitative information such as particle concentrations at discrete size ranges, whereas technologies based on dynamic image analysis (DIA) have been used to provide orthogonal sizing and counting measurements and at the same time produce particle shape and morphology information. (USP <788> and USP <787>). At the present time, the most common approach for subvisible particles has been evaluating them per USP <788> or USP <787>. The quantitative nature of subvisible particle analysis makes it a good candidate for the QbD approach. A complicating factor that one needs to be mindful of is that the traditional light obscuration (LO) method and its associated particle allowance limits do not distinguish protein particles from other particles such as silicone oil droplets or extrinsic environmental particles. The presence of silicone oil as lubricant in prefilled glass syringes provides a challenge to the LO method in the subvisible range especially in the < 10 micron range. Image analysis corrections based on axial ratio exists but cannot totally neutralize the contribution of silicone oil particles to the total number which makes it difficult to define a baseline of < 10 micron particles in prefilled glass syringes.

Protein aggregation as a degradation path must be characterized. The literature has many references on various methods that can be applied (Wolfe et al. 2011a, b; Zolls et al. 2012). For QbD the emphasis should be on selecting those methods that are validatable or at least can be qualified. The use of orthogonal methods is acceptable as long as the data interpretation is based on proper judgment. Due to the complex nature of analytical methods in the micron size range, a quantitative particle baseline that is method or instrument specific should be used for trending using the same methodology. As long as the method is precise, it can serve the purpose even though its accuracy remains uncertain. Precise data will identify trends and are very useful for comparability as part of technical transfers and lifecycle improvements.

To apply the QbD principle, the first step is to understand and document the particle profile of the product. To this end, the typical protein aggregation pattern should be captured as a WIN (what is normal) phenomenon which can be recorded in the Particle Summary Report (PSR) for that product. Since protein aggregation is not a homogeneous property across a production lot and heterogeneity typically increases over time during storage, the PSR should cover this by presenting data summaries relevant to each specific development stage of a drug product. For each stage, properties that might be useful to be included in the PSR include: a qualitative description of the product's visible protein particle appearance such as buoyancy, contrast, shape, translucency, light reflection/scattering, turbidity, edges, etc.; references to forensic data confirming protein identity; reversibility of aggregation as a function of temperature and time; the percent distribution across a lot using a representative sample size;; a quantitative description by particle counts in different

size bins where possible; cross reference of subvisible particle data with visible particle data for possible correlations; kinetics over time at different temperatures during nonaccelerated stability studies; determination of an aggregation plateau as a function of time; impact of freeze-thaw to aggregation; impact of transportation vibration and air-liquid interactions (Bee et al. 2012) by foaming effect on aggregation; impact of aggregation to dose strength with respect to % insoluble protein; representative imaging at a minimum still images or preferred video images of closed containers ideally with background subtraction to remove surface defects or embedded particles or surface exterior particles.

An PSR has many practical uses. For example, it can be used as the basis of the appearance specification; as a training aid to manual inspection by certified inspectors; as a time correcting tool to estimate aggregation during clinical trials; as a background document for investigations on product returns, reject limit out of spec (OOS); as the data for a USP monograph if required to demonstrate bioequivalence after technical transfer and second generation process development including formulation optimization (Sharma et al. 2010). This PSR document must be updated when new information becomes available during the multiyear life cycle of a commercial drug product.

A case can be made through proper justification that a product can be licensed with a controlled level of protein particle formation accompanied by mechanistic understanding and control strategy. The justification process is however entirely prepared by the applicant and the regulator must be convinced on a case by case basis before an application is approved. This scenario gets interesting as potential acceptance of insoluble protein matter by exception, is no license for the presence of foreign matter. The FDA and USP through ongoing discussions are moving toward a monograph-based approach for each protein therapeutic in case of inherent aggregation particulates (Joubert et al. 2011) with support by the Visual Inspection interest group of the PDA.

Different stages in a product's life cycle require different applications of QbD. During the clinical development phase, when applying one of the QbD principles, a number of steps are listed for consideration to mitigate particles and aggregation:

- First, defining the QTPP with information on indication, delivery method, patient population, route of administration, dosing, storage requirements, etc. Next, conduct molecule assessment during lead candidate molecule selection with respect to the optimal choice of the primary amino acid sequence. This is followed by initial testing during process development composed of formulation excipients, container material, transportation route related vibration, and environmental factors.
- Include variability of the raw materials (container components and excipients) and establish effectiveness of in-process filtration across all unit operations. Establish capable forensic capabilities for particle isolation, characterization, and identification. Apply statistically justifiable sampling reflecting aggregation heterogeneity across lot.
- Determine kinetics and aggregate characterization as part of stability during development on material from the commercial-like process using a statistically justified number of samples per time point. Characterize aggregate levels in clinical

material and adjust for kinetics and isolate and establish aggregation misfolding and/or covalent modifications if possible considering recovery limitations. Capture data in the WIN or PSR document as the basis for appearance specifications for FIH and late clinical materials. Perform a risk assessment within the context of the QTPP and rank findings and define whether mitigation is required.

- Correlate degradation pathway to inherent and/or external factors and correlate these factors to critical process parameters (CPP). Attempt to correlate rate of aggregation to range of one or more CPP values using multivariate experiments based on DOE.
- Track aggregation with cGMP stability studies on material from the process performance qualification (PPQ) runs and determine "CpK," a statistical tool to measure the capability of a process to meet quality requirements. Apply predetermined statistical analysis to define the range of aggregation within a confidence interval and set alert/action and reject levels for visible particulates.
- Finally, establish a clinical immunogenicity surveillance program to determine possible antibodies responses. Establish nature of antibodies in terms on neutralizing vs. nonneutralizing antibodies. Interpret data using again a risk analysis.

The resulting design space must demonstrate a manufacturing process that is capable of producing reproducible quality. When producing clinical material one needs to assure that the aggregation level is not artificially reduced to levels not achievable within the context of commercial viability. Under this paradigm, three validation runs can no longer form the basis of validation. Instead, PPQ runs serves as a demonstration of a sufficient control strategy. The greater knowledge gained upfront, the lower the number of PPQ runs needed because the residual risks are less. License application must reflect if the aggregation is an inherent property of the therapeutic entity, and demonstrate ample understanding accompanied by detailed characterization and a control strategy.

During commercial manufacturing, the QbD approach requires that one establish standards ideally of the real (line defects) drug product for comparison and replace them when expired. One can establish an inspection pattern covering begin, middle, and end of filling and capping process in addition to rapid checks for attributes such as weight accuracy and appearance testing. For semiautomated or MVI, one uses the product specific training and standards if possible aided by the appearance report documentation. Next, establish the time window and storage between fill and inspection. Verification of 100% inspection through AQL sampling and testing is required (USP <790>) as part of batch release. One is expected to set alert/action limits on % aggregate rejects based on data obtained from predefined number of lots. Exceeding these limits will be the basis for an investigation with a focus on if the additional particles observed are more of the same or represent new types of aggregates. Plotting data for process monitoring and tracking trends for each particle category enables continuous verification. These inspections can also apply to the annual stability lot evaluation and retention sampling limited testing. Continuous verification by real time in-line testing (PAT or process analytical technologies) for proteins needs to be developed in the future as alternative to rapid off-line testing by human inspection or automated instrumentation. Continuous improvements in

analytical techniques are needed in order to trend data and correlate using a predictive model. The expectation is to not only just aim to pass compendial specifications but also to better them where possible by applying historical data gathered over many lots. These quantitative aggregate measurements can be applied to support bioequivalence, product complaints, process change evaluation, and technical transfer to new sites.

QbD principles require postlaunch activities on protein aggregation complaints from the field. Procedures need to be in place to receive samples in-house under controlled conditions; attempt to image the contents while container is still intact to obtain number and sizes of the particles; next open the container and collect contents by filtration, and establish particle number, size, and identity using forensic methods. If it is a protein particle, compare video/still images to what is expected per the PSR document. The investigation can include different scenarios that include closing of the CAPA (corrective and preventive action). If the complaint observation is not different from what's already documented in the PSR, no further investigation is required after a correction for time dependent kinetics. However, if the observation is different, a nonconformance (N/C) can be initiated (Wen et al. 2013), which could result in inspection of the retained samples of the same lot or even include reinspections. These data provide the ability to connect with attributes of the lots given to the patient in order to perform the risk assessment for that attribute going forward. Note that the accept/reject criteria for the lot must be established in advance with statistical protocols. If the forensic results demonstrate the formation of new types of protein particles that do not match the PSR consideration is given to report a Biological Product Deviation (BPD) by the licensed manufacturer (Guidance for Industry Biological Product deviation reporting for licensed manufacturers of Biological products other than blood and blood components, 21 CFR 600.14(a) through section (e)) and possibly a product recall (www.fda.goc/safety/recalls/industryguidance/ucm129259.htm).

15.4 QbD Case Studies

15.4.1 Case Study on Protein Particle Formation

A new drug product (QTPP as liquid fill in a glass container) was manufactured for clinical studies in a process where the inspection was initiated typically in 1–2 weeks after filling process on containers adjusted to room temperature after intermediate refrigerated storage. No visible protein aggregation was ever observed during manufacturing or during stability studies using a sample size of three containers for each time point. Some clinical lots were held in reserve for up to 6 months. One of those lots was reinspected primarily for external cosmetic defects that are normally considered minor. The expectations for appearance in a new country for clinical trials were zero particles or blemishes on the exterior of the filled drug product container. This second 100% inspection revealed several containers with a few particles that upon extensive

forensic analysis showed to be composed of protein matter. The vast majority of aggregates observed were less than 80 micron in size and present in about 3% of all containers. Of all the detected particles, only about 5% were in the visible size range and limited to about 3% of all the containers. These were new findings that thus far escaped detection because of the low statistical probability of detection. Furthermore, it showed a time dependent phenomena. A risk analysis was performed, followed by tracking of aggregation using a larger sample size which showed that the observations made at 6 month time point remained roughly constant throughout the remainder product shelf life. The aggregates could be removed by filtration but reappeared after 2–3 months in a small fraction of the containers. In order to increase the probability of detection during the stability study, the number of samples per time point was increased and the same vials were repeatedly used. A statistical justification of the improved sampling plan was written and applied across other stability studies as well in order to capture aggregation earlier during the development. The existing protein aggregation report was amended to update its description and kinetics of appearance of visible matter. In addition, the quantitative analysis was extended to other methods (destructive) to track the formation of subvisible aggregates. As these new methods are not yet validated except light obscuration, the information generated is not used for setting product specific specifications but used for trending and setting assessment criteria for comparability. The control strategy is the application of the subvisible aggregation kinetics early on as a surrogate marker for future visible aggregation as a correlation was shown to exist between particles in these two size ranges. In addition, the morphology of the protein aggregates was captured using DIA for future use. The potency or dose concentration was confirmed to meet the label claim even at the end of shelf life in terms of measuring total soluble protein.

15.4.2 Case Study on Foreign Particle Detection

A drug product's (QTPP liquid filled container) AQL failed the preset acceptance criteria after the 100% manual inspection. Evaluation of the AQL visible rejects showed that these particles were difficult to dislodge from the container but once free, relatively easy to detect. Attempts to alter the inspection condition included magnification, increased illumination, increased inspection time, were investigated but to no avail. The only effective approach was the introduction of increased mechanical agitation followed by cessation and immediate inspection. Next it was demonstrated that this increased agitation had no effect on the stability of the drug product or CCI (container closure integrity). This case demonstrates that particle detection is not only a function of its visibility but also of its ability to be placed in motion thus enabling its detection. This example underscores again the role of probability in particles detection as it includes both the human inspection probability (see the discussion on Knapp previously) and probability of particles to be stuck onto a surface or micro niche within the primary container.

15.5 Current and Future Trends on Inspection

The attribute of appearance as a CQA is not aligned between a number of regulatory bodies such as the US (USP), Europe (EMEA), WHO, JP, India, and Chinese Pharmacopeia. With increased globalization and international expansion, it would serve the interest of all parties to align. Though significant progress has been made, the process is slow and arduous, with updates going through extensive reviews including public comments and the involvement of standard-setting organizations. The current FDA expectation is to track aggregation in the range between the SEC and visible particles. Currently, a wide range of alternative methods are available for the analysis and quantitation of subvisible particles in the submicron and micron ranges, although the results thus obtained do not always align or correlate well. As technologies mature and become validatable, it is possible that specifications in the future could include quantitation of subvisible particles in the submicron to 10 micron size range. It is recommended to apply several methods and gather data over a large number of lots combined with a solid sampling plan reflecting heterogeneity. The application of visual inspection as an accurate and nonperturbing example of process monitoring is required and increases process understanding and control. When integrated as part of the design space after risk management, it provides a powerful tool to execute the particle control strategy as part of QbD principles. Visual inspection is just one example of increased on-line monitoring to verify process control and its impact will increase as technology progresses. Finally, the only way to approach a near-particle-free future is the full implementation of automated vision systems as functions of hardware and software advances.

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Chapter 16
Quality by Design for Distribution of Environmentally Sensitive Pharmaceutical Products

Paul Harber

16.1 Introduction

Pharmaceutical companies distribute temperature-sensitive products globally, including drug substances, intermediates and finished drug products. Distribution occurs between pharmaceutical manufacturing plants and from the pharmaceutical manufacturers to customers. Therapeutic proteins are a growing subset of medicinal products. These proteins are sensitive to hazards including temperature and physical stresses in the logistics environment. Quality by Design (QbD) is a valuable tool for development that is referenced throughout the product lifecycle. This chapter highlights the methodologies suitable for biopharmaceutical products. These methodologies can be successfully applied to a wide range of temperature-sensitive and/or physically vulnerable products.

QbD can provide a valuable framework that furthers the understanding of the distribution process and its effects on product quality. The January 2011 revision of the CDER Guidance on Process Validation is one example of regulatory support for the principles of QbD; and the differences contained in the document are a stark contrast to the original guidance. There is no longer a mention of "worst case." Instead it is up to the manufacturer to establish a design space for the product and assure that the process can operate within the established product design space. The Food and Drug Administration (FDA) considers the distribution and holding of the product as a part of the current good manufacturing practices (cGMPs). Thus the principles contained in the process validation guidance can and should be applied to distribution.

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Maintaining control of the network in a worldwide distribution system requires expertise in logistics and product development, as well as qualification of the materials intended to protect the product. In the past, distribution methods have been monitored solely based on outcomes of the transported finished drug product. Regulatory expectations now consider empirical analysis of individual shipments to be unacceptable as a stand-alone metric. New FDA guidelines adopt a lifecycle approach to validation of equipment and operations that support the product. The lifecycle begins in product development and ends with the decommissioning of the process and end of product distribution. The guidelines also support process improvement and innovation through monitoring and control. The controlled-environment logistics system fits into this lifecycle. Implementation of the distribution process is best accomplished with a validation master plan for distribution (dVMP) that delineates the responsibilities of product characterization, operational and performance qualification, and ongoing monitoring and control of the validated system. Ideally, QbD principles should be contained in the dVMP.

Studies that define the design space can be formulation-specific. For example, in frozen formats the dry ice used to maintain a required range might penetrate some container closure systems and shift the pH out of the desired range (Murphy et al. 2013). With protein solutions, physical stability can be degraded from the ordinary shocks and vibration present in the distribution environment. Dosing patients with parentally administered proteins containing aggregation products can have dire consequences that include immunogenicity or anaphylactic shock and death (Schellekens 2002; Moore and Leppert 1980; Ratner et al. 1990). Without establishing a product design space for each critical quality attribute (CQA), the risk assessment exercise is merely a brainstorming session of possible events that could affect the product without being able to rank the criticality of the event. Thus, the events are either ignored, or addressed late in the project cycle when the cost of mitigation has become much greater. Classic stability studies are a critical source of data for chemical and physical stability during production and storage. Data on the physical stability of the therapeutic proteins under conditions relevant to the distribution process, when combined with chemical and physical stability during the full manufacturing and storage process, assures that the product is fit for use at the expiry date at the *end* of the supply chain.

16.2 Scope

The scope of this chapter is an overview of each component of the distribution process and a comparison of best practices in process validation within current industry practices. The purpose of the comparison is to identify the contribution QbD can make to achieve improved practices and standards.

Fig. 16.1 Elements in distribution process design

16.3 Process Overview

Design of the process for transportation of products in the biotechnology and pharmaceutical industry, Fig. 16.1, will be reviewed in five categories: external requirements, quality manual, distribution process, technology transfer, and implementation. The components of a dVMP demonstrate that each of the key areas have requirements that must be met in order to maintain process control. A successful logistics process is created when each of the five areas are integrated to form one validated process. A validated logistics or "supply chain" process combines the quality system with the distribution process. QbD enables a thoughtful analysis by identifying those activities within distribution that may have a significant impact on product quality.

16.4 External Requirements

The design process begins with regulations and guidance documents issued by the regulatory agencies, these publications form the external requirements for process design (Fig. 16.2). Regulatory agencies have issued regulations with oblique references to the QbD principles. The regulations define the quality systems and controls under which products must be manufactured.

Industry groups, e.g., the Parenteral Drug Association (PDA) and United States Pharmacopoeia (USP) develop guidance documents and process improvement approaches that support regulatory requirements. The guidelines from various groups in general require documented processes that are designed to ensure that products are manufactured and shipped in a "state of control." Adoption of QbD in transportation is still maturing. Its promise is the protection of the product throughout its lifecycle. The resources section at the end of the chapter contains links and titles to relevant regulations and guidance documents. An understanding of external requirements is critical to develop a well-defined process that is both documented and science-based, ensuring that product shipping meets the minimum regulatory requirements.

Fig. 16.2 Distribution process design, external requirements

Fig. 16.3 Distribution process design, quality manual

16.5 The Quality Manual

The quality policies set the standards for science-based product quality, assessment and assurance during development, manufacturing, storage, and distribution. The order of quality manual, from Fig. 16.3, reflects the critical nature placed on the firm's understanding of the external requirements and the expected scientific rigor needed for commercialization.

One of the purposes of a company's quality manual is to incorporate the regulatory requirements and guidance documents into the logistics process. The intent of a quality manual is also to present current quality system requirements applicable to all functions that support clinical and commercial production, storage, and distribution.

The chapters in the quality manual describe the process needed for a quality system, divided into elements of pharmaceutical processing functions. The sections that apply to controlled-environment logistics are document hierarchy, process control, training, and audits.

16.6 Quality Document Hierarchy

Quality planning through document hierarchy establishes the quality system. The categories of controlled documents are listed below:

a. Global standards: provides the company's approach to meeting specific regulatory requirements that must be followed at all applicable locations
b. Global standard operating procedure: provides direction on how the regulatory requirements are to be integrated in a multilocation company
c. Local documents: local documents comply with the requirements stated in the quality manual or operational standard. These documents provide instructions for performing a specific function or task and shall be followed, such as:

   - Standard operating procedures
   - Validation protocols
   - Technical reports
   - Guidelines
   - Forms
   - Best practices

16.7 Process Control

The process of shipping product should have efficient and effective distribution controls. This process is divided into four sections to meet the requirements set by the regulatory agencies. Further, the capabilities of each in the following are a source of data used by the development scientist when planning scale-up and tech transfer activities.

1. Receiving (what is the time between delivery and put-away?)
2. Storage (what controls are in place to control and monitor?)
3. Packing (how long is product exposed to warehouse conditions?)
4. Shipping (what is the service level and expected transit duration?)

16.8 Training

All employees are responsible for following applicable laws, regulations or directives, and commitments made to regulatory agencies. Each function shall be sufficiently staffed with competent and appropriately qualified members to achieve its quality objectives. This consistency of operation through training supports the QbD function by assuring that the testing parameters used during product development remains in place in the operation during the product lifecycle. The training can have additional meaning to the employees when the QbD aspect is added to the regulatory mandate. All functions supporting distribution contribute to the control and stability of the process.

16.9 Audit Measurement

The audit program is intended to identify and communicate compliance deficiencies associated with the manufacturing, processing, packaging, holding and testing of key intermediates, active pharmaceutical ingredients (APIs),, and drug products to ensure conformance to regulatory commitments and international regulations as necessary. Sharing the critical to quality attributes based on the product knowledge coming from the QbD exercise adds a context beyond that of compliance and shows how the audit is intended to improve the process in support of the product.

It is the duty of the manufacturer to establish acceptable limits and tolerances and share those requirements in a supplier Fment. Monitoring for the occurrence of these defined nonconformances and working with the supplier to resolve them in a timely fashion fulfills the intent of a supplier quality agreement as driven by necessary requirements including QbD. When it is grounded in product requirements, the improvements that result in monitoring and measurement truly have benefit

Fig. 16.4 Distribution process design, distribution process

for the patient. As an example, in some of the developing countries the operation of the chill room may be somewhat challenging. Managers at some 3PLs consider "colder" better for refrigerated material. Usually, an unreliable power grid is the reason for this practice. As a result, the set points for some of these storage areas are intentionally set low (+1 °C). Normal variation in the storage areas can expose product to temperatures as low as −2 °C, information that should be incorporated into product development and testing.

In summary to the quality manual, QbD should be seen as an enabling tool, not an added task. The quality manual enables the process, and QbD provides the context with specific requirements.

16.10 Distribution Process

The application of QbD requires that the distribution process (Fig. 16.4), example in Fig. 16.5, be widely known across the development and operational components in the company. Currently, few companies have this process in mind during the development of the product. The adoption of a risk-based approach compares and contrasts the capability of the distribution process with the requirements of the product. Knowledge of the network and collection of product data based on the network performance creates efficiencies and is critical to all distribution operations, whether internally sourced, or when contracting with transport service providers.

For an increasing number of products, the traditional approach comes short of recognizing the realities that make up the distribution environment. Here are a couple of examples.

Situation Stability studies support a shelf life of 36 months at +5 °C storage for a solution product in a vial.

Traditional Approach Follow International Conference on Harmonization (ICH) guidelines and conduct stress studies at +25 °C and −15 °C with cycling between the two conditions.

Result: Product fails analytical testing.
Impact: Any thermal exposure outside 2–8 °C will result in disposal.

QbD Approach Data collected from the supply chain network shows capability to keep refrigerated product between 0 and 12 °C with a 99% confidence level. Test the product between 0 °C and 12 °C with cycling between the two conditions. Place samples on long-term stability.

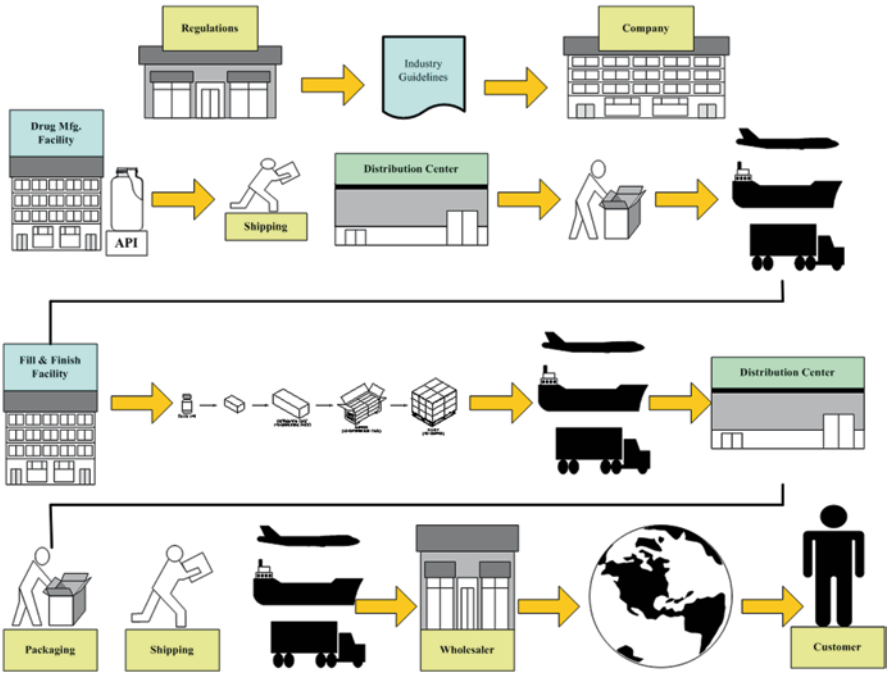

Fig. 16.5 Distribution flowchart

Result: Product passes initial and end-of-shelf-life testing.
Impact: The product design space has been harmonized with the network capability and minor exposures outside of long-term storage conditions have been shown to have no effect on product quality.

Situation Stability studies support a shelf life of 36 months at +25 °C storage for a tablet formulation.

Traditional Approach Perform accelerated studies showing stability at 40 °C and −15 °C for up to one month

Result: Label the storage temperature 20–25 °C.
Impact: Any thermal exposure outside the 20–25 °C will result in disposal.

QbD Approach Data collected from the supply chain network shows capability to keep room temperature product between 0 and 35 °C with a 99% confidence level. Test the product between 0 and 35 °C with cycling between the two conditions. Place samples on long-term stability.

Result: Product passes initial testing and end of shelf-life testing.
Impact: The product design space has been harmonized with the network capability and minor exposures outside the long-term storage conditions have been shown to have no effect on product quality.

Fig. 16.6 Distribution process design, technology transfer

Situation Internal development processes require shear testing for protein solutions to assess formulation suitability for select filling machines.

Traditional Approach Perform shear testing and analyze for particulates.

Result: Filling machines tested are suitable for use with the solution.
Impact: No determination of lifecycle impact has been studied.

QbD Approach Perform shear testing as above with a range of fill volumes, expose the samples to transport conditions of temperature, vibration, shock, and pressure. Study the longer-term effects on aggregation, posttransport.

Result: Lifecycle testing can assure that the product is formulated so that visible and subvisible particulates will be below critical thresholds.
Impact: The product formulation has the robust qualities to proceed through tech transfer and commercialization.

16.11 Technology Transfer

Technology transfer, Fig. 16.6, is the last step in distribution process design. It encompasses development of practical solutions for manufacturing at a new site (and often scale) from the results of development studies and previous manufacturing experience. In order to perform its functions of planning, implementing, and controlling the operations of shipping cold-chain products, the supply chain requires tools to facilitate the shipping process. The supply chain will require an acceptable process tool to design, build, and maintain a compliant biopharmaceutical-controlled environment. Achieving mastery of this "tool" is one of the greatest challenges facing the biopharmaceutical industry. QbD holds the key to this challenge. The work of generating the product knowledge in the development phases is critical. With sufficient data from development, technology transfer can be a direct replication at scale with reliable outcomes. Suitable supply chain partners can be identified based on product requirements. Only those potential partners that can meet the requirements need to be considered. No technology transfer package is complete without a strategy of monitor and control. Critical process parameters must be reviewed to assure compliant operation. The following is a real-life example of a mismatched distribution partner. Drivers of temperature-controlled trucks who had 3-day transit times regularly switched off the engine-driven compressors during their sleep periods. This practice was found out after two consecutive loads were lost. QbD sup-

plies the rigor to the selection process to ask some important questions in advance of some critical incidents.

16.12 Conclusion

Integration of QbD principles into the distribution processes represents a significant opportunity to understand the product and plan for the safe handling of temperature- and transport-sensitive products from the point of manufacture to the patient. It defines the necessary activities and has monitoring and controls in place to assure the critical quality attributes are maintained. Knowledge of the product outside of long-term static storage conditions gives a context to risk assessment and mitigation. Best practices in the industry have led to cost reductions in transportation based on a full understanding of the product stability. Short, managed exposures to small elevations in temperature, for instance, have accounted for much of these reductions. Successful launches of therapeutic proteins are a result of knowing how the physical stability of the molecule withstands the transport environment prior to launch. Many issues are identified and corrected in such a way as to avoid interfering with product launch schedules. There is a measure of skepticism in the industry pressured with "speed to market." The emphasis on creating additional product knowledge earlier in the development process is an investment, and leading companies are learning how to incorporate the means necessary to gain this knowledge. Regrettably, postapproval, most of the development resources are shifted to new projects, so the best time to generate the data is when the whole team is assembled. As successes are shared and replicated, QbD processes will be incorporated in product development across the industry. For now, the benefit is enjoyed in only a subset of the industry. In future, it will be an expectation not only within companies for the obvious cost savings, but more broadly from investors and partners when they evaluate promising new programs.

16.13 Resources

16.13.1 The PDA

The mission of the PDA is to advance pharmaceutical and biopharmaceutical technology internationally by promoting practical and scientifically sound technical information and education for industry and regulatory agencies. The PDA Quality and Regulatory Affairs Department monitors both international and domestic regulatory landscapes for new guidance that affects PDA members. The Regulatory Affairs Department notifies members of these guidance's via the PDA website, PDA emails, and the *PDA letter*. Relevant guidelines include quality systems, risk management, PAT, dispute resolution, manufacturing, chemistry and manufacturing

controls (CMC), and cGMPs. The PDA Quality and Regulatory Affairs Department also publishes news to help PDA members understand and comply with complex global regulatory expectations.

The PDA interacts with the FDA, The European Medicines Agency (EMEA), WHO, ICH, USP, and numerous other regulatory bodies around the world. Through the PDA Regulatory Affairs and Quality Committee (RAQC), comments to guidance and original proposals are made to regulatory bodies to promote science-based regulations and harmonization. The PDA Office of Science and Technology (OST) supports the pharmaceutical and biopharmaceutical industries by examining regulatory requirements to assure that they are scientifically sound.

If regulatory requirements are found to be scientifically unsound, the OST will petition the corresponding regulatory agency to amend the requirement. Another function of the PDA OST is to provide industry guidance where none previously existed. The PDA has published many technical reports offering the industry guidance on a vast number of topics that were previously unexplored.

16.13.2 The ICH and USP

The USP is the official public standards-setting authority for all prescription and over-the-counter medicines, dietary supplements and other healthcare products manufactured and sold in the USA. The USP works with healthcare providers to set standards for the quality of these products. USP standards are also utilized in many countries outside the USA. These standards have been helping to ensure proper pharmaceutical care for people throughout the world for more than 185 years. The new section, <1079>*Good Storage and Shipping Practices* specifies:

- Packaging and storage statement in monographs
- Storage in warehouses, pharmacies, trucks, shipping docks, and other locations
- Distribution and shipment of pharmacopoeial articles
- Special handling
- Shipment from manufacturer to wholesaler
- Shipment from manufacturer or wholesaler to pharmacy
- Shipment from pharmacy to patient or customer
- Returns of pharmaceutical articles from patients or customers
- Storage of physician samples handled by sales representatives in automobiles
- Storage of drugs in emergency medical services (EMS) vehicles
- Stability, storage, and labeling
- Statements/labeling of the immediate containers or package inserts

The ICH guidelines for technical requirements for the registration of pharmaceuticals for human use was established in 1990 as a joint regulatory/industry project to improve the efficiency of the processes for developing and registering new medicinal products in Europe, Japan, and the USA through process harmonization. The final goal of the technical requirements was to make products available to patients with minimum delay.

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Chapter 17
Quality by Design for Primary Container Components

Fran DeGrazio and Lionel Vedrine

17.1 Primary Container

The main function of a primary container is to protect a drug during its shelf life, which includes all steps during manufacturing, transportation, storage, and use. Depending on whether it is used in combination with a delivery system, various functions are expected from the primary container. The functions include:

- Sterility barrier
- Drug stability
- Functionality
- Delivery to patient
- Ease of use

Each of these functions needs to be characterized, and their critical attributes identified.

There are different types of prefilled containers, the most common being vials, prefilled syringes, and cartridges for injection pens. Prefilled syringes are unique because of their dual function to contain/protect and also deliver the drug, alone or in combination with an autoinjector, whereas vials and cartridges require that other delivery systems (plastic syringe or pens) are used.

The general concepts of Quality by Design (QbD) have been applied to devices for years, under terms such as Design for Six Sigma or Robust Engineering, but these concepts have not been widely used in combination with a drug product. However, for combination products, looking at the primary container and delivery

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system independently does not address the critical interactions between the two systems and has not been a consistently successful approach to development. Recent issues affecting combination product quality have triggered drug and primary container manufacturers to look more deeply at quality attributes and their impact to the functionality/performance of the primary container, including critical interactions with the drug product. Such a holistic approach to understanding the impact of critical quality attributes (CQAs) and critical material attributes (CMAs), independently as well as in combination, has proven to be the key to success for the primary container and combination product.

The QbD approach is to look at the different functions of the primary container (drug product protection, device interface, secondary packaging) in parallel with the delivery system (user interface, primary container protection). The main functions of the primary container are to:

- Preserve the drug product over its shelf life,
- Protect the drug product from an aggressive environment (light, contamination, bacteria, etc.), and
- Deliver the content (for prefilled syringes: accuracy, route of administration, completeness, etc.).

The main functions of the delivery system are to:

- Protect the primary container (shipping stresses, prevent activation),
- Deliver the content (accuracy, route of administration, completeness, etc.), and
- Ease of delivery (end user interface).

Many tools are available for identifying critical attributes. These include risk management, application failure mode and effects analysis (AFMEA), design failure mode and effects analysis (DFMEA), process failure mode and effects analysis (PFMEA) (Stansbury and Beenken 2011), tolerance analysis with Monte Carlo simulation, mathematical modeling, noise analysis (Taguchi), and robust engineering. Guidelines for building a framework for QbD using such tools are given by the appropriate ICH Guidance documents, which help provide the foundation for execution. These are ICH 8 (Pharmaceutical Development), ICH 9 (Quality Risk Management), and ICH 10 (Pharmaceutical Quality Systems). Some important excerpts from these guidance documents are given below:

Q8 R2 Pharmaceutical Development At a minimum, those aspects of drug substances, excipients, container closure systems and manufacturing processes that are critical to product quality should be determined and the control strategies justified. Critical formulation attributes and process parameters are generally identified through assessments of the extent to which their variances can impact the quality of the drug product. (ICH 2009)

Q9 Risk Management Evaluation of risk to quality should be based on scientific knowledge and ultimately linked to the protection of the patient. The level of effort, formality and documentation of the Quality Risk Management process should be commensurate with the level of risk. (ICH 2005)

Table 17.1 Application of process performance and product quality monitoring system throughout the product lifecycle

Pharmaceutical development	Technology transfer	Commercial manufacturing	Product discontinuation
The process and product knowledge generated, and the process and product monitoring conducted throughout the development can be used to establish a control strategy for manufacturing	Monitoring during scale-up activities can provide a preliminary indication of process performance and the successful integration into manufacturing. Knowledge obtained during transfer and scale-up activities can be useful in further developing the control strategy	A well-defined system for process performance and product quality monitoring should be applied to ensure performance within a state of control, and to identify improvement areas	Once manufacturing ceases, monitoring such as stability testing should continue to completion of the studies. Appropriate action on marketed product should continue to be executed according to the regional regulations

Q10 Lifecycle and Knowledge Management Table I: Application of Process Performance and Product Quality Monitoring System Throughout the Product Lifecycle [as shown in Table 17.1]. (ICH 2008)

The selection of an appropriate container closure is multifaceted, and includes the following considerations: protection, compatibility, safety, and performance. The responsibility of the sponsor is to ensure suitability and control of the components used in the container closure system; typically, this is achieved by developing a functional relationship with the supplier to understand their manufacturing processes, supply chain, and capacity to enable lifecycle management. Figure 17.1 maps the scope of qualification for packaging components. Items highlighted are examples of CQAs for a packaging component.

17.2 Vial

17.2.1 General Overview of Glass Vial Composition

Glass vials are typically the basic container used in the laboratory during drug development, early stage clinical trials, and are often used for the commercialized dosage form. In order to understand the potential impact of glass vials on the final quality of a drug product, it is helpful to have a basic understanding of glass composition and the vial manufacturing process.

The basic constituents of glass are:

- SiO_2, which is the base material;
- Na_2CO_3 or K_2CO_3, which are used as fluxing agents to lower the melting point of glass;

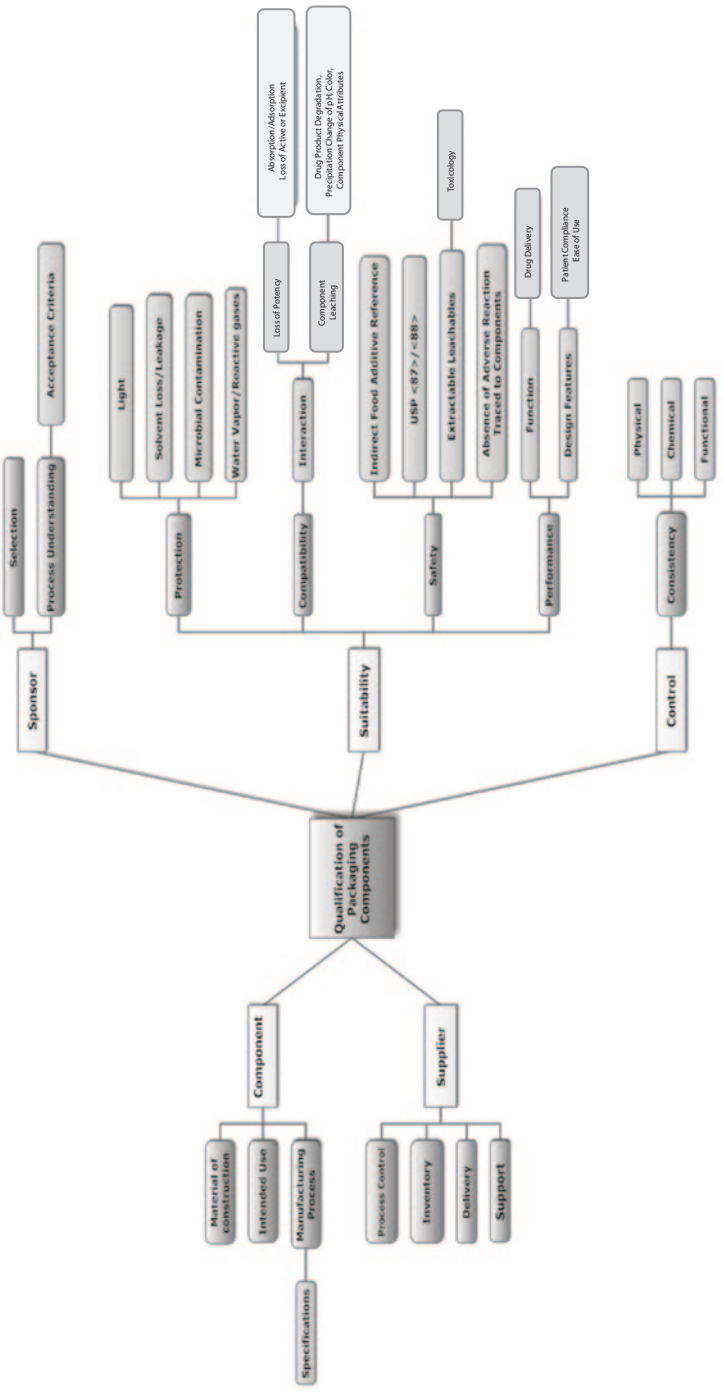


Fig. 17.1 Considerations in understanding packaging component qualification. (Reproduced from DeGrazio 2011)

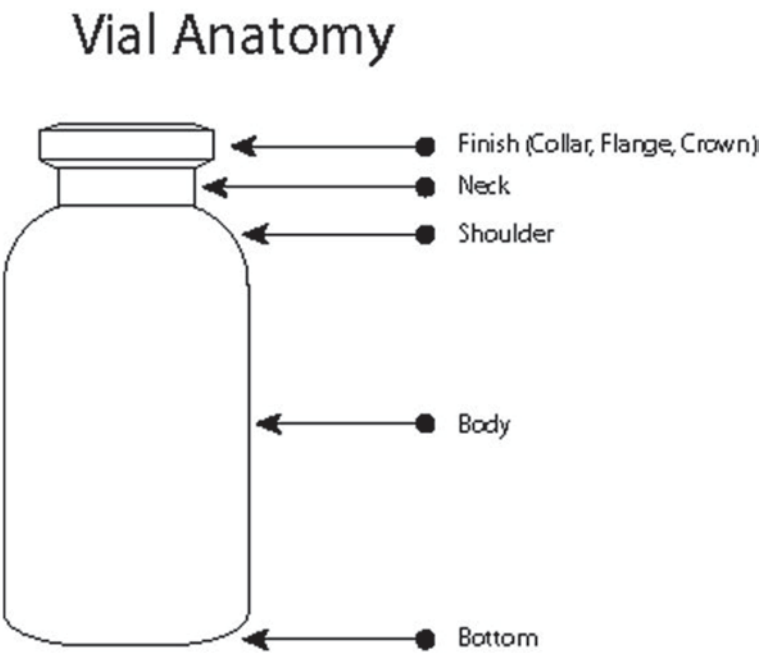


Fig. 17.2 Example of different vial features. (Reproduced from Schott Pharmaceutical 2000)

- $CaCO_3$, Al_2O_3, or B_2O_3, which are stabilizers; and
- Various coloring agents, which may be used to give amber or other hues.

Borosilicate-type glass is more durable and typically composed of 70–80 % SiO_2 and 7–13 % B_2O_3, with the remainder being additives. Type I pharmaceutical glass is the standard for parenteral drug products, including biologics. For biologic products, coloring agents and other additives would typically be avoided unless absolutely necessary, in order to minimize potential extractables.

17.2.2 Vial Design

An example of different vial features is shown in Fig. 17.2. Vial design and compatibility with the closure system chosen are critical for efficiency in machinability and functional issues, such as container closure integrity (CCI). From a QbD perspective, critical attributes related to vial dimensions may include the following examples:

- Minimum bottom thickness and maximum concavity—the ability to lyophilize efficiently is impacted by these attributes
- Crown inner diameter—critical to fit with the stopper to ensure CCI
- Crown outer diameter—important to ensure the aluminum seal or flip-off seal that keeps the integrity of the entire system fits together appropriately

17.2.3 Vial Dimensions

Glass Packaging Institute (GPI) and ISO have defined standard vial sizes for the industry; GPI vial dimensions are widely used in North America, while ISO standard vials predominate in Europe. Although both are in use currently, there is a trend in North America toward European dimension vials driven by a desire for global consistency and platform concepts, including preapproved packaging standards to be used globally within an organization. In addition, the European dimension vials benefit from a broader set of closures while retaining the same CCI benefits. Together these are the chief drivers for the move toward European glass across the industry. Of course, any change to the dimensions of a product not only has implication in the final container, but also affects the ability to machine and assemble. Therefore, adjustments will need to be made to ensure line speeds and quality during implementation.

17.2.4 Vial Configuration, Fill Volume, and Stopper Design Considerations for Freeze-Drying

When choosing an appropriate vial, it is important to consider whether it will be used for a liquid fill or a lyophilized product. If used for a freeze-drying application, the shape of the vial is critical in minimizing breakage, assuring CCI, and maximizing heat transfer in order to optimize the lyophilization process. Historically, a flat bottom glass vial was considered to be the best configuration for lyophilization (DeGrazio et al. 2010). Recently, however, research has been conducted (DeGrazio et al. 2010) that contradicts this historic assumption. In this work, the flat shape of the bottom of a standard vial was changed to a more "champagne-like" geometry and, as a result, improved heat conduction and enhanced mechanical stability were achieved (DeGrazio et al. 2010; Hibler et al. 2012).

Fill volume is another important consideration in selecting the appropriate vial for a frozen or lyophilized drug product. Significant rates of vial breakage during freezing have been reported for formulations containing crystallizing excipients, such as mannitol (Jiang et al. 2007a) or sodium chloride/sucrose (Milton et al. 2007). The phenomenon has also been reported for amorphous formulations, particularly at high protein concentrations (Jiang et al. 2007b). Fill volume has been shown to be a critical attribute in all cases. It is generally not advisable to fill more than 35 % of the vial capacity (Bhambhani and Medi 2010), which also has implications for fill line throughput and secondary packaging.

The choice of the appropriate stopper design is also dependent on whether the drug product will be provided as a liquid or lyophilized powder. Although specific design features and dimensions can vary; in general, lyophilization stoppers are designed with slots for transfer of water vapor and other gasses, as well as a means of positioning the stoppers above the vial during the drying process. The two basic stopper designs are described further in Sect. 1.3.2.1.

17.2.5 Manufacturing Processes

There are two basic methods to produce vials. USP Type 1 glass should be used for pharmaceutical applications of vials produced by either method. The first process is molding, in which molten glass is poured into a mold and then cooled over time to form or set a physical shape. Although molded vials are still available today in all sizes, typically they are used mainly for larger sizes in the 50–100 mL range. The other, more commonly used technique for small volume parenterals (SVPs) is to form vials from long tubes of glass. Vials produced by this method are called tubing vials. The tubing process utilizes heat to pierce the bottom of a closed-end glass tube with a flame. Heat is then applied to form the neck of the vial, and finishing tool is used to complete the formation of the neck and mouth of the vial. Heat is then used to form a narrow area that will eventually separate the vial from the tube, which then becomes the bottom of the vial. (It is this final step which limits the applicability of this manufacturing method for the larger vials.) Careful temperature regulation is required throughout to maintain dimensional control.

There are several advantages to using tubing vials instead of molded vials. Most significantly, tubing vials have a better wall and finish dimensional consistency. In addition, tubing vials have no seams, weigh less, and are typically easier to label. For these reasons, tubing vials are typically preferred for SVPs. However, because additional heat is applied during the forming process, tubing vials are more prone to glass delamination under certain conditions (Ennis et al. 2001). Glass delamination can potentially lead to glass particulates being shed from the internal surface of the vial into the drug product. Other factors can also affect delamination rates including manufacturing processes, such as terminal sterilization (Iacocca et al. 2010), formulation conditions such as alkaline pH and/or phosphate or citrate buffers (Sacha et al. 2010), and storage temperature (Iacocca and Allgeier 2007). A series of sterile-product recalls related to glass delamination, including at least two biologics (Hylenex May 2010 and Epogen and Procrit Sept. 2010), led to the 2011 FDA advisory to drug manufacturers on the formation of glass lamellae in certain injectable drugs (FDA 2011). A number of recent publications have reported accelerated delamination test methods as well as improved methods for examining vial inner surfaces (Wen et al. 2010; Guadagnino and Zuccato 2012). These examples clearly illustrate the sometimes complex interplay between the drug product and primary packaging, and emphasize the need to consider the critical attributes of the drug product and the primary packaging, in parallel.

17.2.6 Glass Vial Surface Treatments

The surface of glass vials can be reactive with the materials that are placed inside the vial. There are various glass-drug interactions that can take place. In addition to glass delamination discussed in Sect. 1.2.5, there is also the potential for inorganic substances to leach from the surface of the glass into the drug prod-

uct. Extractable and leachable components of glass are discussed in Sect. 1.2.8 (Walther et al. 2002).

There are several approaches to minimize the reactivity of the glass. The first is control of the manufacturing process. Heat input is critical to the reactivity of the glass surface. Heat input and process control during vial production can vary from manufacturer to manufacturer. This is a critical process parameter (CPP) that leads directly to control of a CQA for the glass vial (Haines et al. 2012).

A second approach to consider is the use of a surface treatment to reduce glass reactivity. This approach may be used in conjunction with an improved manufacturing process. The most common treatment is glass dealkalization using a sulfur treatment in which an ammonium sulfate solution is sprayed into the vial. This scavenges metal ions in the glass and converts them to water soluble salts. These salts can then be easily rinsed from the vials (Markovic 2009). The reactive surface, however, can recur over time as this treatment has limited depth of penetration.

A third option is the use of vial coatings. These are plasma treatments applied to the internal surface of the vial to form a barrier film of pure silica between the reactive surface of the glass and the drug product (Schwarzenbach et al. 2002; see Fig. 17.3 for an illustration).

Fig. 17.3 Example of SiO_2 coating applied to glass surface. (Reproduced from Schott Pharmaceutical 2000)

17.2.7 Glass Vial Defects

In 2007, the Parenteral Drug Association (PDA) released Technical Report No. 43: *Identification and Classification of Nonconformities in Molded and Tubular Glass Containers for Pharmaceutical Manufacturing*. This document, which was assembled by a cross functional team of industry experts, is the de facto guide on glass defects. It provides a complete overview of potential defects for tubing and molded vials, and covers sampling strategies, specification development, and defect lexicons (PDA 2007).

Assuring high-quality glass vials is the responsibility of, both, the supplier and the drug manufacturer. Controlled vial processing to minimize defects and ensuring effective packaging are critical and should be the key expectations for the vendor. The drug manufacturer needs to be knowledgeable on quality criteria and should actively participate in the development of comprehensive specifications that ensure product quality.. The other critical aspect for the drug manufacturer is to understand high risk areas in its process that could impact product quality. A thorough PFMEA should be completed to mitigate risks within the sterilization fill/finish and packaging processes. Details about glass vial quality have been published elsewhere (Loui 2011).

17.2.8 Chemical Compatibility: Extractables/Leachables

Glass is not inert. There are a series of potential interactions that can occur between the glass and its contents (Paskiet et al. 2010). The following is a list of these reactions which should be considered:

- Ion exchange: Li, Na, Mg, Ca, Al
- Glass dissolution and ion exchange
  - Acidic/neutral (ion exchange): Na^+ (glass) + H_3O^+ (solution) ↔ Na^+ (solution) + H_3O^+ (glass)
  - Basic (dissolution): 2 OH^- (solution) + $(SiO_2)_x$ ↔ SiO_3^{-2} + H_2O
- Pitting: reaction with EDTA
- Adsorption: materials, such as insulin, albumin, epinephrine, and atropine sulfate can stick to the surface of the glass vial; and therefore, not be available for use
- Precipitation: formation of $MgSiO_3$, $BaSO_4$, or similar materials

Several of these reactions are due to the potential of glass constituents or additives to leach and react with the constituents of the drug product. Table 17.2 provides a summary of the types of extractables from glass, and the conditions under which they are most likely to leach.

As with any material used in the storage of a biopharmaceutical, extractable-leachable studies must be conducted to characterize product-related degradation or impurities resulting from the long-term interaction of the drug product with the glass vial. Each individual drug product may have specific sensitivities. As an ex-

Table 17.2 Extractables from glass

Extractables from glass		
Major	Minor	Trace
Si^{+4}	K^{+1}	Mg^{+2}
Na^{+4}	Ba^{+2}	Fe^{+2}
	Ca^{+1}	Zn^{+2}
	Al^{+3}	
Potential glass reactions		
Ion exchange	Glass dissolution	
Occurs at pH < 7	Occurs at pH > 8	

ample, pH shifts have a significant impact on product stability and/or the ruggedness of many biologics. If this is critical, then pH shifts due to leaching from the glass vial must be identified as a CQA, which must be mitigated by understanding the CMAs and CPPs that influence this property. Part of a QbD process in drug development should be the use of a risk assessment process, such as an FMEA, to understand the highest risks from packaging that could negatively influence the drug. For more information, Wakankar et al. (2010) provide an example of a general program for assessing extractables and leachables, including the primary container.

17.2.9 New Technologies: Plastic Vials

As stated earlier, majority of the vials used for sterile, parenteral applications are made of glass. A newer trend in the industry, however, is the use of plastic vials. Although, historically, the only type of products stored in plastic have been simple liquids, such as water for injection or saline solution; introduction of new plastic resins may allow these materials to play a more significant role as primary containers for biological drug products in the future.

One of the major reasons that "traditional" plastics, such as polyethylene or polypropylene, have not been used is that these materials cannot withstand the heat from sterilization. In addition, the permeation/transmission rates for oxygen and moisture are significantly greater for these traditional plastics than for glass containers. Also, these materials are not fully transparent, leading to problems during the visual inspection for particles that every vial must undergo as a part of quality control release. The vial must be clear to facilitate this inspection whether by an automated vision system or by a human inspector. Finally, the traditional plastics lack the "pharmaceutical elegance" associated with a fully transparent "glass-like" appearance.

With the advent of more advanced plastic materials, such as polycyclic olefins, many of these negatives have been minimized, and the benefits of these materials over glass may be realized. The development of polycyclic olefin materials has raised the bar in the qualities of plastic vials due to their properties including their glass-like transparency. As shown in Table 17.3, these materials provide many key benefits but also have some drawbacks when compared to glass vials (Vilivalam et al. 2010).

Table 17.3 Features of cyclic olefins for parenteral drug delivery

Key benefits	Drawbacks
Glass-like transparency, sterilizable (via autoclave, radiation, and ethylene oxide), high break barrier, excellent moisture barrier, biocompatible (inert, low binding, and ion extractables), design flexibility and excellent dimensional tolerances, good chemical resistance	Gas and moisture barrier properties are less than glass but better than other plastics, sensitivity to scratches, short-term discoloration due to radiation

Currently, a number of small- and large-molecule drugs contained within these high performance resins have been approved in Japan, Europe, and North America; and many biologic drugs in the new plastics are being evaluated.

17.2.10 Key Attributes

The critical attributes of a container closure system reflect the multiple functions of the primary container to protect the drug product during its shelf life, including providing a sterility barrier as well as preserving the integrity and stability of the product. In addition, under the integrated QbD approach, potential interactions between the drug and the container system are also considered in developing a set of CQAs. Examples of CQAs that can be applied to the drug and container system:

- Protect product sterility
- No formation of glass flakes/delamination
- Compatibility with drug
- Minimize particles in solution
- Minimize pH shift
- Reduce container breakage
- Pharmaceutical elegance of the package

As with all aspects of drug product development, the specific CQAs for a particular system will vary based on design-space considerations, product knowledge, and the risk assessments for the individual product.

17.3 Components for Vials and Prefillable Syringe Systems

17.3.1 Elastometric Components

Elastomeric components are rubber-based materials that are used to seal containers. In this case, the primary container may be a vial or a prefillable syringe system. In the case of a vial, the closure is called a stopper (Fig. 17.4), and it has

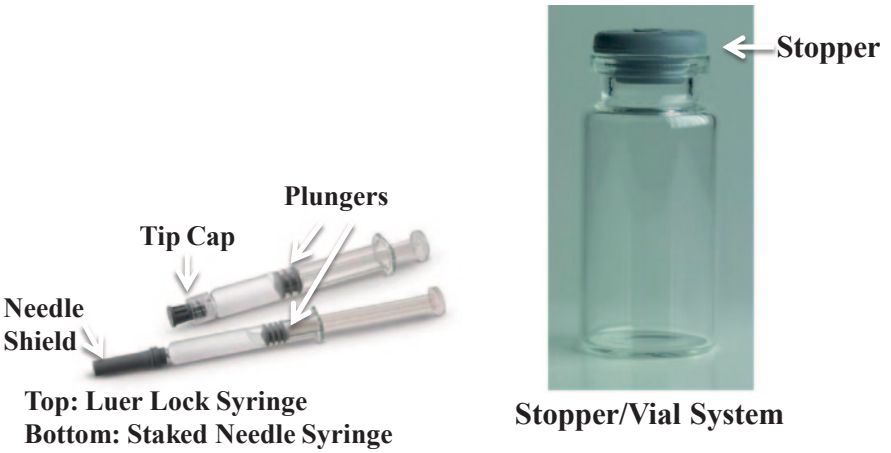


Fig. 17.4 Systems used for parenteral packaging **a** stopper/vial system, and **b** syringe systems

multiple functions, such as assuring sterility and allowing needles to access the medicament and reseal. In a syringe application, there are typically multiple elastomeric components, as shown in Fig. 17.4. The most critical is the piston (ISO term) or plunger (common term), which is inside the barrel of the syringe. This component has constant direct contact with the drug product, so its compatibility from a chemical standpoint is critical. It also has multiple functions as it must slide down the barrel of the syringe, and help to express the drug while maintaining the CCI of the system. At the end of the syringe, whether it is a Luer-lock design or a staked-needle system, there is another elastomeric component that seals the end of the syringe. This component is a tip cap (for Luer systems) or a needle shield (for staked-needle systems).

17.3.2 Closure Configurations

17.3.2.1 Stopper Configurations

There are many different stopper designs; the most critical factor in stopper design is its application to a liquid or lyophilized product. A stopper for a liquid product is designed to ensure sterile containment, allow needle penetration with resealing, and work smoothly with the stoppering equipment on the manufacturing line. A lyophilization stopper requires additional features to allow positioning over the vials during lyophilization with unobstructed sublimation of water vapor or other solvents. Figure 17.5 shows examples of stoppers that are appropriate for use in these types of applications.

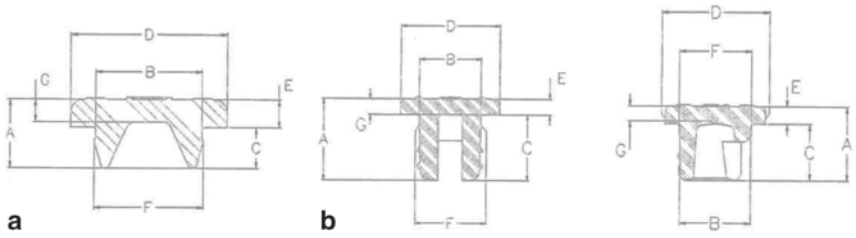


Fig. 17.5 Example stopper configurations **a** serum stopper and **b** lyophilization stopper *A* overall height, *B* plug outer diameter, *C* plug height, *D* flange outer diameter, *E* flange thickness, *F* no-pop ring, *G* diaphragm thickness

17.3.2.2 Syringe Component Configurations

The piston/plunger has a critical dual role, as it needs to balance the features of breakloose and extrusion with the feature of CCI, meanwhile assuring chemical compatibility and drug stability.

Typically, silicone oil is used on the inside of the syringe barrel and on the plunger in an effort to optimize the functionality. Other types of lubricity or barrier coatings can also be used, such as B2 coating, a polymerized silicone, and various materials that are applied through vapor deposition. Each of these variables can impact the appropriate interference fit between the plunger and the barrel.

Examples of various plunger designs are shown in Fig. 17.6. The dark area at the face of the stopper indicates a fluroelastomer film coating, which is commonly used as a barrier between the rubber and drug product (West Pharmaceuticals 2012).

The use of these types of film minimizes the potential for soluble extractables to migrate into the drug products; thus, minimizing the impact of leachables into the drug. The use of a West FluroTec® fluroelastomeric coated plunger was one of the corrective actions used in the Eprex case (Boven et al. 2005). Currently, a majority

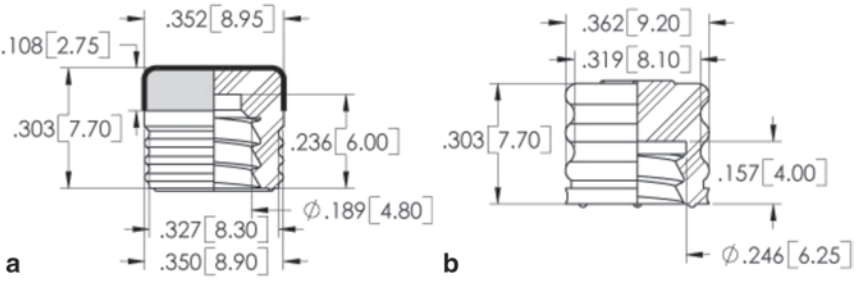


Fig. 17.6 Examples of **a** film coated and **b** uncoated plungers

of biologic products utilize FluroTec® coated stoppers and plungers as a standard for risk mitigation.

17.3.2.3 Elastomeric Formulation Composition

Multiple ingredients are blended together to produce a rubber component. These ingredients not only aid in manufacturing but also give, both, physical and chemical characteristics to the finished elastomer component. Typical materials used in the rubber component are listed in Table 17.4, and an example of a typical rubber formulation's ingredients is listed in Table 17.5.

The basic manufacturing process for elastomer components is as follows: The raw materials are mixed together and calendared or extruded into a sheet; this uncured sheet of rubber is placed into a press, which contains a mold indicative of the design of the closure; under extreme heat and pressure the rubber undergoes cross linking, a chemical reaction that is irreversible.

The overall QbD objectives for the elastomer manufacturing process are: to design a product to meet patient requirements; to consistently meet CQAs; to understand the materials and process parameters; to identify critical sources of variation; and to monitor and improve quality over time (Nasr 2009). With the advent of QbD,

Table 17.4 Rubber composition

Component	Purpose/function
Elastomer	Base material
Curing agent	Forms cross-links
Accelerator	Type and rate of cross-links
Activator	Efficiency of accelerators
Antioxidant	Antidegradant
Plasticizer	Processing aid
Filler	Physical properties
Pigment	Color

Table 17.5 Typical thermoset rubber formulation. (Source: West Pharmaceutical Services Formulation Development Group 2012)

Ingredient	Percentage by weight
Chlorobutyl rubber	52.7
Calcined clay	39.4
Paraffinic oil	4.2
Titanium dioxide	1.1
Carbon black	0.13
Thiuram	0.14
Zinc oxide	1.6
Hindered phenol AO	0.53

this includes defining CQAs at the initiation of a development program through the utilization of design of experiments (DOE) to understand CPPs through the use of process analytical technology (PAT) as part of a control strategy for the container/closure system in combination with the drug product. As advances have taken place, there have been additional developments to improve the quality of these types of products. This includes developing drug formulations and manufacturing to meet predefined product quality attributes. With respect to packaging components, the advances in technologies include developments, such as the addition of barrier films or coatings, which are applied to the surface of the rubber to make it more inert or more lubricious, and the use of inspection technologies, such as automated vision inspection to ensure outgoing closure quality. ICH Guidance Q8R2 directs the pharmaceutical development process from the identification of the CQAs through control strategies (ICH 2009).

17.3.3 Key Attributes

Elastomeric formulation recommendations for, both, stopper and syringe components applications are based on their specific applications. In general, the following holds:

- Stoppers
  - Halogenated or non-halogenated butyls
  - Need very good moisture and oxygen transmission barriers
  - Steam sterilized
- Plungers
  - Halogenated or non-halogenated butyls
  - Need very good moisture and oxygen transmission barriers
  - Gamma irradiation or steam sterilized
- Tip cap or needle shield
  - Polymer blends
  - Need high gas-transmission rates to facilitate sterilization by ethylene oxide or steam while on syringe barrel (applicable to glass only)

17.3.3.1 Coatings and Films

Barrier films, which are based on fluroelastomers, can be applied to, both, stoppers and syringe components to minimize the potential for extractables to migrate, to improve general compatibility with the drug, and to improve functionality. These films, commonly known by their trade names Teflon® and FluroTec®, are applied in the component molding process, and are chemically bonded to the surface of the rubber.

Table 17.6 Common defect categories for elastomeric components

Defect type	Defect category
Critical	Contamination of biological origin (hair or insect)
	Defects leading to leakage and non-sterility
	Film incomplete/missing/cracks
Major	Defects impairing function (non-fill, holes, film wrinkled, etc.)
	Defects impairing processing (trim lips, cut-offs, malform, etc.)
	Embedded foreign matter ≥ 0.2 mm^2
	Embedded fiber ≥ 2 mm
	Embedded foreign matter ≥ 0.05 mm$^2 < 0.2$ mm^2 in solution contact/target/seal area
Minor	Defects not impairing function (non-fill, holes, film wrinkled, etc.)
	Defects not impairing processing (trim lips, cut offs, malform, etc.)
	Embedded fiber < 2 mm ≥ 0.5 mm
	Embedded foreign matter ≥ 0.05 mm$^2 < 0.2$ mm^2 in non-solution contact/target/seal area

Other coatings are also available. These are typically used solely for the purpose of adding lubricity for machinability or functionality. The most typical coating is polydimethyl siloxane fluid, which is commonly known as silicone oil.

17.3.3.2 Elastomer Defects

Table 17.6 depicts common defect categories for elastomeric components and gives an example of critical, major, and minor defects that are typical for elastomeric components. It is only a portion of the complete list that a component manufacturer could supply to a customer and selected on the basis of the fact that these are common defects of high importance to the end product. The PDA Task Force for Elastomeric Closures and Seals Defects is developing standards for elastomeric defects. The current target for completion of the draft technical report is till the end of 2014.

Maintaining Drug Quality through the Closure System

Ultimately, one of the most important considerations in a total package is the use of a "clean" closure. The responsibility for this is best driven upstream from the drug manufacturer, as there are many factors that contribute to this "cleanliness" factor, and most are best controlled at the closure manufacturer.

Closure cleanliness comprises three segments: particle cleanliness, chemical cleanliness, and biological cleanliness. Table 17.7 gives examples of CQAs in relation to the individual components of a system for each segment of cleanliness. It is important that there is an understanding of these criteria for each component; this applies not only to the formulation and how it was manufactured, but also to its configuration, preparation, and sterilization.

Table 17.7 Examples of critical quality attributes (CQAs) of elastomeric components relating to cleanliness

Particle cleanliness	Chemical cleanliness	Biological cleanliness
Endogenous	Soluble extractables	Pyrogens
Exogenous	Volatile extractables	Hemolysis
Visible	Blooming	Microbes
Subvisible	Process residuals	Hair
Abrasion		
Fibers		

17.3.4 Extractables and Leachables from the Primary Container

Primary container suitability for the drug product and patient in the regulatory environment is defined as compatibility, safety, protection, and performance (US Department of Health and Human Services 1999). In this context, the issue of extractables and their relationship to leachables, specifically with respect to patient safety, is critical. Extractables, which are defined as species that can migrate from the packaging under stressed conditions, can impact drug product quality directly, impact compatibility, and even impact drug analysis due to test method interference or related issues. The migration of extractables into the drug product during production, storage, shipping, and handling is known as leaching; leachables are considered a CQA of the finished drug product in its package. As leachables are often a subset of extractables, both qualitative and quantitative correlation of leachables to extractables is the ultimate goal. Given that these leachables could be injected into a patient, there is direct correlation to patient safety (Markovic 2006).

QbD concepts for extractables and leachables can be addressed through a systematic approach, which includes the following:

- Understanding the extractables from the primary package during drug development
- Understanding sensitivities of the specific drug product (e.g., metals, oxidizers, etc.)
- Risk-based assessments, which involve defining CQAs, gaining material and process knowledge, and performing controlled extraction studies
- Identification and measurement of extractables and leachables, development of acceptance criteria, and assurance of control (Wakankar et al. 2010)

17.3.5 Key Functional Characteristics

In addition to CCI, there is an assortment of considerations that need to be understood in relation to a vial/stopper/seal system. For a stopper, some of the most critical concerns are the issues of coring and reseal. Coring is the characteristic of a stopper formulation in a certain configuration to withstand multiple injections with

a needle without fragmenting. Fragmentation can take the form of small bits of rubber that can break off or actual cores of rubber that can fill the cannula of the needle. This could lead to safety, sterility, and quality issues. Reseal is the characteristic of the rubber sealing back following multiple injections. Both, coring and reseal methods, can be found as part of the European Pharmacopoeia Reference Standard 3.2.9: Rubber Closure Containers for Aqueous Parenteral Preparation, for Powders and for Freeze-Dried Powders.

In addition to these basic tests, there are other functional tests that should be considered depending on the final application of the drug. These are considerations such as needle penetration or spike penetration. This is the force at which the needle or spike would pass through the stopper. In all cases, functionality testing should be conducted both before and after sterilization, as the addition of any energy, whether it be steam, gamma, or e-beam irradiation, can impact the rubber elastomer and change functional characteristics. Basic functional testing methods can be found as a part of USP<381>and EP 3.2.9 protocols.

The other critical aspects of the elastomer that are specific to drug application are the characteristics of moisture vapor transmission, oxygen transmission, and moisture vapor absorption. Typically, the data for vapor and oxygen transmission can be provided by the elastomer supplier. Butyl-based elastomer formulations are typically recommended for pharmaceutical and biopharmaceutical applications because they are good barriers to moisture and oxygen (Bhambhani and Medi 2010).

The characteristic of moisture absorption, however, is formulation specific. Moisture absorption is the capacity of a formulation to absorb and retain water following an autoclave cycle. This is important in relation to lyophilized products. Following steam sterilization, if a stopper is not dried sufficiently of internal moisture, then the moisture can transfer from the stopper to the freeze-dried cake over the storage time period, potentially impacting the cake structure and/or stability of the protein drug. On the other hand, stopper drying cycles can be extensive, adding cost to a process, and extended heat can damage the stopper by making it tacky or by increasing its crosslink density, which could have functional implications. Therefore, it is important to avoid overdrying (Wolfe et al. 2004; DeGrazio et al. 2010).

QbD techniques can be used to optimize and understand the drying process and its impact on moisture retained in the stopper following processing. This is an excellent example of how a DOE can be used to optimize the drying cycle of a closure to ensure desired moisture levels and to minimize the time necessary to perform drying. Figure 17.7 provides an example of CPPs related to the key CQA of coring of a closure system.

Extrusion passes and curing temperature are process parameters that are being evaluated in an effort to understand the CQAs of stopper coring.

17.3.6 Compatibility of Vials, Stoppers, and Seals as a System

- The most critical issue that needs to be understood is the dimensional compatibility of the total system. The typical interference fit of stopper to glass is 3–4%.

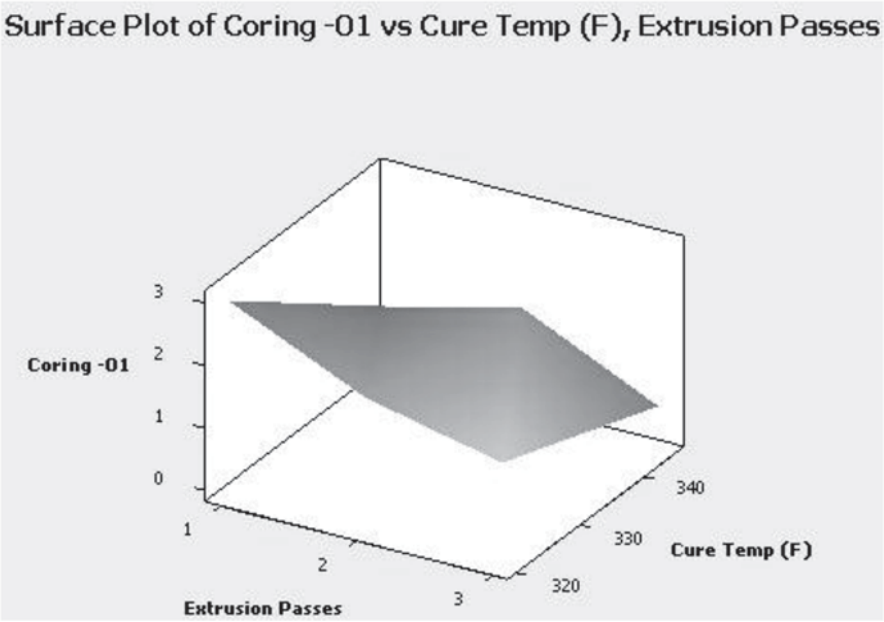


Fig. 17.7 CPP example: relationship of coring, cure temperature, and extrusion passes

The system is visualized in Fig. 17.8. Interference fit is the percentage of stopper outer diameter that interferes once inserted into the inner diameter of the neck of the vial. If there is not enough of an overlap, CCI can be impacted. If there is too much interference, the stopper will not insert into the vial or may pop up.

17.3.7 Container Closure Integrity of System

QbD for the vial/stopper/seal system starts with the development of a design specification. This specification is based on previous knowledge, risk assessment, and continuous learning. These container closure designs are specific to each drug and its intended application. In addition to the system design specification, there should be a reference to CQAs for each individual component. CQAs should relate to end drug product quality and patient safety, and to the potential impact in the drug manufacturing process, itself.

The product development approach by the pharmaceutical industry expects that market/user/regulatory requirements will be translated into measurable, technical, quality criteria for the proposed product(PDA Letter 2010). Table 17.8 provides an example of CQAs related to CCI, which are further described briefly below.

Table 17.8 Example of critical quality attributes relating to container/closure integrity

Stopper	Glass
Outer diameter of plug	Presence of blow back
Stopper flange thickness	Inner diameter of neck

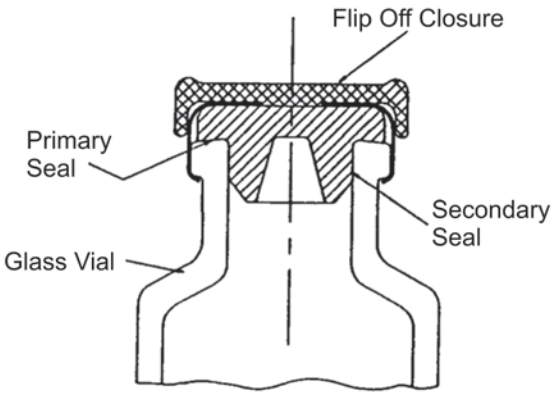


Fig. 17.8 Vial closure system diagram

17.3.7.1 Container Closure Integrity of a Stopper/Vial/Seal System

Looking at a total system, one of the most critical aspects that can have direct impact on the safety of the end patient is CCI, a term that covers the aspect of assuring sterility as defined by blocking microbes from moving into the vial. It also applies to the assurance that gas or vapor is contained, as appropriate. It is a measure of the ability and quality of a system to provide protection and maintain efficacy and sterility during the shelf life of a sterile drug product. Each system is only as good as the sum of its parts; therefore, it is critical that the individual components fit together appropriately. Individual components design and the processing of the components may have an impact on this feature. In addition, the assembly process is critical. Aspects of assembly, such as capping pressures and crimping technique, can all have impact on CCI.

To truly understand CCI, one must first have an understanding of leak rates and an understanding of the barrier that needs to be maintained. Many drug products are moisture or oxygen sensitive. Moisture or oxygen can enter the vial by permeation or by leakage. The transport rate for a gas (permeation) is determined by the size and amount of holes in the system. If the correct types of closure and vial have been chosen to minimize permeation, then the focus should be on minimizing leakage. Leaks can be categorized as gross or fine. In general, many standard test methodologies are appropriate for the detection of gross leaks, including the following (Guazzo et al. 2010):

- Helium leak
- Laser adsorption headspace
- Residual seal force

- Vacuum decay
- Dye immersion
- Microbial ingress

In selecting the appropriate methods, it is critical that one understands the capability and sensitivity of each in order to ensure that both gross and fine leaks are addressed (Kirsch et al. 1997a; Kirsch et al. 1997b).

17.3.7.2 Container Closure Integrity Points to Consider

Seal integrity techniques can be used in lieu of sterility testing per the FDA Container Closure Systems for Packaging Human Drugs and Biologics Guidance (FDA 1999). This allows for dye penetration (weight loss and bubble testing can only detect gross leaks) and ensures that helium leak rates are well correlated to microbial ingress and can be validated.

17.4 Prefilled Syringes

Glass prefilled syringes have been the fastest growing primary container over the last 10 years, with an expanding range of applications such as high concentration formulations, viscous solutions and combination products (e.g., autoinjectors). This has brought new requirements for which prefilled syringes were not originally designed.

The main issues observed to date comprise three major categories: drug product syringeability and injectability, particularly for high concentration or higher viscosity formulations; drug product stability and compatibility with the prefilled syringe components; and performance of the prefilled syringe in the injection device. Syringeability and injectability may be a significant concern particularly for high viscosity drug products, such as PEGylated proteins or high concentration antibody formulations (Shire et al 2010). In the area of drug stability and compatibility, issues can include drug instability due to tungsten, interactions with silicone oil, and leachables from the prefilled syringe components, particularly the plunger or needle-glue assembly (Adler 2012). Issues impacting performance in the injection device may include suboptimal silicone distribution and glass breakage (Rathore et al. 2012). As the market has matured, many of these issues have been addressed and improvements have been made to prefilled syringes. For example, additional staked-needle configurations, including thin-walled needles, have been developed for higher viscosity products, low- and no-tungsten syringes are available, silicone distribution and silicone quantity have been improved by utilizing diving nozzle technology, and glass breakage has been minimized

through improved controls on the forming process and better understanding of the device requirements.

The following subsections describe the different components/processes and packaging of prefilled syringes, and the key quality attributes that need to be addressed.

17.4.1 Quality by Design Applied to Prefilled Syringe

The attributes listed in Table 17.9 may be part of the design space investigation:

17.4.2 Nested Packaging vs. Bulk Packaging

Syringes can be delivered from the manufacturer in either nested- or bulk-packaging formats, as shown in Fig. 17.9.

> Nested packaging: in sealed boxes where the syringes are washed, siliconized with the tip cap, or needle-shield assembled, then ethylene oxide sterilized.
> Bulk packaging: in trays where the syringes are not processed. The washing, drying, siliconization, needle-shield or tip-cap placement, and sterilization (steam for staked-needle syringe and dry heat/steam for Luer syringe) are performed just before filling by manufacturer or contract filler.

The current market trend is toward nested packaging. This trend is supported by the flexibility, ease of processing (no washing, siliconization, sterilization), lower

Table 17.9 Potential key quality attributes for a prefilled syringe

	Design space investigation		
Attributes	Factors	Responses	Comments
Needle	Internal diameter	Flow rate	End-user force (human factor), spring design (autoinjector)
	External diameter	Pain perception/penetration force	Patient acceptability
Silicone	Quantity	Gliding force	End-user force (human factor), spring design (autoinjector)
		Drug stability	Subvisible particles, change in drug substance
	Distribution	Gliding force	End-user force (human factor), spring design (autoinjector)
Glue	Percent polymerization	Extractable/leachables	Drug stability
Tungsten	Quantity of W, W oxide, W salt	Particles (aggregates…)	
Flange design	Design, diameter	Breakage force, drop test	Autoinjector design, needle safety design

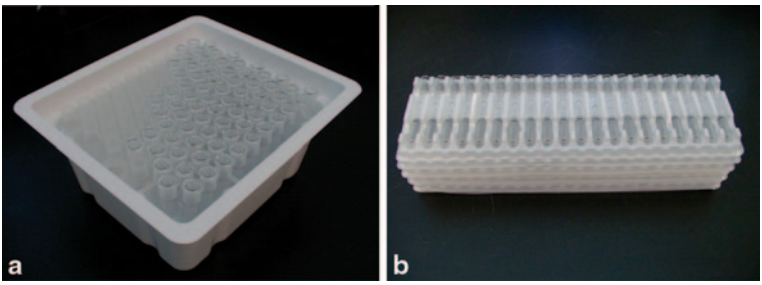


Fig. 17.9 Examples of **a** nested and **b** bulk syringe packaging

capital investment for filling equipment, and the consistency of quality (facility running 24/7 on the same format). A few years ago, only two manufacturers were able to deliver nested syringes; however, currently almost all suppliers are providing the nested format.

For bulk-packaged syringes, there are several different sterilization methods that can be used after washing and siliconization: dry heat tunnel to cure the silicone and sterilize, ethylene oxide, or steam to sterilize. Dry heat (>200 °C) is applicable only to tip-cap or Luer configurations because the adhesive currently used in staked-needle syringes is not resistant to high temperatures.

17.4.3 Design

Prefilled syringes are fundamentally different than vials and the plastic disposable syringes used with vials or other applications. The prefilled syringe contains the drug product during its shelf life, and it is designed to contain and deliver an accurate amount of drug product. Two main types of prefilled syringes are available: staked needle and Luer syringes.

17.4.3.1 Staked-Needle Syringe

Figure 17.10 represents a glass prefilled syringe with staked needle (needle glued to the syringe body). It has a needle shield to close the fluid path and maintain needle sterility; it is filled and closed with a rubber stopper and plunger rod assembled. Some can have printed lines to enable dose adjustments. Prefilled syringes with staked needles are available with different needle lengths and gauges.

In a prefilled syringe with a staked needle, the needles are maintained in the syringe body by adhesive, which is cured, usually by UV light. New fabrication techniques for plastic syringes are attempting to eliminate the adhesive, which can be a source of extractables and leachables, by utilizing polymer overmolded around the needle. So far, this solution has been reserved for specific applications and is, in

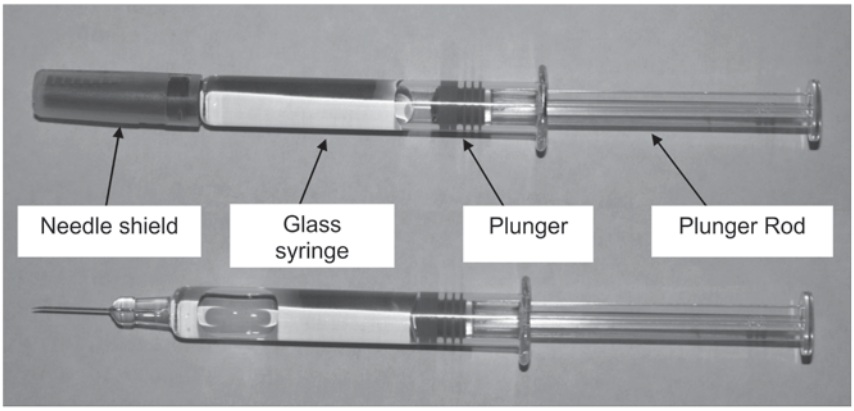


Fig. 17.10 Staked-needle prefilled syringe

any case, not available for the more common glass prefilled syringes. The cannula, itself, is made of stainless steel and complies with ISO 9626 (ISO 1991).

Staked-needle syringes have some unique features that can impact drug stability and compatibility, including tungsten deposits from the needle insertion process and the needle-glue assembly. In addition, the range of needle options is more limited. Nevertheless, the use of staked-needle syringes is increasing. Drivers for the increased uptake of staked-needle syringes, include ease of use, greater dose accuracy, reduced holdup volumes, and reduction of external contamination (no needle connection). In addition, the staked-needle syringes are the standard primary container for autoinjectors to maximize the convenience and ease of use of these devices.

17.4.3.2 Luer Syringe

Figure 17.11 represents a glass prefilled syringe with a Luer-lock connection. It has a tip cap to close the fluid path and maintain sterility; it is filled and closed with the rubber stopper and plunger rod assembled. Some also have printed lines for dose adjustment. Luer-slip solutions are also available when locking threads are not required. Luer syringes offer more needle gauge and length options than do the staked-needle syringes, as they can take advantage of the wide range of available needles.

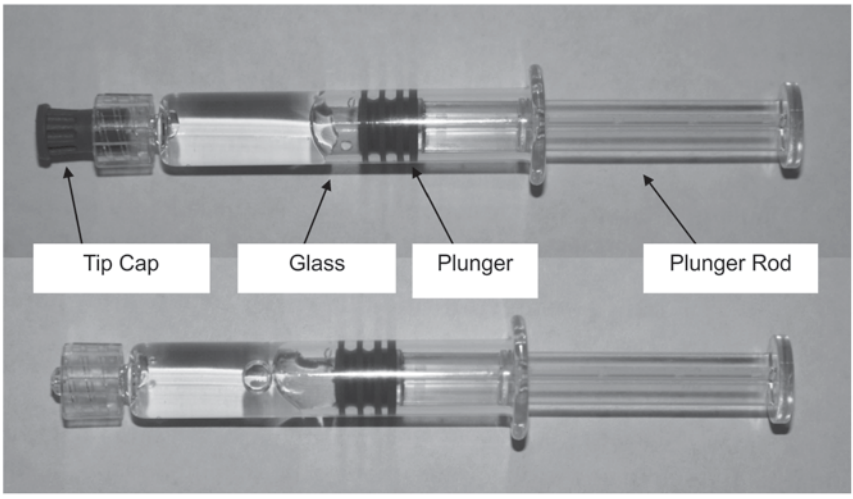
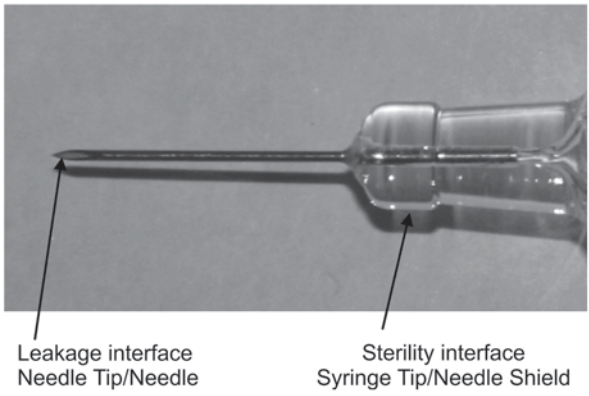


Fig. 17.11 Luer syringe with tip cap

Fig. 17.12 Syringe tip and needle shield interfaces



17.4.4 Needle Shield and Tip Cap

The needle shield (staked-needle syringes) and tip cap (Luer syringes) are designed to ensure CCI of the drug, that is, ensure sterility and prevent leakage. They are made of elastomeric materials, including newer latex-free formulations. The tip cap functions similar to a stopper to maintain CCI for Luer syringes. Different designs are available to form a tight seal with slip or locking-thread syringes. The needle shield is designed to be pierced by the tip of the staked needle to ensure tightness. Between 1–2 mm of the tip of the needle is embedded into the rubber to close the fluid path. Due to this design, the elastomeric formulation has an impact on needle

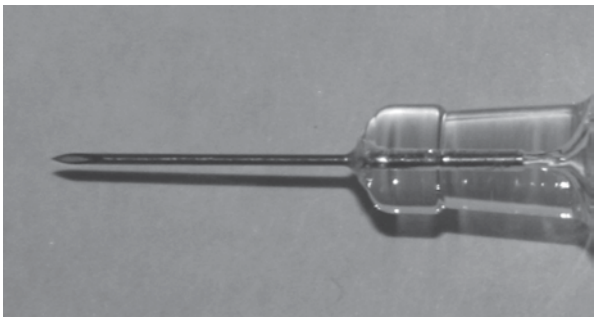


Fig. 17.13 Staked needle

sharpness, and some suppliers have developed special formulations to protect the sharpness (Vedrine et al. 2003). The sterility of the needle is ensured by the interfaces of the syringe tip and needle shield, as shown in Fig. 17.12, and not by the needle embedded into the rubber as was commonly thought.

17.4.5 Needle

The needle represents the ultimate interface between the patient and the syringe. Needle characteristics impact the injectability of the drug product as well as patients' perception of pain or discomfort and are, therefore, an extremely important aspect of the prefilled syringe design.

A needle (Fig. 17.13) is characterized by its length, gauge, internal diameter, and bevel design.

Length: defines the injection depth and the route of administration. Standard needle lengths for various applications are given below:

- Intradermal: 1.5 mm
- Subcutaneous: 4–12.5 mm (1/2 in.)
- Intramuscular: 15.8–55 mm (5/8–2 in.)

Gauge
Refers to the external diameter of the needle, and is defined as the number of needles that fit within a defined diameter; the larger the gauge, the smaller the external diameter of the needle. Gauges exist from 33 to 16 G or larger. The gauge drives pain perception, whereas the internal diameter is the main contributor of flow.

Internal Diameter
Determined by the needle gauge and wall thickness. In an effort to improve the injectability of higher viscosity drug product solutions, such as high concentration antibodies, thin-walled needles have recently been developed with reduced wall thickness and increased internal diameter for a given needle gauge. The advantages of the thin-walled design, in terms of perceptions of patient comfort and product

flow, have to be balanced with the disadvantage of an increased tendency to bend or deform during handling and use.

Bevel Design
Beveling refers to the angled cut of the needle tip, with the purpose of improving the ease and comfort of the injection. A wide variety of bevel designs are available for disposable needles, which can also be selected for use with Luer-tip prefilled syringes. The most common bevel design today for staked-needle prefilled syringes is the standard 3-bevel design. However, 5-bevel designs have recently become available, which are intended to further reduce the perception of pain/discomfort during injection.

17.4.6 Quality by Design Applied to Needles

The internal diameter of the needle is a key attribute in combination products. It ensures adequate flow of the drug product solution. The nominal value drives the flow based on the viscosity; whereas, the tolerance drives the variation. Based on Hagen-Poiseuille equation for laminar flow, the internal needle diameter is the main contributor:

$$F_{HP} = \frac{128 Q_u L}{\pi D^4} \times A$$

where, F_{HP} is the force due to viscous effect (need to add stopper friction force), Q is volumetric flow rate, μ is dynamic fluid viscosity, L is total length of the needle, A is the cross sectional area of the syringe, and D is the internal needle diameter.

Tests are not required because the model is quite accurate, and a sensitivity analysis will show the main contributors and the variation based on the tolerance on different factor (see Fig. 17.14). A sensitivity analysis is a method used to understand the contributing factors impacting the response when they vary within tolerance. In Fig. 17.14, the first factor is the main contributor, which means a small variation of this factor will have a large impact on the response so more attention to this factor is required. This type of model can be used in QbD to understand and define limits of the key factors.

17.4.7 Adhesives

For glass syringes, the standard adhesive is urethane methacrylate. The adhesive is delivered between the cannula and the glass tip, and it is then cured with UV light using a wavelength that is able to penetrate the glass and initiate polymerization. This process is similar for glass and plastic prefilled syringes (except the overmolded plastic option, which is intended to eliminate the need for an adhesive).

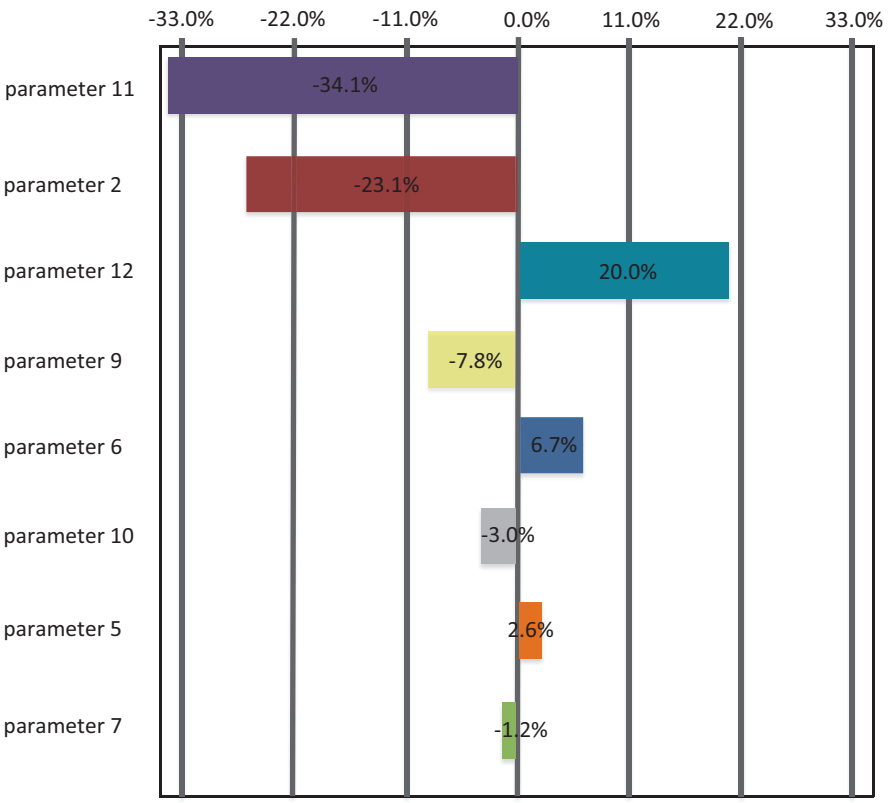
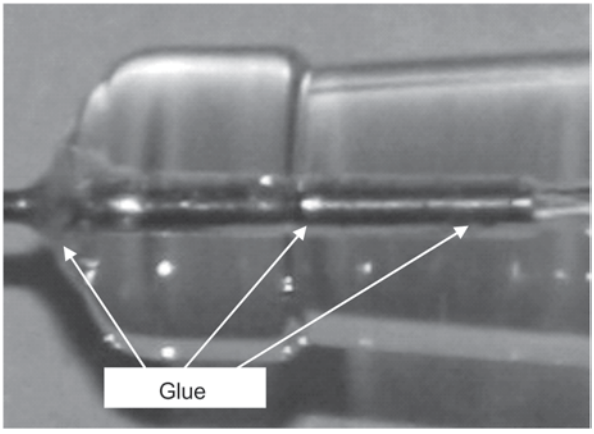


Fig. 17.14 Example of a sensitivity analysis illustrating the contributions of multiple factors to injection force

Fig. 17.15 Location of adhesive along the fluid path



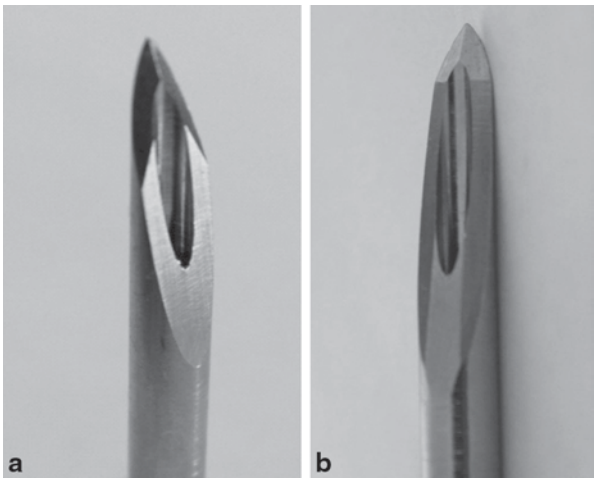


Fig. 17.16 Examples of **a** 3-bevel and **b** 5-bevel needle designs

Because the adhesive is in the fluid path (see Fig. 17.15) and it is also a drug/primary container contact during storage, it should be investigated from an extractable/leachable/stability point of view. Information on the components of the adhesive, including the photo initiator, is available from the manufacturer. (See Wakankar (2010) for more information concerning evaluation of extractables and leachables.)

17.4.8 Needle Design and Sharpness

There are two major needle point designs in the market: the 3-bevel needle and the 5-bevel needle (Fig. 17.16). (A third design, called V-bevel (4-bevel design), also exists but there are no published clinical data at this time.) The 3-bevel needle is older and can be considered the current standard in the industry with a large body of information to support its use. The 5-bevel needle is a more recent innovation, designed to optimize the point resistance and sharpness. Several clinical studies have been published to support the design (Jaber et al. 2008; Hirsch et al. 2012). In addition, a clinical study that was performed by Merck Serono compared various syringes and showed that the point design, gauge, and needle shield material can improve patient comfort (Bozzato and Jaber 2004).

17.4.8.1 Disadvantage of Bench-Top Penetration Test

Needle point design is challenging due to the duality of sharpness and point resistance. The design of point geometry is difficult because the bench test to assess

penetration force (on film strip, rubber, etc.) is not a good predictor of needle sharpness. A study comparing nurse perception (clinical study) and bench testing demonstrated that the bench test is not a good predictor of the sharpness perceived by nurses when inserting the needle into a patient's skin (Vedrine et al. 2003). These differences come from the complexity of human skin, its thickness, and the needle geometry. Bench tests could be useful for comparing needles within a batch for damage or defects (e.g., hooks), but they should not be used for the selection of needle point design without the support of clinical studies. Some suppliers offer specific needle-testing machines that use sound or force when piercing film, but standard pull/push bench machines are adequate for detecting differences in point damage.

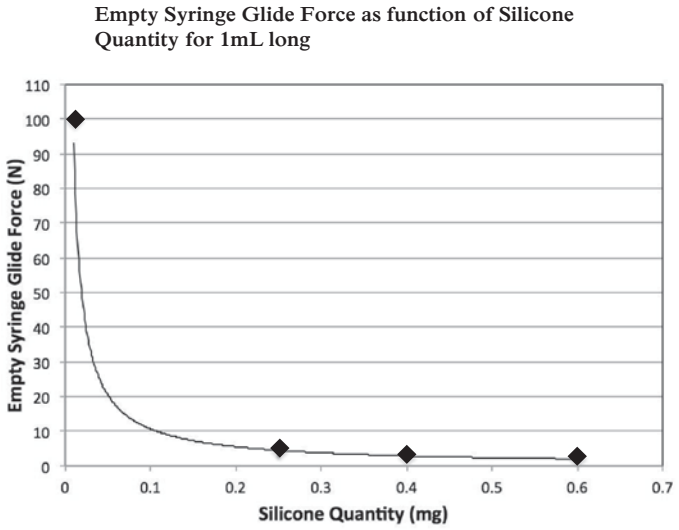
17.4.9 Syringe Siliconization

Silicone oil is a key component of the prefilled syringe. It is used to lubricate the inner side of the syringe and allow the stopper to glide into the barrel. The silicone used is of a medium viscosity (< 500 cP) and is sprayed into the syringe after it is washed. For staked-needle syringes, the silicone is not cured; however, for a Luer syringe it may be cured (baked) by processing in a high temperature oven. To address products that are highly sensitive to silicone oil, suppliers are also developing newer coatings for glass syringes (Majumdar et al 2011). In addition, some of the newer plastic syringes are designed with specific stoppers and do not require siliconization.

The appropriate level of siliconization has to balance the opposing requirements of sufficient silicone to ensure low and consistent gliding force for functionality, while minimizing the potential impact on drug substance stability by maintaining the overall level as low as possible. The consistency of the silicone application is also critical from the functionality perspective. In addition, it may be important to evaluate the potential impact on performance of loss of silicone during shelf life due to leaching into the drug product solution. (Issues related to the impact of silicone on drug product quality and stability are covered elsewhere in this book.).

17.4.10 Quality by Design Applied to Syringe Siliconization for Functionality

As silicone can affect drug substance characteristics, minimizing the level of silicone is a current market trend. Figure 17.17 shows the relationship between silicone quantity and gliding force (author's unpublished data). As the graph demonstrates, the quantity of silicone is not directly linearly correlated to gliding force. An op-



Based on 1 mL glide force data collected using syringes at 0.6, 0.4, 0.25 mg and unsiliconized.

Silicone extraction is performed using organic solvent and quantification by AAS (atomic absorbance spectrophometry).

Source: Author's unpublished data

Fig. 17.17 Effect of siliconization level on gliding performance

timum silicone level can be determined, at the beginning of the flat portion of the curve, above which additional silicone does not further reduce the gliding force. Using this information, the silicone level can be minimized while ensuring that functionality over the shelf life is not compromised.

Silicone distribution is a key parameter to ensure good gliding performance across the length of the syringe and avoid higher force at the end of the stroke. The gliding force measurement can be used as an indirect measurement of silicone distribution. A variety of optical and spectroscopic methods have also been reported (Wen et al. 2009; Chan et al. 2012).

From a QbD perspective, the siliconization design space can be defined by testing gliding force with different levels of silicone over shelf life. It is highly recommended that testing should start early in development to provide an understanding of, both, the drug formulation sensitivity to silicone and the removal of silicone from the syringe surface due to leaching by the formulation. The edge of failure (lowest quantity but still having the required gliding performance) should be defined as part of these studies. Study variables may include different levels of silicone, surfactant concentrations, temperature, and the response in the measurement of the gliding force at the right speed to detect variation.

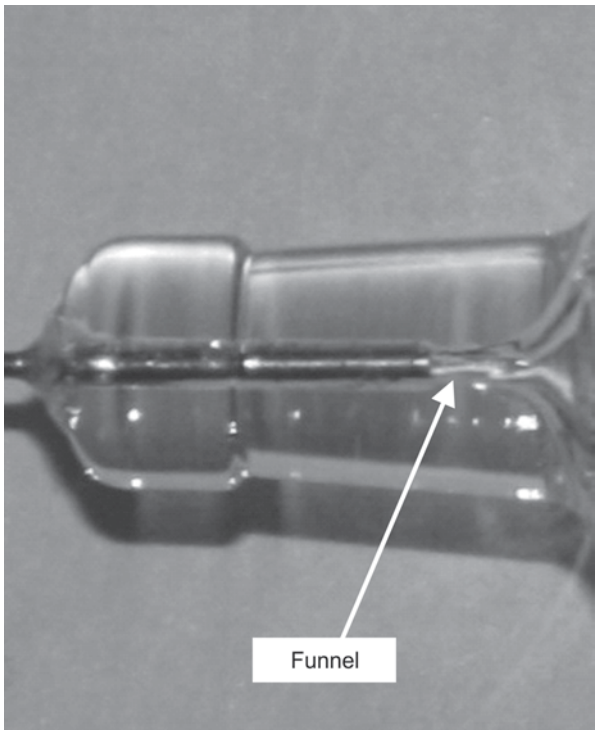


Fig. 17.18 Fluid path at syringe tip

17.4.11 Tungsten

During glass syringe forming,, glass is handled at very high temperatures (> 1000 °C). For Luer or staked-needle syringes, a funnel (fluid path, see Fig. 17.18) needs to be produced at the tip of the syringe. The funnel is used to assemble the needle or expel the drug for the Luer syringe (Faulkner 2006).

This feature is produced using a pin that can withstand those high temperatures when the glass is pressed around. Platinum is often used in glass forming/molding because platinum is traditionally considered not to be a highly reactive material, and it does not stick to the melted glass. Platinum pins can be used for Luer syringes but not for staked-needle syringes because platinum is too soft to produce the small diameter required for needles. In addition, even if considered as inert, platinum can still induce unexpected results, as platinum is used in chemistry for its catalytic property (Jiang et al. 2009).

Tungsten pins are most commonly used in forming staked-needle syringes due to its physical properties. Comparing tungsten to other metals, it has the highest melting temperature (3422 °C), the highest tensile strength, and the lowest coefficient of thermal expansion (Narhi et al. 2007). However, at high temperatures and in the

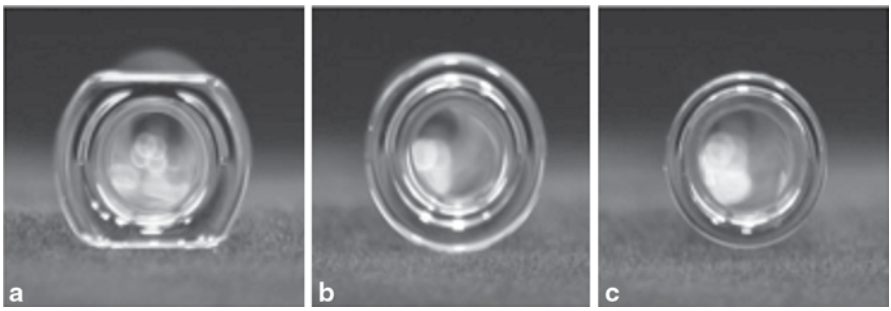


Fig. 17.19 Examples of flange designs **a** clipped, **b** round, and **c** small round

presence of air, a layer consisting of different tungsten species (water soluble or not) is deposited in the syringe funnel. This area may have a high, localized tungsten concentration.

It has been documented in recent years that high tungsten residue levels can result in increased protein aggregation and precipitation. Studies have shown that the precipitate is composed of, both, protein and tungsten. Following these observations of tungsten-induced protein precipitation, syringe manufacturers have updated their processes (including forming temperature and time) to decrease the tungsten residues left inside syringes. Over the past years, certain glass syringe suppliers have succeeded in decreasing the tungsten level from thousands of parts per billion (ppb) to a few ppb by better understanding the tungsten/glass forming interaction and controlling factors, such as forming temperature, tungsten pin life, and other forming parameters.

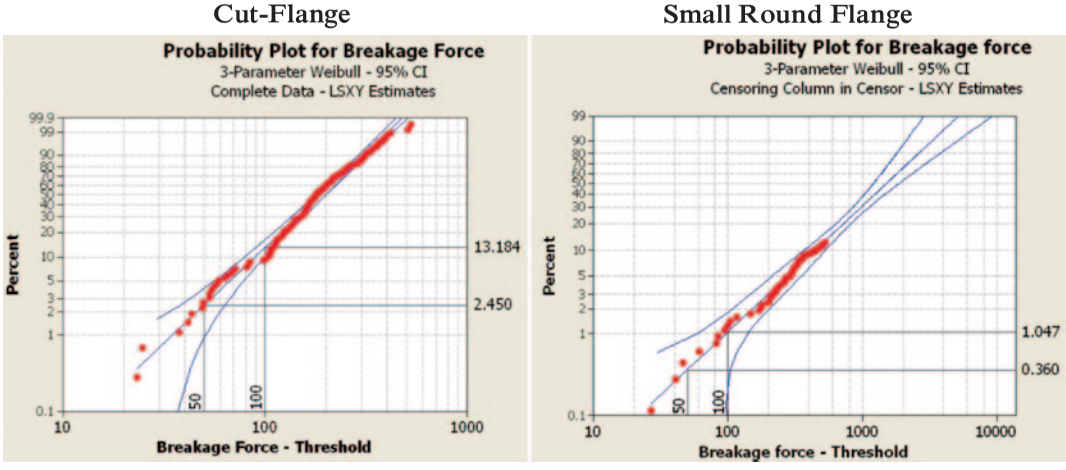
17.4.12 Flange Design

A variety of flange designs are available for prefilled syringes; these include the standard flange (clipped or cut to avoid roll), the large round flange, and the small round flange. Figure 17.19 shows an example of each design. .

Selection of the most appropriate flange design requires consideration of the final dosage form. If the prefilled syringe is intended to be used on its own (i.e., no autoinjector) the clipped or large round designs may have advantages in terms of ease of grip and/or avoiding roll. Add-on flanges or flange extenders can also be designed to aid patients in making injections. If the prefilled syringe is intended for use in an autoinjector, breakage becomes a major consideration.

When the three types of flange designs were submitted for a noise experiment, the small round flange outperforms the other designs alone or in combination with a safety needle or autoinjector. The standard cut flange has the weakest breakage point and the small round flange the strongest one (2–5 times stronger for the 1 mL PFS size; see Fig. 17.20) (author's unpublished data). The flange design is a good

Estimation of Syringe Breakage



Small round flange reduces the probability of flange breakage by a factor of about 10.

Breakage Limit (N)	Estimated breakage Cut-Flange	Estimated breakage Small Round Flange
100N	13.18%	1.05%
50N	2.45%	0.36%

Source: Author's unpublished data

Fig. 17.20 Effect of flange design on strength

example of QbD applied to devices, where choosing the most appropriate design up-front can minimize potential issues in the field.

17.5 Cartridge

The cartridge is a primary container in use for many years and less complex than prefilled syringes. From a QbD perspective, the main design space investigations concern siliconization (gliding and impact on drug product), septum resealability, and tolerance analysis for dose accuracy when assembled into a pen injector. The studies to define the design space and CQAs described in the prefilled syringe section are applicable for cartridges.

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Chapter 18
Devices and Combination Products for Biopharmaceuticals

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18.1 Introduction

The market for biopharmaceutical therapeutics has experienced remarkably strong growth during the past decade due to the advancement of translational research that has led to the introduction of a wide variety of therapies based on macromolecular drug molecules. According to a new report, entitled "Global Protein Therapeutics Market Forecast to 2015," protein-based therapies have been demonstrated to be clinically effective for treating a wide spectrum of diseases ranging from cancer to metabolic disorders and may reach the mark of US$ 143.4 billion (RNCOS 2012). Biopharmaceutical sales were US$93 billion in 2009 and are expected to grow twice as fast as small molecules through 2015 (Kline 2010). By 2011, the global protein therapeutics market is estimated to have reached around US$105 billion and is likely to expand at a compound annual growth rate of around 8% during 2012–2015 (RNCOS 2012). As the biologics expand, a growing number of products require chronic administration at relatively frequent intervals, driving increasing adoption of self-administration for those products whose safety profiles support use in the home setting. In addition, the biologics markets have become increasingly competitive with, in many cases, multiple products within the same therapeutic class from different manufacturers (e.g., insulin, human growth hor-

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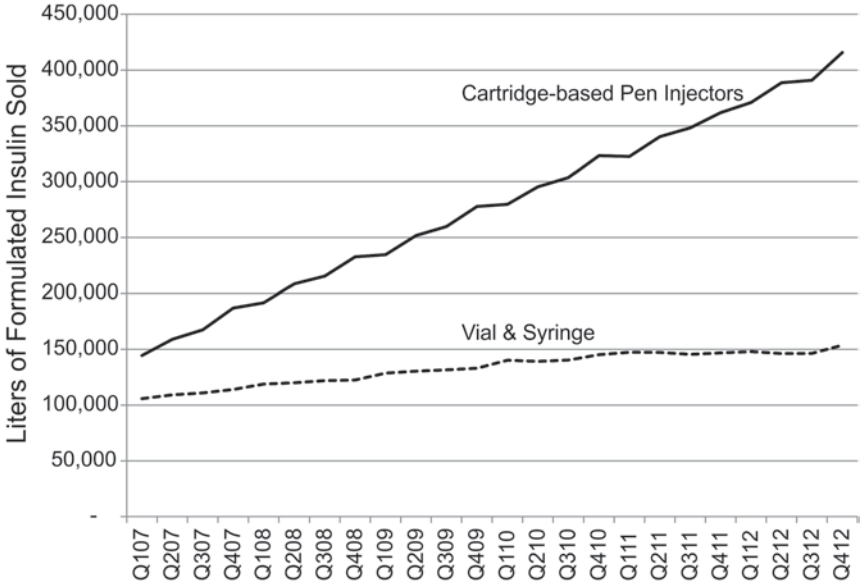

Fig. 18.1 Global volume (liters) of major insulin analogs by delivery presentation (2007–2012): Global growth rate of pen injectors over vial and syringe format

mone, anti-TNFα, etc.). This rapid growth and increased competition has occurred despite the fact that biotherapeutics typically require injection through a needle to ensure that the product is delivered chemically and physically intact to the targeted site. Among all forms of administering medication, injection via needle is probably the least favorable for patients as it involves delicate manipulation, the administration itself can be time-consuming, and many patients experience varying degrees of aversion to needles or injection. To address these issues as well as meet the specific needs of particular patient populations, biopharmaceutical companies are increasingly partnering with medical device manufacturers to add value to vital life-enhancing medicines and to also manage the life cycle of these respective drug products.

Recent innovation in the development of drug delivery systems results in benefits such as reduced preparation steps, enhanced ease of use and the potential for improving end-user compliance (French 2007). Insulin, one of the oldest biologics, with a history that predates recombinant DNA technology, provides an excellent example of innovation in drug delivery. Pressures to reduce rising healthcare costs (by minimizing hospital and healthcare provider office visits) along with a rapidly expanding patient population and intense competitive pressures in the marketplace have prompted a long-standing interest by both patients and biopharmaceutical companies to introduce accurate, easy-to-use, adjustable dose injection devices for these products. Figure 18.1 demonstrates the accelerating growth rate in the use of pen injectors relative to the more modest increase in the traditional vial and

syringe image for delivery of major insulin products (insulin glargine, insulin detemir, insulin lispro, insulin aspart, insulin glulisine) in major worldwide markets (Source: IMS MIDAS™ data). The convenience benefits, along with equivalent (and in some instances, improved) dosing accuracy (Luijf and DeVries 2010; Pfützner et al. 2013), have induced prescribers and providers to encourage patient use of pen injectors for their treatment.

The injectable TNFα inhibitors provide another example of the effect of competitive market pressures in driving the development of injection devices and, conversely how these devices have been leveraged for drug lifecycle management. Enbrel®, the first subcutaneously injected TNFα inhibitor (initially indicated for the treatment of rheumatoid arthritis (RA)), was originally launched in 1998 in a lyophilized vial configuration, requiring reconstitution prior to administration. The preparation involved the addition of a diluent followed by a mixing step for complete dissolution of the drug. Relatively complex transferring steps had to be followed before injection, which further complicated the process and increased the chance for preparation and dosing errors. Despite these drawbacks, however, it took almost 5 years before a more convenient vial adaptor presentation was introduced. In 2003, a competing product, the Humira® PFS, which contains the medicine in a liquid form prefilled into a syringe with a staked needle, was introduced. This ready-to-inject product eliminated many of the 15-plus steps needed to prepare lyophilized Enbrel® for administration. It also reduced chances for error during product reconstitution and lessened patients' reliance on healthcare providers. The Enbrel PFS became available in 2004. In 2006 two auto-injection products, Enbrel® SureClick™ and Humira®Pen, were launched in the same month to provide further convenience to the users and to minimize accidental needle stick. The auto-injector has since become the predominant product presentation of TNFα inhibitor products. More recently with the introduction of additional effective treatments for RA, the rate of introduction of delivery devices (as well as a drive toward less frequent administration) has accelerated rapidly as highlighted in Fig. 18.2. Today, over 90% of Enbrel is sold in PFS and auto-injector form, and the annual sales of TNF inhibitors reached US$ 26 billion by 2012 with the vast majority in a PFS or auto-injector presentation (Thomson 2012).

Integrating therapeutics through injection devices is a cost-effective and lower-risk approach for the biopharmaceutical industry. In addition, product differentiation through the application of end-user aids for vials, prefilled syringes (PFS), and injection devices is becoming increasingly prevalent in the marketplace. However, in order to minimize the time to market and as a part of the life cycle management strategy, lyophilized biologic drugs in vials are often the first-to-market presentations, which are subsequently followed by more convenient dosage forms such as liquid formulations in PFS or cartridges. This product evolution may also incorporate the use of more complex drug delivery devices such as auto-injectors with the PFS, or pen injectors with cartridges. A large number of proteins and monoclonal antibodies (mAbs) are in the pipelines of numerous biopharmaceutical companies (PhRMA 2013), and many require high doses which result in high solution viscosities (Shire 2009) if being delivered in subcutaneous volumes. These applications

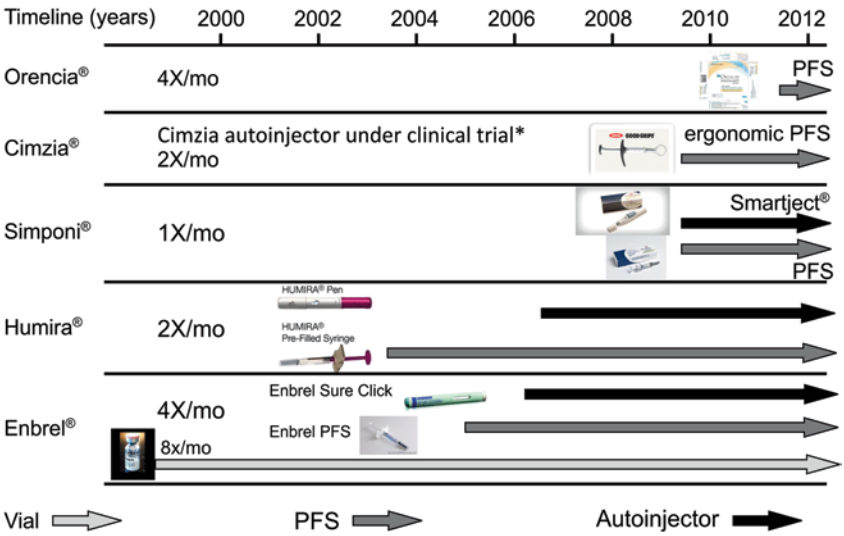

Fig. 18.2 The rate of introduction of devices to aid injection of the TNFα inhibitors accelerated significantly with intensifying market competition among multiple commercial products

pose additional delivery challenges and call for novel means of delivery, which necessitate the development of drug product formulations, primary containers, and delivery devices in tandem to ensure compatibility over the full range of properties of both formulation and device.

There is no doubt that rapid growth and increased competition in the biopharmaceutical industry will lead to increasing therapeutic opportunities as well as intensifying competitive pressures in the marketplace, fueling continued innovation in delivery technologies for injectable drugs. The recent development of subcutaneous versions of Herceptin and Rituxan/MabThera are recent examples of continuing innovation through leveraging a combination product approach to improve the ease of administration; in this case converting from intravenous to the simpler subcutaneous route of administration (Roche 2013; Shpilberg and Jackisch 2013).

Many biopharmaceutical companies are already responding to these challenges by making investments in novel drug delivery technologies (formulations and devices) as well as life cycle management of the existing drug molecules through second and third generation delivery devices. The challenge, however, is to ensure the safety and effectiveness in delivery for these advanced biologic therapies by a broad patient population in the home setting without routine nurse or physician oversight. Therefore, both intuitive to use and highly robust combination products are necessary to ensure compliance in use. This is where a "Quality by Design" framework becomes foundational for the biopharmaceutical companies in development of satisfactory combination products. It is essential to approach the integration of the drug product, primary container, and drug delivery device in a scientific, risk-based, and comprehensive manner, with thorough consideration in the development

and manufacturing of a drug-device combination product, to ensure it is safe and effective for its intended use. In this chapter, an overview of the device landscape for biopharmaceutical drug products will be given. A brief overview of the regulatory framework of medical devices and combination products, as well as the implications of Quality by Design (QbD) for device development, will be examined. More importantly, a framework for self-injection device development via QbD approaches will be provided.

18.2 QbD for Combination Product Development

The biopharmaceutical industry has made significant progress in coming to terms with the nuances of FDA's risk-based approach to current good manufacturing practices (cGMPs) and the International Committee on Harmonization (ICH) Q8/Q9/Q10 guidance. The industry has also made great strides in identifying a path forward within the product development, risk management, and quality infrastructure for drug substance and late stage drug product development via the QbD paradigm.

For years, the medical device industry has followed design-for-six-sigma (DFSS) and FDA's Quality Systems Regulation (QSR; 21CFR820 2013) which is well aligned with the basic framework of QbD. QSR establishes clear requirements for understanding the device's user requirements early in its development lifecycle. As product development progresses through the clinical phase, the design controls serve to identify key considerations in moving the product development toward commercialization. The development of a medical device routinely integrates product risk analysis at the outset of the design phase, followed by a series of failure modes and effects analyses (FMEA), as the design, process, and user performance are refined. The QSR for medical device development demands a clear documentation trail through the product development process as the device history file is created. Design for manufacturing, risk assessment, and reliability are routinely performed prior to commercialization. These considerations mirror the requirements advocated in the QbD approach described in ICH Q8 (ICH 2009) and the integration of risk analysis and management into product lifecycle prescribed within ICH Q9 (ICH 2005) and Q10 (ICH 2008).

Recently, the challenge faced by pharmaceutical companies has been the search for a common ground to satisfy the requirements of both 21 CFR 820 (device) (21CFR820 2013) and 210/211 (drug) (21CFR210 2013; 21CFR211 2013). The terms "specification error allocation analysis" and "gauge R&R analysis" were relatively new or even foreign to traditional biopharmaceutical development. With the gradual addition of biologic-device combination products to biopharmaceutical companies' portfolio, the gaps between device verification and biopharmaceutical qualification (IQ/OQ/PQ) and validation exercises are narrowing. Risk management tools have become an expected component of any product and process development and/or troubleshooting exercise. Unfortunately, large gaps remain when the theoretical recognition of these concepts is put into practice.

It is collective belief of the authors of this text that by adopting a holistic QbD approach across drug substance, drug product, primary container, and device development, the biopharmaceutical companies and their device partners can navigate much more smoothly through the complex regulatory framework, ultimately delivering products with improved quality, and offering benefits to patients with minimized risk and enhanced performance. This chapter illustrates how the basic QbD principles could be applied to the development and manufacturing of such biologic-device combination products.

18.3 Biopharmaceutical Device Categories

One important fact to note is that improvement in safety and convenience does not have to come in the form of complex device designs. An appropriate delivery device, even as simple as an add-on finger flange to a syringe may significantly improve the benefit to the patients. Due to the large number of devices available for fulfilling different needs, and the general lack of standardization among similar options, we will provide only a brief overview of the more prevalent devices in this section.

18.3.1 Pre-filled Syringes

Recent learnings and development challenges for the PFS image is discussed later in case studies, along with commentary on the opportunities for more integrated application of QbD approaches. Only the most salient features of PFS (in terms of the integration with the auto-injector) are briefly presented here.

Historically, the PFS was developed to be used by healthcare professionals for manual injection, and these applications existed long before the practice of self-injection and the advent of the auto-injector which houses the filled PFS. Significant redevelopment of the specifications and the manufacturing quality of the PFS was therefore required to ensure functional compatibility with the auto-injection device when self-injection became a health-management necessity and the auto-injector was introduced into the market place. The needs for these changes were not immediately recognized, however. In fact, until very recently, the syringe of a PFS product was treated mainly as a primary container, and the medicinal product presented in the PFS format was registered as a drug or biological product in all major markets (rather than considering the whole system as a combination product). In 2013, FDA issued a final rule on combination products (21CFR4 2013) with an accompanying guidance that clearly designated the PFS format as a combination product. This regulatory designation brought a refreshed and intensified focus to the quality attributes of the syringe.

By nature, the syringe of a PFS product is both a primary container and a delivery device. Thus, its quality attributes encompass considerations applicable to both. First, as a primary container, the syringe barrel plus the plunger stopper, the attached needle, and the needle shield must provide adequate protection of its content drug such that stability and sterility are ensured. Quality attributes such as leachables, container closure integrity (CCI), and oxygen or light transmission (for oxygen sensitive and light sensitive drug respectively) need be studied and controlled to ensure long-term chemical compatibility with the filled drug or biologic product. Sources of detrimental leachables can include additives in the plunger stopper or the needle shield, constituents of the needle-glue, silicone on the syringe barrel or the stopper, the glass or plastic barrel itself, and tungstates from the glass barrel (Markovic 2011).

CCI is primarily controlled by the quality of interface between the plunger and the syringe barrel, between the needle and the needle cone, between the needle and the needle shield, and between the needle shield and the needle cone. Of course CCI also relies on integral primary components including pin hole-free or crack-free glass syringe barrels, and defect free elastomeric plunger stoppers and needle shields.

From the viewpoint of the PFS as a delivery device, appropriate mechanical performance criteria need to be developed and the device's performance should be consistently maintained. Quality attributes such as glide force profile as a function of injection rate, needle-stick risk, and strength of relevant regions of the syringe need to be studied and controlled. Control of these critical attributes is particularly important when the PFS is intended to be housed in another device such as an auto-injector.

The glide/injection force profile is dependent on numerous PFS properties including the inner diameter (ID) and length of the needle, the uniformity of siliconization of the barrel, consistency of the plunger stopper-glass barrel interaction, degree of interference fit between the outer diameter of the plunger stopper and the ID of the glass barrel, the rate of injection, and the viscosity of the drug product. The strength of the syringe depends on the geometric design of the syringe, the residual stresses in the syringe, the extent of surface damage to the glass barrel, and the mechanical stresses to which the syringe is subjected. Moreover, strict control measures for the various critical dimensions for physical interaction between the syringe and the injection device (e.g., auto-injector), and between the syringe and the formulation filling equipment must be put in place via quality agreement with the supplier.

Most PFS are made of glass (with the exception of those marketed in Japan). Approximately 80 % of PFS sold in Japan are made of plastic (Constable 2012). There has been increasing interest in molded plastic PFS in the Western world due to some of the quality challenges described above. Having the potential of being silicone-oil-free, tungsten-free, and adhesive-free, the plastic PFS has been under development as an alternative to the traditional glass PFS. Concerns regarding extractables and leachables associated with molded plastic syringes have largely been resolved (DeGrazio 2011). In addition, the more precisely controlled dimensions and higher

break-resistance of molded plastic PFS render it an attractive alternative for the auto-injector application. Higher gas permeability, static charge build-up, and cost (relative to the glass) will continue to be considered as shortcomings of the plastic syringe until further technological advancements are made.

18.3.2 Add-On devices

Add-on devices are typically relatively simple plastic components that help the user prepare the medicine for injection, perform the injection or minimize the risk of needle-stick after injection. These devices can be disposable and/or reusable. Finger flange extension parts and manual needle guards are examples of such devices. When finger flange extension parts are used with a glass PFS, consideration of the break resistance and dimensional tolerance of the syringe flange may demand heightened tolerance criteria to ensure consistent compatibility with the add-on finger flange extension component. Commercial products such as Enbrel® and Humira® PFS are supplied with plastic molded finger flange extensions to increase the ease of use by the patients.

Unfortunately, there has been little effort to standardize the various injection-aide concepts. There has been some success in improving the landscape for glass PFS; but a similar statement could not be made for other containers such as vials, ampoules, or even cartridges. This is partly a consequence of the historical practice of inadequate dimensional standardization of the primary containers. As a consequence, even today, there continues to be some customization of containers made according to standards such as ISO.

18.3.2.1 Vial Adapters

Although the conventional vial image is considered a commercial disadvantage for new biologics when there are competitive products in the format of PFS and/or injection devices, it is sometimes favored when time-to-market dictates very rapid product development. To overcome some of the inherent shortcomings in ease-of-use and user safety, a variety of vial adapter designs have been developed and used commercially for the reconstitution of lyophilized drugs and for the transfer of liquid products from the supplied vial into injection devices such as the syringe. Since there are often some variations in detailed dimensions among nominally similar vials, many of these adapters may need further customization for a specific product. Consequently, off-the-shelf deployment of vial adapters should be evaluated thoroughly and carefully before such a decision is made.

18.3.2.2 Needle Stick Protection (NSP) Devices

According to Congressional findings detailed in the Needlestick Safety and Prevention Act (2000), the estimated accidental needle stick injuries to healthcare workers occur at a rate of 600,000 to 800,000 a year in the USA alone. Many blood-borne pathogens that can be transmitted by accidental needle stick injury and studies show that a lot of needlestick injuries are never reported (Johns Hopkins Medical Institutions 2009). Many countries have subsequently passed worker safety laws that include needle stick prevention. Following the awareness campaign and the regulatory actions across the globe, interest in and use of NSP devices has been on the rise.

There are several different types of safety syringes, generally termed "active" or "passive" types, for shielding the needle post injection. The active device requires specific manipulation by the user to activate the protection mechanism in addition to the normal injection action, while the passive device is activated without additional user action. Depending on the situation, either approach could be satisfactory in providing the intended protection (Dierick 2011). However, the active approach typically uses a true add-on such as a sheath/shield or a flip-on/hinge cap. While they meet the legislation's requirements, they can place the healthcare providers' fingers dangerously close to the exposed needle. As a result, the so-called passive safety syringes with integrated retractable needle mechanism have gained much adoption in recent years among the pharmaceutical companies. New technologies that integrate the safety features directly into the PFS are also appearing on the horizon.

18.3.3 Pen Injectors

Pen injectors have typically been used for frequent self-administration of multidose drugs or drugs requiring weight-based or condition-based dosing. For example, the diabetes and human growth hormone (hGH) deficiency markets have been using pen injectors for decades. Pen injectors typically require the patient to attach a separate pen needle, prime the device and then dial their prescribed dose. Insertion of the needle and injection of the drug is typically manual with these systems. The primary container employed within pen injectors is typically a glass cartridge with a crimp sealed septum on the neck end, and a stopper inserted into the open end. These cartridges and associated components are designed to permit multiple punctures and expulsion events creating a multiuse delivery system. Current pen injectors have the capability to deliver dosing volumes as low as 5 µl, up to nearly 1 ml. Most pen injectors afford dosing flexibility of 10 µl increments.

Pen injectors can accommodate liquid or lyophilized formulations, which is an advantage over some other types of devices that are limited to liquids. The newest and most advanced developments in pen devices today include automated injection (through internal springs) and electronics which permit delivered dose memory and

dosing interval timing. These devices have entered the multiple sclerosis, osteoporosis, and reproductive health markets in addition to the prevalence in the diabetes market referenced earlier. There even exist safety pen needles which incorporate a simple sheath/shield that hides the needle prior to injection and permanently locks after a single use to prevent subsequent needle sticks.

18.3.3.1 Reusable Pen Injectors

Pen injectors have historically been designed as reusable devices which require the end-user to periodically replace the drug cartridge when empty. There exist some strong regional differences in preference (either driven by patient, insurance or government) for the reusable pen injector configuration over the disposable pen injector. Countries such as Germany, Canada, Poland, China, and the Netherlands demonstrate significant adoption of the reusable configuration (relative to the disposable pen injector) for insulin delivery (Perfetti 2010). The use of reusable pens versus disposable pens is also dependent on the dosing regimen and indication.

Reusable pen injectors are regulated as pure medical devices obtaining CE marking or 510k authorizations. The manufacturer will design, manufacture, and test the injector to remain within performance specifications for in-use periods of 2–5 years; therefore, durability of the inner mechanics and outer surfaces of the injector become a core design requirement. It is important to note, however, that a single reusable pen injector is rarely compatible with cartridges and products across manufacturers. Even small differences in cartridge or component dimensions across suppliers or manufacturers can produce device malfunction and/or dosing accuracy issues.

18.3.3.2 Disposable Pen Injectors

Disposable pens (for insulin administration in particular) have become notably more prevalent commercially in the past decade (as presented in Fig. 18.3; Source: IMS MIDAS™ data). These systems are supplied irreversibly preassembled with the cartridge containing the drug product and typically regulated as drug-device (or biologic-device) combination products obtaining approval along with the submitted regulatory dossier for the drug/biologic product. Strong regional preference again exists in acceptance and utilization of disposable pen injectors where the US pen injector market for insulin injection is nearly entirely in the disposable configuration.

The disposable pens present virtually the same functionality as the reusable pens in terms of variable dose selection, pen needle attachment, and multiuse until the cartridge is empty. The entire disposable pen injection device is discarded after the drug contents contained within the preassembled cartridge have been used, typically within a period of 1–4 weeks, depending on the dosage. Engineering resins are used

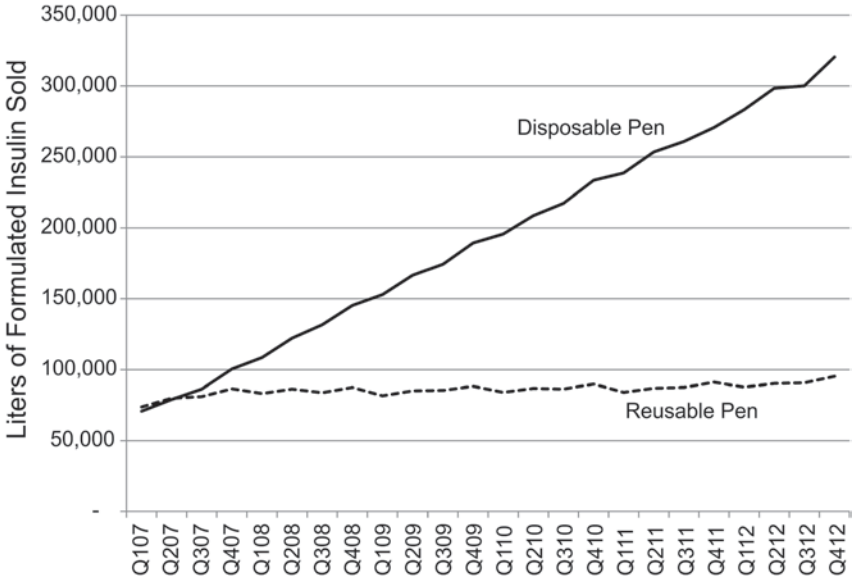

Fig. 18.3 Global volume (liters) of major insulin analogs by delivery presentation (2007–2012): Global growth rate of disposable and reusable pen injectors

in the manufacture of the parts as tight tolerances are typically required to achieve the dosing accuracy and low-injection force requirements typically expected. Performance, aging, and stability studies are conducted to ensure the lifetime/expiry of the product is limited by the stability of the formulation, not the device. However, as contrasted to the reusable injector format, disposable pens are not designed to be highly durable with typical lifetimes of less than 1 year of usage.

18.3.4 Auto-Injectors

Auto-injectors in combination with the PFS bring an additional ease of use and safety, with the fully disposable auto-injector being the gold standard for infrequent injections (weekly or less frequent) for self-administration. In order to integrate a drug delivery system containing a PFS and an auto-injection device, the auto-injector must be developed around the existing PFS. The auto-injector is designed to empty the full contents of the syringe. After activation is initiated by the user, all functions are typically passive and occur in a sequence until the drug delivery is completed and all other mechanical parts of the injector have reached their final mechanical positions. The integrated needle is commonly hidden within the device prior to injection and protected within the device after injection by an integrated needle shield.

18.3.4.1 Reusable Auto-Injectors

Reusable systems are suitable only for self-injecting applications. Reusable auto-injector systems have become particularly well established in the MS market. Reusable systems are a cost-effective option for frequently administered products. These auto-injectors require a significant amount of end-user training and manipulation to perform the injection accurately. Consequently, the complexity of use has limited their prevalence. Drug containing PFSs or cartridges are loaded into the reusable auto-injector each time before the injection. Once triggered (typically accomplished with the push of a button or pushing the device against the injection site), the auto-injector uses an automatic mechanism to insert the needle into the SC/IM tissue, and force the liquid drug out of the syringe through the needle.

18.3.4.2 Disposable Auto-Injectors

The current trend in biopharmaceutical drug delivery is predominantly in the direction of single-use, fixed dose disposable auto-injectors without performing a specific dose setting function by the user. Of course, the nature of the biologic molecule must typically support a once-weekly or less frequent dosing regimen, as well as a broad enough therapeutic window to eliminate the need for dosing titration. Therapies requiring daily injection typically render the disposable auto-injector format impractical from a cost of goods perspective.

The single-use, disposable auto-injector was originally used in the emergency medicine markets over 3 decades ago (circa 1980), and entered the anemia and oncology markets with therapeutic proteins in 2005. These systems are supplied preassembled with a PFS containing the drug. They are inherently easier to use than reusable systems and can have integrated NSP functionality, which makes them flexible for use in clinical and home administration settings. Single-use, disposable auto-injector functionality is highly similar to the reusable device except that drug containing PFS is preassembled in the device by the manufacturer.

18.3.5 Patch Pumps

The diabetes market leads the way with patch pump delivery devices. As envisioned, diabetics will wear the patch pump which provides a basal infusion rate of insulin over the course of the day. These pumps are battery operated and require the user to periodically load insulin cartridges into the device. Many medical device manufacturers are advancing this technology to provide the convenience, comfort, and control demanded by the patient for management of their disease (Schaepelynck et al. 2011). Recently, patch-pumps are being developed to respond to the unmet needs of the large dose mAbs. The ability to self-infuse subcutaneously makes this delivery option attractive.

18.3.6 Needle-Free Jet Injectors

Needle-free jet injectors enable the administration of drug product without the use of a needle. Typically, a high pressure gas or spring energy source drives the drug product through a small orifice directly through the skin. To date, needle-free injectors have not made major commercial inroads into the marketplace. The major needle-free self-injection devices marketed by the biopharmaceutical industry are reusable systems used for administration of growth hormone therapies and vaccines. Reusable systems offer dosing and formulation flexibility but, as is typical of all reusable devices, they are more difficult to use than disposable versions. In the past few years, single-use disposable needle-free devices have been commercialized for migraine relief markets and are under development for products in the areas of hematology, multiple sclerosis, and rheumatoid arthritis. While the jet injection technology has existed for several decades, there still remains a limited number of marketed products employing jet injector which have been approved by the FDA as of 2013 (Gratieri et al. 2013). This may be due to factors entailing general design complexity and consequent cost of goods, human factors evaluation requirements to demonstrate safety and effectiveness in use, as well as stability of the biologic molecule undergoing the high-velocity jet injection event.

18.4 Regulatory Complexities for Combination Products

FDA has defined four different types of combination products. Under 21 CFR3.2(e) (2013), a combination product may be comprised of:

i. Two or more regulated entities that are physically or chemically combined into one integrated product, such as a drug-eluting stent.
ii. Two or more separate products of different types packaged together in a single package, such as a drug packaged together with a delivery device.
iii. A new drug, device, or biologic labeled for use with an already approved, separately marketed drug, device, or biologic, such as a delivery device labeled for use with an already approved, separately marketed drug or biologic, when upon approval of the new product, the labeling of the approved product would need to be changed (e.g., to reflect a change in intended use, dosage form, strength, route of administration, or significant change in dose), and
iv. Two investigational products where both are required to achieve the intended effect.

These different types of combination products are compared and contrasted in Table 18.1, with relevant examples for current biologic products.

Table 18.1 Comparison of various combination product formats

Single-entity combination product	Co-packaged combination product	Virtual (separately packaged) combination product	Virtual (separately packaged) investigational combination product
Drug-device Biologic-device Drug-biologic Drug-device-biologic	Drug-device Biologic-device Drug-biologic	Drug, device, or biologic packaged separately … *for use only with* … approved individually specified drug, device, or biologic	Investigational drug, device, or biologic packaged separately … *for use only with* … individually specified investigational drug, device, or biologic
Physically, chemically, or otherwise combined or mixed and produced as a single entity	Two or more separate products packaged together in a single package or as a unit	Both products required to achieve the intended use, indication, or effect; upon approval of the investigational product the labeling of the approved product will need to be changed	Both products required to achieve the intended use, indication, or effect, according to the proposed labeling
Examples: mAb in PFS; PFS in auto-injector	Example: vial co-packaged with vial adapter and disposable syringe	Example: PFS designated for injection using a reusable auto-injector	Example: Investigational drug administered with specified reusable auto-injector

18.4.1 Regulatory Framework

As the previous sections illustrate, the current scope of medical devices used in drug delivery is varied and complex. The incorporation of drug products, including biologics, into medical devices as combination products adds additional layers of complexity and potential regulatory requirements to the mix.

Historically, the regulatory rules, guidelines, and organizational interfaces involved in product development and commercialization have been determined by the existing statutory framework of the individual components that are involved with the overall therapy; namely drug, biologic, or device. Three main bodies of medical device regulations, the US FDA 21 CFR Part 820 (i.e., the QSR), ISO 13485:2003, and the Global Harmonization Task Force (GHTF) guidelines, establish the baseline requirements for each of the elements or "constituent parts" of a combination product (Combination Products Coalition 2007).

For combination products which combine different types of products and cross the boundaries of multiple centers, the additional question arises as to which agency will take the lead in reviewing the product. As stated within the FDA Final Rule on Definition of Primary Mode of Action of a Combination Product (21CFR3 2005), the lead center is determined by the primary mode of action, defined as "the single mode of action of a combination product that provides the most important therapeutic action of the combination product…." A formal decision-making algorithm

is provided for those situations in which the modes of action make it difficult to determine the primary mode of action, and ultimate responsibility for assigning combination products to a lead agency center is assigned to the Office of Combination Products (OCP), which was created statutorily in 2002.

18.4.2 Cultural Dichotomies of Devices and Biopharmaceuticals

Although devices and drugs are both regulated by the FDA, the drug and device industries differ in fundamental ways. FDA considers the cGMP (21CFR210 2013; 21CFR211 2013) and the QSR (Quality System Regulations, 21CFR820 2013) to be similar, and they are meant to achieve the same goals. cGMP and QSR have considerable overlap, yet differ in many details, as each set of regulations is tailored to very different product characteristics. It is important to note that quality is not defined by the drug company; it is defined by both the regulators and the buying public. There is a general distinction between a drug/biologic and a device: drugs/biologics are to be discovered, in part, by trial-and-error; product quality is all about manufacturing to a standard. Devices on the other hand are designed to accomplish predefined functionality and are manufactured to required specifications. This difference is reflected in the way the FDA approaches the cGMPs and the QSRs. Combining the drug/biologic and the device into a combination product requires the resolution of these differences.

The cultural dichotomies of chemical/biologic and mechanical worlds cause additional challenges. Drugs/biologics and devices have very different lifecycle and development processes. It is not uncommon for drugs or biologics to be researched and developed in a timeframe of over 10 years before market launch while the device company cannot accept such long product development cycles. Drug/biologic product development is very costly and usually has a much lower probability of success to reach the market. The cost per dose for an innovator drug is usually much higher than that of the device. Once approved, drugs tend to remain on the market unchanged for decades; this is particularly true for biologics. In contrast, medical devices tend to evolve rapidly through innovation and continuous improvement (CI). Older drugs can retain healthy profit margins (at least until patent expiries), but static device designs are prone to obsolescence. This ability to change is governed by the different regulatory constraints. Drug companies have little latitude in making drug product or labeling modifications without getting FDA approval, even for improvement in instruction for use of the device constituent.

18.5 Case Examples from Industry Experience

Despite dramatic growth in combination products in the biopharmaceutical space, there have been examples where holistic QbD was not sufficiently applied to combination product development, possibly due to under-appreciation of the complex

interactions between the drug product, primary container, and injection device. This section highlights some of the challenges encountered in combination product development and commercialization for purposes of sharing the learnings and for motivating the biopharmaceutical commercialization efforts to incorporate systematic QbD development practices.

As a first example, it has been reported that ill-defined glass syringe flange dimensions resulted in significant delay in the market introduction of a reusable auto-injector during the early years of such combination products (French 2010). Weeks before the launch of the product, it was found that a high percentage of devices "failed to complete injection" in clinical field testing. An ensuing comprehensive investigation led to the realization that a large variation in the flange diameter of the syringe rendered many of the syringes incompatible with the syringe holder in the auto-injector. Failure to recognize the flange diameter as a critical attribute by both the syringe supplier and the drug company sowed the seed for this "manufacturability" problem. This oversight resulted in lack of suitable specifications and controls on the syringe-flange diameter, and led to the mismatch between two critical component attributes, resulting in performance failure of the combination product. The syringe supplier ultimately modified the measurement methods during the inspection stage in order to provide a high level of assurance that flange geometry is in the re-aligned specification ranges (French 2010).

Other examples of combination product development challenges include the numerous auto-injector recalls which have occurred due to long injection times or stalled injections (PMR Publications 2006). The root cause for this undesirable performance characteristic begins with the lack of historical appreciation and evaluation of critical component properties and their links to device performance. Both the ID of a needle and the silicone oil distribution along the syringe barrel have a strong influence on the spring force and time needed for completing the injection. The finished needle ID is typically referenced by its nominal ID due to the inherent variation from the welded-and-drawn process. For example, the standard nominal ID of a 27-gauge needle (regular wall) can range approximately from 0.19 mm to 0.21 mm (some even up to 0.22 mm). The needle ID is a critical component property for auto-injector combination products, since the force to push the fluid through the needle is inversely proportional to the fourth power of the ID. Therefore, small changes in the ID of the needle can result in large changes in the required force to advance the plunger in the syringe and expel the liquid product.

Poor silicone oil distribution has also been determined as a root cause for the stalling of the syringe plunger stopper within the auto-injector due to the limited and fixed force which is applied by the spring (Hwang 2008). Once again, this issue arose as an artifact of leveraging the existing PFS image which was not initially designed for use with the auto-injector. In the standard PFS format, the uniformity of silicone oil distribution was not a critical attribute as the user is capable of applying the needed force to overcome any changes in resistance during the injection stroke. However, the early auto-injectors were initially designed with standard coiled compression springs which exhibit a linear decay in force as they are activated and expand. Unfortunately, the traditional syringe siliconization process incorporated

fixed siliconization nozzles, which did a poor job of depositing silicone oil toward the neck/needle end of the syringe. The combination of lower spring forces during the later stages of spring expansion coupled with low levels of silicone at the lower portion of the glass barrel resulted in a situation in which plunger stalling likelihood was dramatically increased. As a consequence of multiple instances of stalled injections for marketed auto-injector combination products, enhancements have subsequently been made within the industry to the syringe siliconization stations such that the nozzles are designed to dive into the syringe barrel to ensure silicone is more uniformly distributed.

Another rare but severe incident has been the occurrence of glass breakage inside the auto-injector during the early launches of some of the innovator pioneer auto-injectors. Recalls and supply interruption have been reported due to cracked, shattered, or otherwise broken glass syringes originating either from the flange or the body (e.g., FDA 2011). One of the most severe reported cases implicated 2,948,741 Enbrel syringes back in 2010. It was reported the lack of assurance of sterility due to "syringe barrel flange that slightly deviated from the center line of the syringe barrel, resulted in broken or cracked syringes" inside the auto-injector (FDA 2010). During the early days of these first auto-injectors, glass syringes were manufactured with fairly large variations in the geometry and dimensions in the barrel, nose or tip, and flanges. Historically, such variations were acceptable when existing solely as a PFS format, where their impact on success of injection was mitigated by the dexterity of the human hand. However, dimensional tolerance requirements become much more stringent when the syringe was placed in a device with fixed dimensions and mechanical features. Mismatch in dimensions or strength requirements, or even misalignment has resulted in problems either during manufacturing or actual use by the patients. The root cause of this performance failure can be attributed to both the lack of understanding of the critical component attributes (CCAs) and their links to design principles, as well as the lack of sufficient risk assessment and subsequent risk management schemes for controlling these risks.

The main message of the above examples is clear. The underlying engineering principles and physical phenomenon of hydraulic resistance and tribology are well established and understood by themselves. Additionally, the design features of the glass syringes were initially satisfactory when utilized purely as a PFS format. However, due to the lack of an organized and holistic methodology for integrating the formulation, syringe and device into a combination product, the critical significance (and manufacturing tolerance control) of the syringe properties on device design was missed with serious consequences.

18.6 Device Development via QbD

Self-injection devices such as auto-injectors and pen injectors have helped realize many benefits to both the patient and the broader healthcare industry. With the advent of self-injection devices, not only can patient convenience and compliance be

improved but the overall healthcare costs can also be reduced through the elimination of frequent visits to the physician office or the hospital for chronic treatment. Through intelligent industrial design and early consideration of human-factors engineering, safe and easy-to-use self-injection devices simplify the administration of subcutaneously and intramuscularly delivered drugs. This is accomplished through reduction of the number and complexity of the user steps prior to injection relative to the traditional product image of vials and syringes. Self-injection devices thus enable many users to dose themselves despite possible dexterity or mobility impairments that may make it difficult to administer the very treatment that they rely upon to ease their condition.

Self-injection devices can be designed to offer other patient advantages over traditional vial and syringe product images. The prefilled auto-injector hides the needle before, during, and after the injection. Not only does this design help minimize the risk of unintentional needle sticks but it also offers a significant benefit to needle-phobic patients. By virtue of its design, the system may reduce the fear of needles, which can be a deterrent for some users from starting a biopharmaceutical protein treatment. Additionally, these self-injection devices are preloaded with the full prescribed dose to reduce the possibility of under- or over-dosing, which is possible when unskilled users attempt product withdrawal from the traditional vial using a syringe. Injection depth consistency is accomplished by means of mechanical design of the injection device. Moreover, many injection devices have incorporated visual and/or auditory signals to confirm to the user that the full dose has been delivered.

To fully realize the benefits mentioned above, a systematic methodology and process must be followed to ensure consistent "manufacturability" in commercial production and satisfactory performance of the drug-device combination product in the hands of the user. This organized approach is particularly critical during the development stage, similar to the criticality for the drug product itself, since any major deviation in commercial production could be very costly to fix and be detrimental to patient care due to product shortage. In the following discussion, we present this holistic and organized approach under the umbrella of the QbD methodology.

As mentioned in the opening section of this chapter, the medical device industry has historically followed DFSS, EU Medical Device Directives, and FDA's QSR. Thus, we will try to align the language and practice between the drug industries and the device industries to better elucidate the relationship between product quality attributes and their impacts on the safety and efficacy of the combination products.

The essence of the QbD approach is included in ICH Q8, Q9, and Q10 (ICH 2005, 2008, 2009), which start with the criticality of a proper target product profile (TPP) and the definition of critical quality attributes (CQA) for the product at the outset of the development program. Medical device development is framed by the "Waterfall Design Process" which is principally equivalent to the QbD framework; whereby, the user needs and device input requirements are defined (comparable to the TPP and CQAs from ICH). Application, device design, manufacture and assembly risk assessments in the forms of failure modes and effects analyses exercises

then follow in a similar approach to that defined in ICH Q9 in steps of risk identification, risk analysis, and risk evaluation within the assessment phase.

Guided by QSR, one historically would qualify the component manufacturing and assembly process through the production of devices for testing. The molding and assembly processes are validated by demonstration of full functionality against the design input requirements (DIRs). The device along with the developed packaging, labeling, and instructions for use is then "validated" through human factors testing with the target patient population to demonstrate safety and effectiveness in actual use.

Under QSR, the design controls requirement demands design verification in which devices produced from commercially representative manufacturing and assembly operations are tested against the performance measures set out in the initial DIRs. A QbD approach would further require the development of multivariate design spaces, encompassing both process parameters and raw material properties, to ensure robust manufacturing. Such effort on the development of a design space is currently not widely adopted for device development due to timeline and cost considerations. Moreover, a thorough QbD approach calls for establishment and linking of in-process manufacturing (molding and assembly process) controls to the risk assessment already executed within the design control process to complete the risk management.

The criticality of a rigorous approach to device and combination product development can be illustrated via a brief review of the published history of recalls and device performance issues summarized in the case studies portion of this text. The conventional cGMP approach focuses on demonstration of reproducibility via a prescribed validation protocol of the manufacturing process with three initial batches representative of commercial scale production. The goals were to confirm that the assembled auto-injector products consistently met the finished product specifications with little deviation in the three-batch sequence similar to traditional drug-product development practice prevalent in the pharmaceutical industry. In other words, it was a test-centric approach. Such an approach may overlook the lot-to-lot variations in critical component and material attributes which have significant impact on the CQA of the final combination product. In the authors' experience, incomplete application of the holistic QbD framework embodied in the traditional approach could be argued as a contributing factor to the many incidents of device malfunction, manufacturing supply challenges, and product recalls experienced by these pioneer drug-device combination products.

Clearly, critical QbD concepts such as TPP, CQAs for supporting the TPP, development of design space for tying CCAs, and critical process parameters (CPPs) to CQAs had not been articulated and applied during the design and development these "pioneer auto-injectors." As historical events have demonstrated, certain deviation from those poorly understood parameters in a conventional auto-injector development process may result in loss of control of the product quality during post launch commercial manufacturing (Hirshfield 2010). Moreover, because of the limited knowledge of design spaces, any changes in the manufacturing processes would require costly and long regulatory approvals.

Table 18.2 Hypothetical TPP for an auto-injector combination product

Target	Example
Disease or therapeutic area and indications	To be identified based on the mechanism of action and clinical development plans for the drug or biologic
Patient populations	Adults (could specify further details regarding age or comorbidities)
Efficacy and safety	Superior to market leader X (details left out)
Users of the auto-injector product	Patient, caregiver, and healthcare professional
Product images (i.e., presentations)	Market A: 0.6 ml clear liquid formulation containing 10 mg API in prefilled glass syringe contained in auto-injector Market B: 0.3 ml clear liquid formulation containing 5 mg API in prefilled glass syringe contained in auto-injector; etc
Route of administration	Subcutaneous (may have more details)
Dosing frequency	Single dose once monthly
Storage condition and expiry	2 years at 25 °C/60 % RH

Much of the discussion here focuses on syringe-based auto-injector combination products. Similar statements could be made about cartridge-pen injectors and other types of injection combination products; the quality-by-design principles and method of application are equally valid.

18.6.1 Target Product Profile

Similar to the QbD methodology described in ICH Q8, Q9, and Q10 (ICH 2005, 2008, 2009), the development of an injection device starts with a clear definition of the TPP for the drug, along with the DIRs for the device. It is highly encouraged, and often required, to perform formative human factors studies with the target user population using prototype devices or other stimuli to extract the true DIRs. Some of the requirements may be gleaned from the performance of devices already marketed for treatment of related indications. Table 18.2 illustrates typical areas of consideration when one develops the TPP of a combination drug-device injectable product.

Due to the unique features of the injection device, additional areas of consideration can be added to the TPP or captured in the device DIRs document which includes the key functionality and performance measures of the device. Table 18.3 provides examples of these additional considerations.

The DIR should be developed in stages with increasing levels of details and rigor, as a collaborative effort among the stakeholders including technical, commercial, clinical, and regulatory representatives. One general approach is to start with the TPP, preferably an expanded version that includes product characteristics from the user's point of view in terms of preinjection preparation, injection, and

Table 18.3 Additional considerations for developing the TPP of an auto-injector combination product

Target	Example
Duration of injection once activated	3–10 s
Torque to turn dose knob to set dose	25–40 N-mm
Force to inject	Not more than 15 N
Passive or active skin penetration	Auto-insertion of the needle
Passive or active needle retrieval at completion of injection	Automatic protective needle shroud extension
Indication of start and completion of injection	Audio, visual, tactile, or combination
Pre-use visual inspection of drug product	Shielded window for unobstructed examination of the liquid product
Needle-stick prevention or risk alleviation postinjection	Various means and rigor
Overall size and shape of the injector	No larger than device for market leader X

postinjection activities; then list what the device must do to satisfy these TPP requirements. As mentioned earlier, the (ranges of) mechanical and geometrical characteristics of the primary container such as the PFS or cartridge must be clearly defined as an input into this consideration. Insufficient attention to this latter critical area has been a sour point historically, and has been known to result in not only project delay but also numerous postlaunch investigations and corrective actions.

Another area which has historically received incomplete attention by the pharmaceutical industry is the so-called human factors assessment. Ideally, during the concept/design development of the device, prototypes of various mechanical and geometrical designs should be evaluated early in development (as part of formative human factors studies) via testing with users to tease out designs that approach fail-safe at use. These studies would be termed "formative" under the latest FDA guidance (CDRH 2011) as they inform the DIR for the injection device. Examples of failure at use may include premature "firing" of the device, incomplete injection, failure-to-inject, and incorrect "orientation" of the device. Naturally, not all misuse can be prevented; but a good industrial and mechanical design minimizes such opportunities. Note that conventional "market research" that addresses mainly user preference is typically inadequate for bringing out a design with minimal risk to the patient due to mishandling of the product. The handling and use of the device by the user should be as intuitive as feasible to eliminate risk to the patient, especially if the product is to be used under stressed situations. FDA now fully expects human factors and usability engineering reports as part of design control for device development with key content below.

1. Intended device users, uses, use environments, and training
2. Device user interface
3. Summary of known use problems

Table 18.4 Hypothetical CQAs for an auto-injector combination product

Attributes	Example
Intact container closure integrity	No liquid leakage
Correct indication and function of lock/unlock status	Suitable mechanism and accurate labeling, assembly
Problem-free removal of needle shield	Needle shield removed as designed
Activation of injection-start as designed	No premature activation of injection mechanism
Injury-free completion of injection as designed	Fail-safe indication of needle end Injection completed within the target time duration Needle retraction and shield mechanism completed postinjection

4. User task selection, characterization, and prioritization
5. Summary of formative evaluations
6. Validation (summative) testing (simulated test or clinical evaluation)
7. Conclusion (of safety and effectiveness)

18.6.2 Critical Quality Attributes

Once the TPP and DIRs are defined, prior knowledge or new knowledge gained through initial development efforts on the requirements for safety, efficacy, satisfaction of user needs, or otherwise facilitates identification of CQAs in support of the TPP and DIR. Table 18.4 illustrates device related (nondrug) CQAs specific to an auto-injector combination product.

18.6.3 Risk Assessment

With the CQAs defined based upon the TPP and DIR, the design and development of the injection device can proceed under the guidance of any systematic approach as highlighted in, for example, US FDA's Medical Device Quality Systems Manual. To strengthen the QbD aspects of the development, the authors strongly recommend adoption of the DFSS (El-Haik and Mekki 2008). It is customary within medical device development to begin the design optimization efforts with execution of user or application failure modes and effects analysis exercise. This activity critically examines the user–device interface with the goal of highlighting possible user actions that could pose unsafe or ineffective use of the device. The development team should then attempt to mitigate the highest risk items through redesign of the device interfaces and functionality. Where redesign is not sensible or feasible, possible use errors should be addressed in the development of device instructions for use.

One of the next steps is to complete, in collaboration with the device designer and developer, a product tolerance analysis of the parts that comprise the injection system. This exercise aims to identify the dimensions that are critical to the CQAs, and the required dimensional tolerances during parts manufacture for ensuring satisfactory functional performance as defined in the DIR. It is important to consider part dimensions and tolerances which directly impact operation/function of the device, as well as those which impact ability to successfully assemble the components into a functional mechanism. Once again, risk mitigation strategies based upon the highest risk items arising from the FMEA exercises should be defined to maintain the desired device CQAs. These strategies often entail in-process monitoring and control measures or final product sampling and testing plans.

The traditional cascade of risk assessment activities for medical device development, completed against the prescribed requirements and manufacturing process capabilities, is well detailed in the FDA QSR, and is completely synergistic with the quality risk management process outlined for drug product development in ICH Q9. The framework of the above risk management scheme has been described in the ICH guidance, and the gist of high-level steps is reproduced in Fig. 18.4 for illustrating the iterative nature of this exercise.

18.6.4 Design Space, Critical Component Attributes, and Critical Process Parameters

During the design and prototyping stage, an iterative loop is often adopted to explore the impact of design features, process parameters, and material/component attributes on the CQAs. These sometimes lengthy but necessary exercises lead to the selection of CPPs and CCAs and their respective design space. The ability to leverage prior knowledge plays a critical role during this stage of development; the prior knowledge could reside in the pharmaceutical company, the component suppliers, the device designer, or the assembly machine supplier. Establishing a business process for effectively integrating knowledge from those collective sources is essential.

The critical molding and assembly process parameters should be identified initially as part of the process FMEAs to ensure achievement of all of the critical component dimensions. Once again, dimensions can be critical to delivering the desired device function, or may be critical in ensuring appropriate and complete device assembly. Selection of materials with properties suitable for the intended application must draw heavily from prior knowledge on materials (such as synthetic polymers) and the processes for making the parts (such as molding). Incomplete effort on these issues historically often resulted in project delay (Deacon 2013).

The use of auto-injector enables the automatic insertion of needle and injects the drug into a patient by releasing the potential energy from a compressed spring.

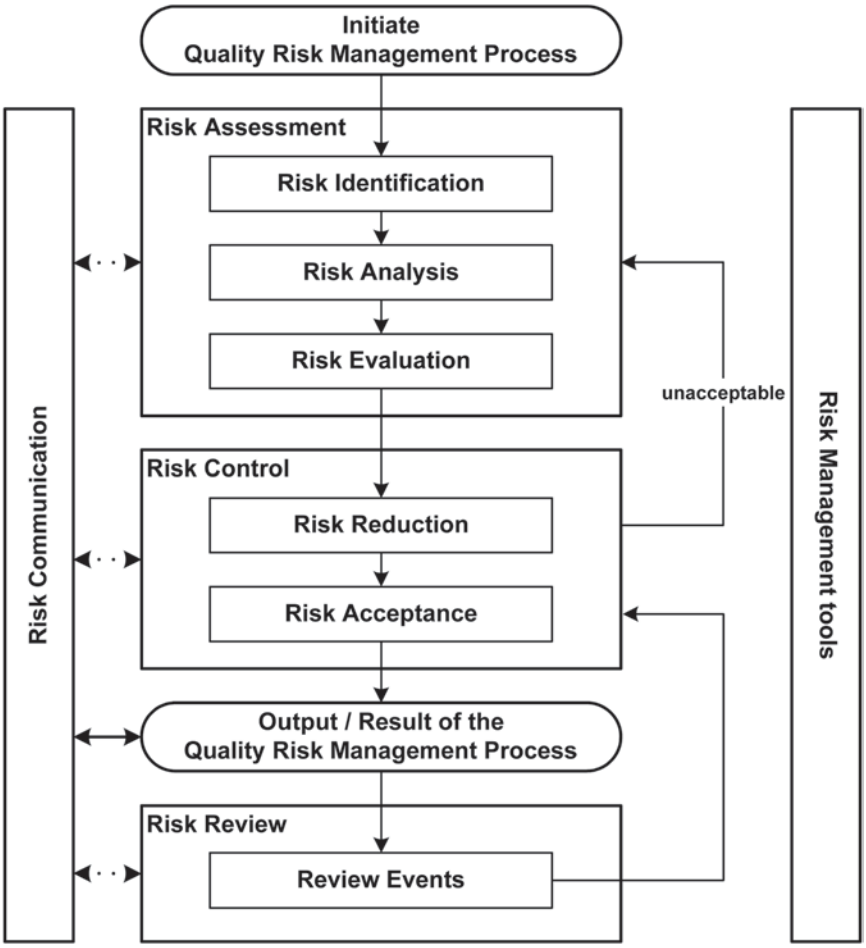

Fig. 18.4 Quality risk management process diagram. (Reproduced from © ICH Q9 guideline 2005)

The plastic component that carries the syringe will subject to mechanical impact to come to a sudden stop for a fixed injection depth. The selection of robust plastic material and proper gating of components to reduce the residual stress are essential to the robustness of the device and to avoid the failure of plastic components.

Thorough application of QbD development principles will draw the connections between the device CQAs and the CPPs/CCAs. The design space development phase entails all the molding and assembly process development through which ranges of process parameters (identified through the FMEAs) capable of yielding acceptable product are established. Ideally, this development work would be conducted in a multivariate fashion such that interdependent process parameter ranges are established across all relevant raw material, molding, and assembly process variables for each device-related CQA.

18.6.4.1 Prefilled Primary Container

As stated previously, we will use the PFS in the ensuing discussion as an example to illustrate the critical QbD concepts with respect to ensuring complete compatibility with the auto-injector. Similar principles are applicable to other types of primary containers such as the cartridge. The PFS itself should be considered as a "component" for assembly into the final combination product, and its characteristics, similar to other critical components, must be clearly defined, controlled, and aligned with the device design. Historically, there had been a lack of effort or even lack of recognition of the criticality of developing an understanding regarding the mechanical characteristics of a PFS. The principles described in this chapter should also be applied to the development of the PFS since the PFS itself is considered a drug-device combination product by FDA (21CFR4 2013, comments on the proposed rule, comment 8, page 19–20).

A common mistake in the authors' personal experience has been to fix critical aspects of injector design before the range of properties of the PFS was fully understood due to an assumption of the lack of criticality or device development time constraints. The PFS characteristics are dependent on the nature of the formulated product, the syringe barrel, the plunger stopper, the needle, the needle shield, and the interaction between the drug product and the syringe components. Historically, the dimensions and lubrication of the syringe components were controlled within fairly large ranges. One must determine whether the design of the device can accommodate these variations, or whether the specifications should be tightened. In some cases, changing the design and specifications of the components, or improving the manufacturing control by the component-supplier may be necessary. Incomplete understanding of the physical properties of the drug product or the capability of the filling process could also result in failure of the device design or lengthy corrective measures.

Glass syringes sourced from different suppliers are not exact replicates dimensionally and functionally in spite of availability of "standard designs," due to the nature of the glass material, the associated syringe-forming manufacturing process, and proprietary trade secrets[1]. Therefore, most auto-injectors have to be "custom-made" for the specific PFS product of interest even if many detailed "considerations" can be leveraged from one design to the other. A detailed description on the proper characterization of PFS can be found in several publications (Rathore et al. 2011). Briefly, one must have access to representative ranges of properties of the PFS components and use them to guide the design and testing of the auto-injector. These properties include (1) critical dimensions of the syringe (barrel, flange, needle, and needle shield) and the plunger stopper, (2) the break-loose and glide force profiles of the matching barrel and plunger stopper (for the drug product or an equivalent placebo) at various volumetric rates and temperature, (3) the me-

[1] The situation for plastic syringes may be somewhat better due to better dimensional control of the molding process; but proprietary factors may still render true standardization difficult.

chanical strength of the syringe under relevant stress scenarios[2], (4) force needed for removing the needle shield, and (5) filled volume and location of the plunger stopper. Based on prior knowledge and new studies conducted specific to the auto-injector system under development, complete design spaces for the various critical CCAs and CPPs can be established, at least in principle when time and resources are available. One should strive to work with the syringe component suppliers to obtain "limit of specification" components for each critical property and then complete evaluation of device performance through design of experiments approaches. In reality, often one will have to accept somewhat incomplete knowledge of the design spaces due to various constraints; however, risk assessment exercises should prioritize the syringe component parameter evaluations which are most critical to safe and effective device performance.

Some of the above properties could turn out to be CCAs and not well controlled, e.g., syringe mechanical strength, needle ID, silicone oil distribution. These properties, if not adequately controlled, have the potential to adversely impact the performance of an auto-injector combination product as discussed in the previous sections of this chapter. Some CPPs, for example, insertion of the plunger stopper by a vent tube, if not well-controlled, could pressurize the PFS to cause the loss of drug upon the removal of the needle shield and impact the performance of an auto-injector. Some of the properties could have high impact but are well controlled. For example, if the range of gliding force profile or the plunger stopper dimensions are well within the respective design space delivering appropriate device performance, neither variable would be designated as CCA (or CPP if process variable).

18.6.4.2 Injection Device Components and Device Assembly

Since a wide variety of injector features are available, the authors will not attempt to provide a full set of representative attributes of the injector *per se* to illustrate the QbD principles. An injection device typically comprises two separate assembled parts; in essence, one provides the "power," and the other accommodates the body of the primary container (e.g., PFS or cartridge). To identify the CCAs, one needs to consider the role of a given part, and analyze the risk of failure due to intrinsic material properties and/or the need for dimensional control to ensure functional compatibility with other interacting parts. Examples include the selection of a reliable metal spring design and wire gauge (if spring is the chosen power source), a strong and dimensionally stable plastic for load-bearing parts (during assembly or shelf life), and plastic parts exhibiting low surface friction coefficient when moving relative to one another.

Depending on the injector design, there will likely be at least several critical dimensions or CCAs that ensure the internal components within the "power pack" of the device to move freely and in sequence as designed. Not only the nominal

[2] For example, strength of the syringe flange or the nose, depending on how the prefilled syringe is supported during triggering and injection.

dimensions, but also the tolerance on those dimensions should be specified for critical dimensions. Stack-up tolerance analysis is typically performed for each device function examining the components within the load/actuation path for the given function (Hurlstone 2014). As noted earlier in the chapter, specific component dimensions may either be critical to accomplishment of a particular device function, or critical to ensure proper and complete assembly of the final product. In the following text, the authors discuss each briefly in turn.

With the critical dimensions (and associated required tolerances) on the relevant device components defined, one proceeds to translate them into the injection mold and tooling design. From a QbD perspective, theoretical and laboratory based engineering analyses considering the component stress states during device operation, as well as the material properties should be conducted to select the appropriate resin. The authors believe that opportunities exist for development of design spaces around the injection molding process to ensure routine achievement of all critical dimensions of the molded components. Multivariate design-of-experiment (DOE) should be run on the relevant molding process parameters (e.g., packing pressure, barrel temperature, cooling hold time, etc.) around the expected upper and lower bounds of each process parameter to both define the design space of acceptable components quality, as well as the required rigor of process parameter control measures. Any molding parameters showing strong correlation to variations in the established critical dimensions of any component may be designated as CPPs.

Proper device function is dependent not only upon achievement of all critical dimensions from the molding process but also upon proper assembly of the molded components and assembly with the primary container. Many injection devices require precise positioning of internal components relative to one another, as well as application of specific loads or displacements applied to specific component features to ensure complete assembly. The commercial volume of many injection devices is large enough to drive assembly towards semi- or fully automated processes. To ensure all key features are appropriately positioned, engaged, or loaded, the development team must leverage the process FMEA previously executed to appropriately design the automation equipment. Assembly process parameters essential for ensuring proper assembly of the two parts, and assembly of the final drug-device combination product (as assessed through either the FMEA exercise or engineering studies), may be designated as CPPs where control ranges are established. Furthermore, in-process monitoring or in-process control measures should be incorporated where appropriate to reduce risks to proper assembly. For example, reproducible alignment of the PFS and the auto-injector parts is critical; ideally, some kind of feedback control should be in place before force is applied (especially if orientation of the parts is necessary), and these alignment parameters could be candidates for CPPs.

Other considerations include the storage and shipping properties. Preassembled device may be stored at the device companies before shipping to the drug companies which impact the shelf-life of the overall combination product. The device components also often will be shipped and manipulated prior to final assembly with the primary container. Risk assessment certainly should be carried out to ensure that

the packaging and tray design, the packaging materials, and the packaging/shipping methods are robust (i.e., relatively large design space compared with the controlled space). The authors do not advocate shipping of PFS for auto-injector assembly if it can be feasibly avoided as the plunger stopper could move slightly if the surrounding air pressure changes are large enough. When syringe shipping is inevitable, care should be taken to accommodate the stopper movement in the injector design, inspect for stopper location before assembling, or select shipping conditions that avoid stopper movement.

18.6.5 Control Strategy, Continuous Improvement, and Knowledge Management

Once the DS, CPPs, and CCAs are defined, risk assessment tools are applied again together with process-capability studies to facilitate development of a control strategy (CS). Historically, this has been the weakest link in the supply chain of drug-device combination products. Customer complaints, supply shortage, or even recalls had occurred due to loss of control on one or more of either the CPPs or CCAs; often the culprit CPP or CCA was not even recognized until the adverse event was investigated. It was reported in the 2008 PDA conference that siliconization process had impacted silicone oil distribution in PFS and auto-injector performance. Upon the improvement of the siliconization process (deemed a CPP) by the syringe supplier, the silicone oil distribution within PFS, and consequent auto-injector performance were improved (Hwang 2008). In a 2011 PDA publication, it was reported that needle ID variation impacted injection force and time, as expected (Rathore et al. 2011). However, it is the authors' understanding that, in general, the industry lacks a generally applied CS in silicone oil distribution and needle ID. It is critical that the sponsor of the combination product makes effort to ensure alignment on the CS (including change-control for processing steps or components) across all the stakeholder organizations and with each supplier that provides critical components.

Prior to product launch, an organized CI system should be established (as directed by the CS) for leveraging experience gained from commercial production and feedback from the market place postlaunch. The improvement could be de-risking and removal of a CPP, or expansion of the design space of a CPP or CCA. Historically, process analytical technology (PAT) has not been thoughtfully applied in the development of injection device combination products. Nevertheless, target application of PAT should benefit the control of some CPPs or CCAs. As stated previously, assembly machines could be equipped with feedback control loops for ensuring alignment of parts before forces are applied instead of relying on simply mechanical setup before a run. Modern high speed metrology inspection sensors could be installed for accurate 100% inspection of critical components in lieu of the inherently flawed sampling plan which is hardly amenable to a "six sigma outcome." For example, it has been shown that nondestructive silicone-oil imaging on filled glass syringes is possible through Schlieren Optics which makes the lubricant visible (Wen et al. 2009). It was also discussed at the 2008 PDA conference that

injection device performance is well correlated with the silicone-oil imaging results. Lot disposition correlated with a 99% confidence interval on 30+ lots of data retrospectively. Individual syringe performance correlated with a 99% confidence interval in prescreened lots (Law 2008). It was recently demonstrated at Achema held in Frankfurt (June 2012) that 100% in-line inspection of silicone oil distribution was feasible at 600 syringe/min.

A rigorous and easy-access knowledge management (KM) system will complete the thorough QbD methodology. As prescribed in the FDA Medical Device Quality Systems Manual, clear and rigorous documentation during a device development is actually a regulatory requirement. Under the QbD umbrella, a KM system enables on-demand access to not just design history but all relevant info that could be leveraged for timely decision-making, CI, and future reference.

18.7 Summary

The flow chart presented in Fig. 18.5 captures the major QbD components to be reviewed in this chapter. The figure has been constructed to highlight the interconnectivity between typical device development process and QbD principles, as articulated through ICH Q8/Q9/Q10 (ICH 2005, 2005, 2005). Many of the well-established and traditional device development activities, milestones and studies are depicted in the center column with connection to the relevant phase of device work, as well as to the equivalent phase within the QbD development paradigm. This illustration highlights the fact that the conventional device development process is rooted in the same tenets of the QbD paradigm.

The traditional QbD flow diagram has been adapted here (left column) to firstly highlight the criticality of the interactions between the drug substance, drug product/formulation, primary container and device at all phases of work. Historically, it can be argued that pharma/biopharma companies had not adopted this holistic approach of considering the interactions across all four development efforts. The authors contend that appropriate delivery device development aligned with QbD principles cannot be achieved without heavy consideration of these cross-functional interactions.

Figure 18.5 has further been constructed to highlight which customary device activities (in the center column) are germane to both the waterfall device development process and the QbD stages. For example, defining the demographics and capabilities of the target patient population is one of the core elements of defining "User Needs" in the waterfall diagram, and this work should be completely integrated within the development of the Quality TPP for the product. Further, a thorough and holistic application of QbD to combination product development would exhibit feedback between the customary device Design-FMEA and the drug substance, drug product and primary container risk assessment activities. Information gained around expected variations in physical dimensions of components of the primary container, filling process tolerances around plunger insertion depth or variations in

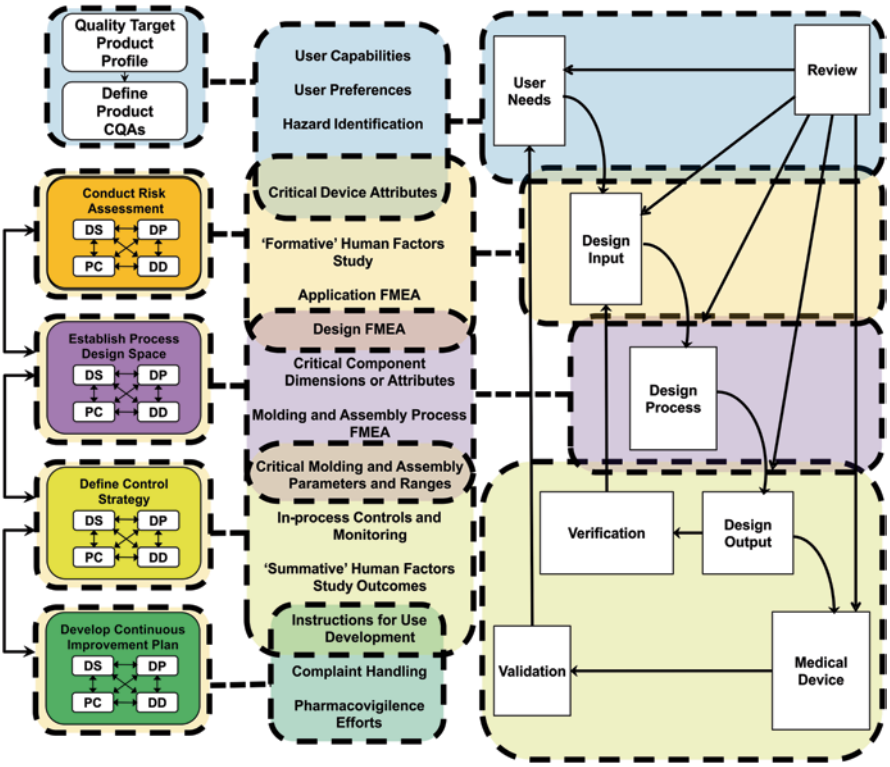

Fig. 18.5 QbD process flow chart as applied to combination products. *DS* drug substance, *DP* drug product, *PC* primary container, *DD* delivery device

initiating or sustaining force of plunger movement over the shelf-life of the product must be fed into the Design-FMEA exercise to ensure the device components are appropriately specified to ensure adequate performance.

While many of the traditional device development activities and stages are highly linked to the QbD development principles, the authors believe the greatest opportunities to achieve a holistic application of QbD to combination product development include enhanced interaction between the DS, DP, primary container and device risk assessment efforts plus integrated design space development efforts across these four development arenas. As was noted or otherwise implied earlier in this text, there are clearly opportunities to more directly connect device component specifications, as well as molding and assembly process variable design spaces, to the specifications and process capabilities of the drug substance and drug product attributes. Additionally, it is likely there exist opportunities to better justify control strategies for DS, DP, primary container, and device production processes based upon the control strategies of the other elements. It is thus our opinion that the major improvement or advantage of the QbD approach lies in the rigor of effort focusing on design space and steps taken on risk assessment and management.

While some of the principles elaborated in this chapter may seem laborious for some applications, the readers are encouraged, and challenged, to draw as much as feasible from prior knowledge and simplify the execution of these principles judiciously. Regardless of the rigor of the QbD principles the developer decides to apply, one will reap the profit of bringing a biologic-device or drug-device combination product to the market in a timely fashion through application of a systematic and thoughtful QbD process. The company will then be further afforded the luxury of being able to conduct rapid and efficient troubleshooting when issues arise, and standing on firm ground for executing continued improvement throughout the life cycle of the product.

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Chapter 19
Applicability of QbD for Vaccine Drug Product Development

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19.1 Introduction

Vaccines are by far the greatest accomplishment in biomedical science, preventing disease and death from many infectious diseases. Historically, vaccine manufacturing was a low-cost, low-tech industry and the production of vaccines was essentially unchanged despite considerable breakthroughs in pharmaceutical science (Streefland et al. 2007). There are many vaccines under development today that are exploiting novel mechanisms of action, adjuvants, and delivery systems for both prophylactic and therapeutic use. Newer technologies include the use of recombinant proteins, viral vectors, and DNA plasmids to immunize humans. Investment in vaccine research and development by pharmaceutical companies such as Sanofi Aventis (Pasteur), GlaxoSmithKline (GSK), Merck, and Pfizer has increased, and from 1995 to 2008, the total number of vaccines in development pipelines more than doubled from 144 to 354 (Davis et al. 1995–2008). Formulation processes and manufacturing technologies employed for vaccines are becoming more current, robust, and efficient to ensure safety, production consistency, and compliance with global regulatory requirements (Dellepiane et al. 2000).

Development of formulation processes for vaccines has more challenges than those for therapeutic proteins or small molecules as the regulatory oversight is more

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stringent. The basis for the stringency comes from many factors. First, the tolerance for side effects is very low, driven by the fact that most vaccines are administered to healthy infants, children, and adults to prevent diseases, rather than the typical drug that is used to treat an existing health condition. Second, most drugs (including both protein therapeutics and small molecules) have functional activities which can be measured by biomarkers unlike vaccines which do not have any inherent activity other than to elicit immune responses. It is often difficult to discriminate between highly efficacious and less efficacious lots of vaccines (potency) due to a lack of in vitro or in vivo preclinical models that correlate to the human response. Third, licensure of prophylactic vaccines requires extensive and expensive clinical trials to demonstrate vaccine efficacy, in part due to the variability in infection rates from year to year. For example, Prevnar®, which is a vaccine against a pathogen with a moderate rate of infection, had nearly 36,000 infants enrolled in phase 3 clinical trials. In contrast, for therapeutic drugs/vaccines clinical trials, each patient has the condition and the trials can be much smaller.

The need to deliver vaccines worldwide has led to additional challenges including minimizing costs to allow access of these vital products to the developing world and emerging markets. Additionally, manufacturing within these markets (e.g., India, China, and Brazil) may be required, which necessitates successful technology transfer. To ensure a successful launch in these new markets, it is important that a robust manufacturing process has been established and the critical quality attributes (CQAs) are clearly defined and controlled (Kristensenand and Zaffran 2010).

The development of an effective vaccine requires a proper vaccine formulation and delivery system, which not only ensure the stability of the vaccine but also increase the vaccine immunogenicity. As vaccine markets continue to expand, other important attributes for vaccines need to be investigated. For example, thermostability profiles of vaccines (Chenand and Kristensen 2009; Brandau et al. 2003) entering into emerging markets and the developing world may require refrigeration, but effective cold-chain systems may not be available which can result in increased temperature excursions during shipping, handling, and distribution of the product to patients. By improving formulations to provide better thermostability of the vaccine drug product (DP), the potential impact of temperature excursions during distribution can be minimized. Along with improving the thermostability profile, determining the proper packaging image is critical for program success. This includes the primary packaging (i.e., single vs. multidose, vial vs. prefilled syringe, and liquid vs. lyophilized), the secondary packaging (i.e., 1× vs. 10×, kits vs. individual vials), and tertiary packing (gel packs vs. nano-cooling). Aligning the product with the specific target product profile (TPP) early in development ensures a product that is appropriate for the expanded regions of distribution and is customer focused.

Vaccine formulation development is achieved in three stages: (1) preformulation, (2) formulation, and (3) process development. During preformulation, development includes investigating biophysical and biochemical characterization to better understand the solution stability and identify the main pathways of degradation. In addition, pre-clinical animal studies are conducted to assess immunogenicity of the expected antigens. The need and selection of an adjuvant is also determined based on the desired immune response, along with compatibility and stability of the

vaccine candidate(s) and adjuvants, and any antigen–adjuvant interactions. After determining the lead antigen and adjuvant for the vaccine in the preformulation stage, the program enters a more rigorous formulation development stage. In formulation development, the critical product attributes are investigated and identified. The impact of formulation variables on these attributes are determined and a lead formulation is moved into clinical development. The formulation target profile along with the delivery system for the vaccine candidate is developed based on the proposed dose levels of the antigens, pain management, and route of administration as determined by preclinical research in consultation with clinicians. In the last stage of development, the focus is on the formulation process.

Excipients that are acceptable for vaccine products are limited when compared to other pharmaceuticals. For example, although generally regarded as safe (GRAS) excipients are utilized across vaccines, biologics, and pharmaceuticals, the route of administration for vaccines requires the use of only parenterally acceptable excipients, as most vaccines are delivered by injection. For penetration into certain areas of the world, all animal-derived raw materials and excipients must be removed and replacement options may be limited. Reformulation of legacy products can be more challenging, when required, if the goal is to have global access for vaccines.

Another challenge with vaccine DPs is the complexity of the active ingredients. Currently, a number of vaccines are comprised of multiple antigen components, including Prevnar®13, licensed by Pfizer, which consists of 13 different polysaccharides conjugated to CRM_{197} protein then absorbed to aluminum phosphate adjuvant, Pnuemovax®, licensed by Merck & Co. Inc., a 23-valent polysaccharide vaccine, and ProQuad®, licensed by Merck & Co., a live-virus vaccine comprised of four different live viruses (Measles, Mumps, Rubella, and Varicella). Identifying a formulation that can provide acceptable stability for all antigens involved can be a significant hurdle. The selected formulation has to be compatible with each one of the components and is usually a compromise among conditions optimal for each.

Due to the multicomponent nature of most vaccines, analytical development can also be complex and challenging. The analytical methods must be able to detect differences for each of the antigens so that an acceptable stability profile is met for all while potency assays, *either* in vivo and in vitro, are difficult to develop and time-consuming to conduct. In addition to the multicomponent nature of vaccines, the high-molecular weights of antigens, e.g., the virus-like particle (VLP) used in the human papillomavirus vaccines (>10,000-fold larger than a small-molecule drug) and low concentrations of the antigens (most vaccine DPs contain antigens at concentrations ≤1 mg/mL) may also be problematic. The analytic methods available for separation of high-molecular weight antigens are limited and a low concentration can make it challenging to quantify the individual components when combined in the final DP. For example, each 0.5 mL dose of Prevnar®13 contains ~2 μg of each *Streptococcus pneumoniae* capsular polysaccharide–CRM_{197} conjugate for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, and 23F ~4 μg of serotype 6B (Prevnar). These factors also make the vaccine formulation and fill-finish process much more complex since it can be difficult to measure the process recovery due to the adsorption of small amounts of antigens to surfaces (i.e., tanks, tubing, and filter housings) during scale-up.

As the characterization of vaccines is more difficult than biopharmaceuticals, traditionally an in vivo animal model has been applied periodically to ensure changes to the process and/or formulation do not impact the product. Although in vivo testing is utilized, there are limitations in their ability to predict immunogenicity in humans. The absence of definitive in vitro immune markers that link to human immunogenicity or known clinical correlates of protection, along with any variation in efficacy due to different manufacturing and formulation conditions, may threaten the registration of a vaccine. Changing the formulation or manufacturing process during clinical development may require additional clinical bridging studies to demonstrate comparability, thereby increasing complexity and cost. Thus, it is ideal to determine an optimized formulation and process at an early stage.

In terms of quality control, it has historically been difficult to define the CQAs of vaccines. Vaccine quality is really based on the overall consistency of manufacture and process controls, rather than relying solely on lot release assays. As a consequence, the process of producing the vaccine itself and the formulation define the product. Any changes in either scale or process require reassurance that the product will remain unchanged in terms of its efficacy and safety, and satisfactory proof is required by leading regulatory authorities. As a result, very sophisticated and extensive analytical characterization, and perhaps additional clinical testing, may be needed to prove equivalence between the new and the old vaccine to bridge a change in process, formulation, or scale. This has led the national (or regional in Europe) regulatory agencies to play a critical role in ensuring vaccine quality. For example, the FDA's Center for Biologics Evaluation and Research (CBER), which is responsible for regulating vaccines in the USA, continues to release all commercial lots of vaccines. The FDA also has the right to conduct its own testing of manufacturers' vaccines.

The Quality by Design (QbD) approach has been an evolving process implemented in the pharmaceutical industry since the FDA launched a chemistry, manufacturing, and control (CMC) pilot program in 2005 (ICHQ8 and GMPs for the twenty-first century). QbD requires a thorough understanding of a product and its process of manufacture, such as the variability in raw materials, the relationship between a process and product's CQAs, and the association between CQAs and a product's clinical properties (Rathoreand and Winkle 2009). Compared with other biopharmaceutical products, the process for producing a vaccine is not well understood, the quality attributes are more difficult to measure, and most importantly, the process defines the product for the reasons listed above. At this time it is still not completely applicable to apply QbD holistically to vaccine development, and simply employing QbD during the product development cycle may not lead to regulatory relief during licensure. Rather, as mentioned in recent published work (A-VAX 2012), it is likely that a "hybrid" approach will be adopted for vaccine filings moving forward. A "hybrid" approach will entail parts of QbD being applied (i.e., application to specific unit operations like lyophilization), in addition to the typical development efforts associated with licensure of a vaccine. QbD can be helpful in understanding vaccine

processes and can potentially improve vaccine production by making the process less empirical and more consistent in terms of quality. However, the regulatory agencies have stated that QbD ensures improved product and process knowledge and is good for the company as it can help them to understand their products better.

19.2 Preformulation Development

Vaccines are comprised of various kinds of antigens including live-attenuated or inactivated viruses and bacteria, native and recombinant proteins, as well as polysaccharides or peptides conjugated to a protein carrier (Shi et al. 2004). Each type of vaccine being developed comes with its own layers of complexity. Due to the lack of sufficient characterization methods available for live-virus vaccines, the limited ability to truly understand the degradation mechanisms for them further increases the complexity of their development relative to recombinant protein vaccines (A-VAX 2012). Thus, the approach that is taken to improve the stability, potency, and delivery of a vaccine is dependent on the specific type of vaccine being developed.

Usually in the early stage of development, robust stability-indicating assays are not available. Thus, the preformulation studies involve understanding the physicochemical properties of antigens and adjuvants, along with antigen–adjuvant interactions by biophysical characterization (Hem et al. 2010). Preformulation efforts should employ methods that can give the formulation scientists insight into the degradation mechanisms associated with the vaccine candidate.

One of the major challenges early in formulation development is the lack of "representative" antigens for development, so it is helpful if high-throughput methods and small amounts of antigens are used to screen formulation conditions. Usually, the early preformulation experiments are carried out by stressing the antigens under accelerated conditions (e.g., 25, 37, and 45 ºC) to quickly identify formulation leads.

Preformulation development studies lead to a better characterized antigen and a stable formulation by understanding the potential degradation pathways, and overcoming any inherent physical or chemical instability. They also help determine lead adjuvants for the product and an initial formulation that can be moved forward into in vivo experiments. The early formulations are typically used to evaluate preclinical immunogenicity and potency of vaccines, and for the generation of monoclonal and polyclonal antibody reagents which are critical for in vitro assay development. The final formulation selection should take into account both the stability of antigens and the adjuvants. Preformulation studies conducted using a rational design based approach provides the basis for a QbD approach as the product moves through development.

19.2.1 Biophysical Characterization of Antigens

Biophysical techniques provide preformulation data to understand many aspects of drug substances and adjuvants including thermostability, pI, accessible phosphate groups, effect of pH on thermostability, and antigen–adjuvant interaction mechanisms, which are critical in the early development of a vaccine DP (Hem et al. 2010). These biophysical characterization results can be used to facilitate formulation studies to screen and identify stabilizers that maintain antigen structural integrity (Volkin et al. 1997). Biophysical characterization is also useful for process monitoring and trouble-shooting the purification of the antigens, e.g., it can quantitatively define the solubility limit of antigens in high salt buffers during purification (Volkin et al. 1997).

Calorimeter Techniques

- Differential scanning calorimetry (DSC) is a relatively simple technique that can measure the thermostability of vaccine antigens in various formulations(Le Tallec et al. 2009; Krell et al. 2005). Since the highly cooperative structures of macromolecular entities undergo conformational or phase transitions upon being heated, significant information can be obtained by DSC (Sturtevant 1987). It is also becoming a high-throughput technique with the advent of capillary DSC instrumentation. Rigorous thermodynamic analysis can be performed using DSC when the measured transitions are reversible, but this is rarely observed for complicated, multicomponent particles such as viruses and VLPs (Kissmann 2010). The measured thermal transition midpoint (Tm) has proven to be an exceptionally good indicator of the relative stability of antigens in liquid formulations (Remmele et al. 2005; Richard and Remmele 2005). DSC can save time and resources by eliminating formulations that are likely to fail and help focus efforts on those that are more viable for real time and accelerated stability studies. Although a powerful tool in determining lead excipients and formulations, DSC does have some disadvantages such as a requirement for high-antigen concentrations (~1 mg/mL) and long run times for each sample. One common problem is that vaccine DPs are generally formulated at concentrations lower than 1 mg/mL, and behaviors observed at the higher concentrations used in DSC may not be representative of the lower concentration in DPs. Additionally, at this stage of program development, limited quantities of drug substance may impact the ability to screen a wide range of conditions using DSC. While DSC can be utilized in a high-throughput approach, the overall run time can require days to complete full analysis of multiple formulation compositions. For DPs, spectroscopic techniques that require much lower concentrations are more suitable and are often used to understand thermostability and structural changes.

Spectroscopic Techniques A variety of spectroscopic techniques are applicable for the physical characterization of vaccine materials. Circular dichroism (CD) and fluorescence spectroscopy are among the most versatile and widely implemented,

although high-resolution second-derivative ultraviolet (UV) absorption spectroscopy has also been used for the characterization of vaccines.

- CD, which detects differences between sample absorption of right- and left-handed circularly polarized light, is an excellent method for rapidly evaluating the secondary structure (i.e., α-helix, β-sheet, random coil, etc.), folding and binding properties of proteins when the incident light is from the far-UV region. Changes in tertiary structure of proteins due to mutations in the aromatic chromophores of proteins can also be monitored in the near UV region.
- Secondary structure can also be estimated from Fourier transform infrared spectroscopy of proteins (FTIR) (Heller et al. 1999; Matheus et al. 2006). The use of FTIR to monitor secondary structure content and protein unfolding is well reported in the literature, especially using the amide I absorption region (1600–1700 cm^{-1}) of the IR spectra. FTIR has been generally recognized as more accurate than far-UV CD spectroscopy in estimating the content of β-sheets and turns, while the far-UV CD spectroscopy has advantages for estimating the content of α-helices (Hurtado-Gómez et al. 2005). However, when FTIR spectroscopy is applied to vaccine candidate characterization, it is limited by a requirement for high protein concentrations (~10 mg/mL). Recently, Dong's group (Dong et al. 2006) obtained FTIR spectra of proteins at low concentrations (0.5 and 1.0 mg/mL) in aqueous solutions by adsorbing antigens onto Alhydrogel® and confirmed that their secondary structure was not altered (22). These results show that, under certain conditions, FTIR can be a very useful biophysical characterization tool to look at the antigen structure during vaccine preformulation development.
- Tertiary structure is usually monitored using fluorescence methods. The intrinsic fluorescence of proteins is dominated by the emission of the aromatic side chains of tryptophan, which have a significantly higher quantum yield, while tyrosine and phenylalanine have weaker quantum yields and are not widely used. The excited state of tryptophan residues, which are often located in solvent-restricted domains of ordered proteins, is particularly sensitive to the solvent polarity and its microenvironment. Therefore, the intrinsic fluorescence spectra can be used to study protein folding and unfolding as well as more subtle conformational changes which result from changes in solvent accessibility of amino acid fluorophores. For the antigens that do not have tryptophan residues or the tryptophans are buried inside the macromolecule, extrinsic fluorescence methods can be used. Probes such as bis-ANS that bind to the hydrophobic regions of proteins, or probes that covalently attach to specific amino acid residues, can provide further assistance to detect physical changes to macromolecular systems. With both intrinsic and extrinsic fluorescence methods, subtle conformational changes in various antigens can be detected during the process of formulation development. For example, a decrease in fluorescence intensity and/or shift in the wavelength maximum of a fluorescence spectrum as a function of temperature can be monitored to assess antigen thermal stability in various formulations. Thermostability data usually correlate well with the storage stability

of antigens and compared with DSC, fluorescence methods require much less material and shorter run times.

Since each of the biophysical characterization tools have advantages and disadvantages and assess different attributes of molecules, it is important to explore multiple biophysical characterization techniques during preformulation development of vaccine candidates to avoid a biased interpretation of the results.

19.2.2 Adjuvant Evaluation

The word "adjuvant" comes from the Latin word adjuvare, meaning to help or aid. Adjuvants are not only used to enhance the immunogenicity of antigens but also to help elicit an optimal quality immune response. Choosing a specific adjuvant for a vaccine candidate is determined by multiple factors, especially the adjuvant's mechanism of action to enhance the immune response which also effects how the adjuvant is analyzed. For example, adjuvants may skew an immune response either toward Th_1, eliciting a T-lymphocyte response including cytotoxic T cells and Th_1 subclasses of IgG, or a Th_2 response that is mostly antibody mediated with little cytotoxic T-cell response. They can also help drive a balanced Th_1/Th_2 response or promote antibody responses at mucosal surfaces (Coxand and Coulter 1997; Exley et al. 2010; Hunter 2002). Adjuvants also have economic advantages such as antigen dose sparing and reducing the frequency of immunizations (CHMP 2005).

The most widely used adjuvants are either aluminum hydroxide (alum) or aluminum phosphate salts, as these were the only adjuvants approved to be used up in the USA until 2009. In 2009, the FDA approved GSK's Cervarix® vaccine, which is formulated with GSK's innovative AS04 adjuvant system (aluminum hydroxide with MPL (monophosphorylated lipid A)). Other new adjuvants such as MF59, CpG, ISCOMATRIX®, QS-21, influenza virosomes, and AS03 (oil in water emulsion, MPL, and α-tocopherol) have now been included into vaccines that are being used in clinical studies or licensed for human use in Europe, but they are still not approved in the USA (Harandi et al. 2009). Although aluminum adjuvants have proven their utility in a large number of applications by eliciting a Th_2 type of immune response, they have significant limitations in certain new generation vaccines due to their poor capability to enhance Th_1 and cytotoxic T-cell immune responses (Harandi et al. 2009). From the formulation point of view, aluminum hydroxide may be detrimental to the stability of antigens absorbed to its surface due to its local high-pH microenvironment (Chang et al. 2001) and the tight binding of antigens that is often observed. Thus, to enhance immunogenicity, multiple adjuvants are usually examined simultaneously in preclinical in vivo animal models and early stability studies. Recent novel vaccine adjuvants, like the Toll-like receptor (TLR) agonists (i.e., CpG, MPL), have been advanced to human trials, and pose new challenges in terms of quality control and characterization. Finally, choosing the proper

antigen and adjuvant combination can be impacted by the route of administration and expected safety and efficacy of the vaccine (Coxand and Coulter 1997).

In addition to the adjuvant choice, the particle size of the adjuvant/antigen complex can also be important (Oyewumi et al. 2010; Clausi et al. 2009). The ideal size of the antigen and adjuvant complex should be 10 μm or less for optimal dendritic cell uptake as suggested by Morefield et al. (Morefield et al. 2005). Therefore, the particle size of the antigen/adjuvant is usually one of the CQA for a vaccine DP.

For the detection and measurement of micron-range particles (>1 μm), the primary methods for analysis are light obscuration (HIAC) and microscopy-based methods. The flow-imaging microscopy instruments, which include micro-flow imaging (MFI) by Brightwell Technologies Inc., pump a liquid sample through a flow cell and subvisible particles (2−70 μm) are imaged and counted by a digital camera. The images are analyzed in real time with respect to variations in the transmitted light intensity that results from the particles in solution. MFI allows for qualitative characterization of the nature of the particles using the captured images and has an increased sensitivity to particles in the smaller (1–10 μm) size range when compared to HIAC, the current "gold" standard. Although MFI allows for improved sensitivity in the smaller size range, as particles increase above 10 μm, HIAC has been shown to have better reproducibility and it is still the instrument of choice for measuring particulates.

Another important characteristic evaluated is the zeta-potential (an indication of charge on the surface) of the adjuvant which can aid in determining the binding capacity or affinity for the different antigens in the DP (Clausi et al. 2008; Diminsky et al. 1999). In addition, characterization of the zeta-potential will determine the potential characteristic point of zero charge (PZC), the pH at which the net surface charge on the particles is zero. More details on the application of adjuvant surface charge characteristic will be illustrated in the adjuvant/antigen interaction section below.

Currently, many vaccine manufacturers produce various forms of aluminum adjuvants tailored to their antigens. Transferring these manufacturing processes to emerging markets and the developing world can be challenging. Thus, examining the TPP during development and exploring options for use of commercially available adjuvants may be a viable option. Utilizing commercially available adjuvants (e.g., Adjuphos®, Alhydrogel®, ISCOMATRIX®, and QS-21), may help to increase the success of the product transfer.

19.2.3 Antigen/Adjuvant Interaction Evaluation: Aluminum Adjuvants

Historically, the development of vaccine formulations containing adjuvants has utilized an empiric approach. An antigen/adjuvant formulation developed for one DP usually cannot be extrapolated to another vaccine candidate. The compatibility of the adjuvant(s) with all antigen components in a vaccine should be evaluated for its

potential impact on the immune response in animal studies and through accelerated stability studies.

There are contradicting examples in the literature with different viewpoint regarding the importance of antigen binding to aluminum adjuvants and its impact on the immune response (Chang et al. 2001; Hemand and HogenEsch 2007; Romero Mendez et al. 2007; Clapp et al. 2011). Adsorption between antigens and aluminum adjuvants has been attributed to electrostatic, hydrophobic, and ligand exchange mechanisms (of which ligand exchange is considered to be the strongest absorption) (Iyer et al. 2004; Vogeland and Hem 2003; Levesque and Alwis 2005). While most interactions between the adjuvant and antigen are electrostatic in nature, ligand exchange with phosphodiesters can occur with an aluminum hydroxide adjuvant. This strong interaction can be detrimental to product stability and must be investigated carefully to ensure the necessary stability profile for the vaccine is achieved (Wittayanukulluk et al. 2004; Sturgess et al. 1999). Thus, it is important to determine not only the quantity of antigen binding, but how strongly the antigen is absorbed to the aluminum adjuvant, as examples in the literature show that the strength of binding can impact the immunogenicity of the product (Hansen et al. 2007; Hansen et al. 2009; Levesque et al. 2006; Egan et al. 2009). Nevertheless, from a quality control viewpoint, it is necessary to demonstrate consistent binding within DP lots and throughout the shelf life (WHO 2003; C.f.M.P.f.H.U. (CHMP) 2005).

As protein binding to the aluminum adjuvant is often facilitated by electrostatic interactions (Hemand and HogenEsch 2007), conditions are usually chosen so that the adjuvant and protein are oppositely charged in a solution. By increasing the negative charge of the adjuvant or, alternatively, the positive charge of the antigen, the electrostatic interactions between antigen and adjuvants can be improved (Le et al. 2001). Two commercially available aluminum adjuvants, Adjuphos® and Alhydrogel®, have PZCs of ~ 5.0 and 11.0, respectively. When formulating at physiological conditions, Adjuphos® will have a negative charge associated with the adjuvant, while Alhydrogel® would have a positive charge. Thus, knowing the charge associated with the antigen under physiological conditions will allow the proper adjuvant for adsorption to be chosen (Matheis et al. 2001; Callahan et al. 1991).

pH can be a critical factor in adjuvant/antigen interactions, and there may be a delicate balance between the stability of each antigen and its adsorption to the adjuvant (Chang et al. 2001; Clausi et al. 2009; Jones et al. 2005). For example, the antigens may be stable at a higher pH with a tendency to aggregate at lower pH, while the adsorption to $AlPO_4$ is greater at a lower pH. Hence, a compromise has to be reached between maximal adsorption and antigen stability in terms of pH selection. In cases where aluminum hydroxide is used as an adjuvant, one needs to consider the microenvironment pH changes that can occur on the surface of the adjuvant that could lead to deamidation of susceptible proteins.

As previously noted, some protein antigens absorbed on to aluminum salts can be monitored for structural integrity by using FTIR (Hem et al. 2010). Although changes in protein structure may not alter immunogenicity, it could be an important characteristic to understand product quality (Hem et al. 2010). Implementation of a series of preformulation studies with antigen, adjuvant, and antigen–adjuvant mix-

tures is very useful in optimizing the vaccine formulation, so that robust formulations are used in preclinical and clinical studies (Hem et al. 2010).

19.2.4 Analytical Control Strategy and Method Development

The analytical control strategy for vaccine development should cover all quality attributes of the vaccine, including component interactions. As already discussed, antigens can interact with adjuvants, necessitating the development of methods to analyze the antigens in the presence of the adjuvant, which can be challenging to do without impacting their overall structure. For example, the relatively tight binding of an antigen to some aluminum adjuvants can affect the ability to fully characterize the antigens. For certain adjuvants, such as ISCOMATRIX™, that do not interact with most antigens, the analytical methods should be more straightforward.

The analytical control strategy should be able to evaluate attributes that could affect the safety, identity, strength, purity, and efficacy of the vaccine. However, as the CQAs may not be known early in development, a risk-based approach should be taken in developing the control strategy. The process of developing a final analytical control strategy involves building a knowledge base to determine the CQAs and the formulation and process design space. Accelerated stability studies or forced degradation studies, using harsh conditions such as pH extremes (5.0 and 8.0) that favor oxidation/deamidation or elevated temperature (e.g., 25, 37, and 45 °C) are performed to understand the possible degradation pathways of the product. These studies are very helpful in determining the quality attributes that need to be measured. Concurrent with the identification of quality attributes, the assays and the control strategy should be refined. A separate in-process analytical control strategy should address the process parameters that may impact the final product quality. As the program moves through development (Phase I to Phase III), the biochemical properties and manufacturing process are better understood and the analytical control strategy may be further refined.

Among all the quality attributes, potency is considered one of the most important for DP release and stability. International Conference on Harmonization (ICH) guidelines, Sect. 6B states that "the measure of the *biological activity* using a suitably *quantitative* biological assay (also called a potency assay or bioassay), based on the *attribute* of the product which is linked to the *relevant biological properties*" and "a relevant, validated potency assay should be part of the specifications for a biotechnological or biological drug substance and/or DP." The World Health Organization (WHO) has adopted the ICH guidelines (2003) and also states that "potency tests measure biological activity of a vaccine, but not necessarily reflect the mechanism of protection in human." A potency assay is used to demonstrate that the DP will elicit the desired immune response and can also be used as a stability indicating assay. The US Code of Federal Regulations (CFR) Part 21600.3(s) states that the tests of potency shall consist of either in vitro or in vivo methods, or both. Ide-

ally the results of an in vitro potency assay for a vaccine should correlate with the results of an in vivo assay, which may indicate efficacy in humans. In the absence of this correlation, CBER expects that in vivo potency assays be used for release of DP. Additionally, the potency assay can be used to demonstrate manufacturing consistency and comparability between lots. As the vaccine markets continue to expand, this can be critical as it is likely that there will be multiple sites throughout the world manufacturing the same vaccine.

Various test methods, such as assays of physicochemical properties, antigenicity, immunogenicity, infectivity, and protection against infection or disease, can be used to measure vaccine potency. As discussed above, it is difficult to determine which quality attributes can impact the vaccine's immunogenicity in humans. It therefore can be challenging to develop a potency assay using one definitive test method (i.e., either in vivo or in vitro) as any one assay will have its limitations. A battery of analytical, physicochemical, and immunochemical test methods can be used to control manufacturing consistency and vaccine formulation, but their correlation with protective efficacy in humans is often difficult to establish. If a particular immune response can be correlated with clinical efficacy, then the correlation could be the basis for a potency assay (Petricciani et al. 2007). Thus, unique potency assays measuring various attributes and functional immune responses may need to be developed for the variety of licensed vaccine types (e.g., vaccines comprising toxoids, live-attenuated viruses, polysaccharide conjugates) (Petricciani et al. 2007). For example, for toxoid-based vaccines, measurement of toxin neutralizing antibodies has dominated vaccine potency testing for decades (Hendriksen 2009). An enzyme-linked immunoassay, which measures the amount of antibodies bound to neutralizing epitopes for each human papillomavirus (HPV) type, is used as the in vitro relative potency (IVRP) test for Merck's Gardasil® vaccine. As the clinical results indicate that IVRP is predictive of human immunogenicity, the IVRP assay has been used as the sole potency assay to release Gardasil® (Shank-Retzlaff et al. 2005). While Prevnar 13® and *Haemophilus influenzae* type b conjugate vaccines do not have either in vitro or in vivo potency methods; free saccharide levels are considered a CQA for the DP. Although animal immunogenicity testing is necessary during vaccine development, WHO recommendations state that the testing for *H. influenzae* type b conjugate vaccines should focus on physicochemical tests to monitor consistency of production of the polysaccharide, the protein carrier and the conjugate drug substances (WHO 2000).

19.2.5 Preclinical Animal Studies

Vaccines are thoroughly tested in the laboratory and in animals (preclinical animal studies) before they are administered to people. Currently there is limited guidance for preclinical evaluation programs other than a WHO guidance document, which only provides general principles for evaluating vaccines preclinically, with particular attention to regulatory expectations for new and novel vaccines (WHO 2003).

Preclinical animal studies can provide data that elucidate elicitation of functional responses, mechanisms of protection, and safety (WHO 2003). Again it should be noted that animal models may not predict the immunogenicity and efficacy in humans and protective doses in animals rarely translate into doses in humans.

Completing preclinical animal testing is critical to determine if a correlation exists between in vitro stability assays and in vivo responses. While various formulations are investigated in animal models, one major factor that should be investigated is dose ranging in animals. It is useful to know where the formulations (antigen dose and any adjuvant dose) investigated in the preclinical studies fall on the dose–response curve (between minimum effective dose and overdosing). Thus, studying a wide range of dose levels early in any animal model should be carried out prior to determining a single dose of antigen(s) and adjuvant(s) for future preclinical studies. It may be useful to obtain guidance from biostatisticians in powering animal studies such that they are able to discriminate between the various dose levels used in the studies. However, the number of animals required to obtain statistically significant differences between dosage levels even threefold apart may be impractical. In these cases, animal studies may serve as "disaster checks" for lack of immunogenicity in some vaccines. Due to the importance of preclinical animal studies in the evaluation of a new vaccine candidate, it is crucial to control the quality of formulation samples used in these studies. The formulation should address potential issues such as vaccine stability, and compatibility of adjuvants and antigenic components so that the animal studies can be interpreted and are reproducible. To avoid any stability issues, samples can be supplied frozen, lyophilized, or injected into animals immediately following sample preparation to help overcome these challenges. Since endotoxin is a powerful adjuvant and can mask issues associated with immunogenicity, it is important to have a low endotoxin level in drug substances and DP so the effect of adjuvants can be determined in the preclinical animal studies (Britoand and Singh 2011).

19.3 Formulation Development and QbD Approach

19.3.1 Define Quality Target Product Profile (TPP)

One of the key factors associated with vaccine formulation development, much like other pharmaceuticals and biologics, is to ensure a specific TPP is established early in the program. Utilizing the TPP, the formulation and development teams understand what is required for the final DP image. A representative TPP should have the specific dosage form, concentration of product, route of administration, expected shelf-life, expected markets, and packaging considerations. Defining the TPP has been extensively discussed in the previous chapters of this book. As stated in ICH Q8 R2

The quality target product profile forms the basis of design for the development of the product. Considerations for the quality target product profile could include:

- Intended use in clinical setting, route of administration, dosage form, delivery systems;
- Dosage strength(s);
- Container closure system;
- Therapeutic moiety release or delivery and attributes affecting pharmacokinetic characteristics (e.g., dissolution, aerodynamic performance) appropriate to the drug product dosage form being developed;
- Drug product quality criteria (e.g., sterility, purity, stability and drug release) appropriate for the intended marketed product.

The following table (Table 19.1) is an example of a TPP for a pneumococcal conjugate vaccine from the WHO.

Once the TPP has been defined, the formulation and development scientists must determine the potential CQAs. ICH Q8 (R2) defines a CQA as "A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality." Issues such as unwanted immunogenicity and pharmacokinetics do not apply to vaccines during a risk assessment.

As discussed above, the CQAs affecting the vaccine quality are usually defined on the basis of prior knowledge and the preformulation studies. During formulation development, the effect of various formulation variables on the quality attributes is evaluated to optimize the vaccine quality in terms of stability and immune response.

Table 19.1 TPP for a pneumococcal conjugate vaccine

Attribute	Minimally acceptable profile
A. Vaccine serotypes	The serotypes in the vaccine formulation must cover at least 60% of the invasive disease isolates in the target region, and must include serotypes 1,5 and 14 which are the most-frequent isolates in GAVI eligible countries
B. Immunogenicity	Immunogenicity should be demonstrated in accordance with WHO criteria, which are based on noninferiority to a licensed pneumococcal vaccine as outlined in WHO *Recommendations for the production and control of pneumococcal conjugate vaccines*. (WHO Technical Report Series, No 927, 2005 and any subsequent published guidance)
C. Target population/target age groups	The vaccine must be designed to prevent disease among children < 5 years of age and in particularly be effective in those < 2 years of age
D. Safety, reactogenicity, and contra-indications	The safety and reactogenicity profile should be comparable to, or better than that of the currently licensed pneumococcal conjugate vaccine. Contraindications should be restricted to known hypersensitivity to any of the vaccine components
E. Dosage schedule	Vaccine scheduling must be compatible with national infant immunization programs and consist of not more than three doses in the first year of life. The first dose must be shown to be administrable at 6 weeks of life or earlier

Table 19.1 (continued)

Attribute	Minimally acceptable profile
F. Interference and coadministration with other vaccines	There should be no clinically significant interaction or interference in relation to safety and immunogenicity with concurrently administered vaccines
G. Route of administration	Intramuscular or subcutaneous
H. Product presentation	The vaccine must be available in monodose or low multidose presentations. Monodoses can be either a single-dose vial or a nonreusable compact prefilled device. Low-multidose presentations should be formulated in compliance with multidose vial policy *(The use of opened multi-dose vials of vaccine in subsequent immunization sessions*, WHON&B/00.09)
I. Product formulation	Liquid formulation with a standard volume of 0.5 ml/dose
J. Storage and cold-chain requirements	The product must be stable at 2–8 °C with a shelf-life of at least 24 months and a vaccine vial monitor should be attached as outlined in *Making use of vaccine vial monitors. Flexible vaccine management for polio* (WHO/V.100.14)
K. Packaging and labeling	Name and labeling must be in accordance with WHO *Recommendations for the production and control of pneumococcal conjugate vaccines*. (WHO Technical Report Series, No 927, 2005). Packaging must ensure minimal storage space requirements as not out in Guidelines on the international packaging and shipping of vaccines (WHO/IVB/05.23)
L. Product registration and prequalification	The product must be WHO pre-qualified in accordance with *Procedures for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies* (WHO/IVB/05.19)
M. Postmarketing surveillance	Post-marketing surveillance should be concluded in accordance with national regulatory authorities and WHO prequalification requirements as set out in *Guideline for preparation of the product summary file for vaccine prequalification* (WHO/IVB/06.16), *Guidelines on clinical evaluation of vaccines: regulatory expectations* (*WHO Technical Report Series, No 924,2004*) *and any relevant published guidance*

19.3.2 Liquid Formulation

Liquid formulations have been generally preferred due to the convenience of manufacturing and use. However, it can be very challenging to develop a liquid vaccine formulation that is stable for a pharmaceutically relevant storage time (e.g., 2 years). This is especially true for live-virus vaccines, where degradation rates in liquid can be as high as 10% loss in infectivity per hour, even under refrigerated conditions. With accelerated and real-time liquid stability studies, a sound judgment can be made as to whether a product is able to achieve the desired TPP profile outlined early in program development. If the liquid profile does not meet the necessary TPP profile, development of a lyophilized or frozen product can be initiated. The formulation screening conducted in liquid state would also serve as a good base for lyophilized formulation development, as well as provide feasibility information (i.e., bulk-holding time) for manufacture.

19.3.2.1 Optimize the Formulation Variables (pH, Excipients, Stabilizers, and Process Conditions)

During formulation development of a vaccine product, the effects of various formulation variables such as pH, buffers, excipients, and ionic strength on the QAs are evaluated to optimize vaccine quality and stability. During formulation screening, vaccine candidates are usually subjected to various stressed conditions, such as high temperatures, agitation, multiple freeze/thaw cycles (1×, 3×, 5×), and light exposure to predict potential stability issues that may occur during real-time long-term storage.

As previously mentioned, vaccines may contain multiple antigens, so the formulation screening of each variable should be performed on each antigen individually as well as in combination with any potential adjuvant. By studying the antigens in a monovalent vaccine, the formulation scientist can better characterize the product and potential degradation pathways associated with the multicomponent vaccine. The final formulation conditions for multivalent vaccines are based on consideration of the stability profiles for all antigens, as well as the optimal conditions for compatibility/binding with the adjuvants. For example, antigen(s) binding to aluminum adjuvants can be maximized by controlling the pH of the formulation (see Sect. 19.2.3). If the maximal binding has been determined to be a CQA, the final selected formulation pH would be at a lower range pH value so that maximal binding can be achieved. Even if binding to the aluminum adjuvants is not a CQA, consistency of antigen(s) binding from lot-to-lot and stability of the soluble and bound antigens upon storage must be demonstrated. Thus, one should be very careful in selecting a liquid formulation for vaccine products, unless there is strong evidence supporting that the antigens and adjuvant remain stable during real-time storage. For vaccine DPs that contain both protein antigens and polysaccharide conjugates, in addition to optimizing the stability, the final DP formulation has to be amenable to filterability of conjugates during manufacture.

19.3.2.2 Determine the Design Space of Formulation Components

By utilizing design of experiment (DoE), a multidimensional combination and interaction of input variables (e.g., pH, excipients, and buffers) demonstrated to impact the quality of vaccine product, can be evaluated more efficiently during formulation development. In addition to identifying the critical formulation components and conditions as well as interaction of parameters, a DoE study can also provide the limits of formulation conditions that must be tightly controlled to maintain product stability. The extent of a DoE study is dependent on the stage of development. In early development, where antigens are limited, a low-resolution study design would be used and could examine a broad range for each variable, as shown in Table 19.2 (e.g., pH explored 5.0–8.0). In late-stage development, a more thorough DoE can be designed so that the design space for the specific product and selected variables can be defined effectively.

Table 19.2 Example of DoE factors to examine for vaccine formulation development

Excipient	Range to investigate
Buffer	5–50 mM
Salt	0–300 mM
pH	5.0–8.0
Sugar	1–10%
Surfactant	0–0.5%
Aluminum (adjuvant)	0.2–0.6 mg/mL
Antigen concentration	1–5 μg/mL

19.3.3 Lyophilized Formulations

There are many papers describing the development of a lyophilized formulation for biopharmaceutical products (Carpenter et al. 1997; Pikal 1990; Schwegman et al. 2005). The selection of the stabilizer, bulking agents, buffers, and excipients for the lyophilized formulation need to balance the stability profile for all antigens within the formulation while maintaining lyophilization related CQAs (cake appearance, moisture, reconstitution time, etc.) and enabling an efficient freeze drying process.

Since freezing of aluminum adjuvant suspensions results in adjuvant particle agglomeration, antigens are not usually coformulated with an aluminum adjuvant when lyophilized, and diluents containing the adjuvants are used to reconstitute. The stability of the adjuvant liquid formulation and the reconstitution volume for the DP should be clearly defined. In addition to the common quality attributes for lyophilized formulations, if aluminum adjuvants are used, it is important to understand the adsorption kinetics of antigens to the adjuvants so that upon reconstitution antigens can be quickly adsorbed and the administered vaccine is consistent from lot-to-lot. Stability of the vaccine antigens and adjuvant upon reconstitution at room temperature must be determined and the allowable time for administration clearly stated to ensure the quality of the vaccine.

19.3.4 Multidose Vaccine Formulation

Vaccines are often formulated as multidose products to reduce the cost, packaging space, and packages in the cold chain for distribution. Multidose presentations differ from single-use formulations as they must contain antimicrobial agents (preservatives) to protect them from microbial contamination that could occur during multiple-dosage withdrawals from a single vial. Preservative(s), for example, 2-phenoxyethanol (2-PE), phenol, m-cresol, parabens, are defined as compounds that kill or prevent the growth of microorganisms, particularly bacteria and fungi (Meyer et al. 2007). While thimerosal (merthiolate) has been widely used for over

70 years as the preservative of choice for vaccine products, the FDA has been actively working with manufacturers to eliminate thimerosal from childhood vaccines due to concerns over its safety (Van't Veen 2001). The industry has been working diligently with the WHO to identify alternative preservatives and one such example, 2-PE is being utilized to develop a multidose Prevenar® 13 formulation for the developing world (Khandke et al. 2011).

The addition of preservatives can present significant challenges in the development of multidose formulations. They can interact with proteins and negatively impact the stability of the vaccine, they may be inactivated by the presence of surfactants which are common in vaccine formulations (Bontempo 1997), and can also absorb to the stoppers, all of which can compromise their long-term antimicrobial efficacy (Lachman et al. 1962; Akers 1984; European Pharmacopoeia (Ph. Eur.) 2009). Thus, identifying a formulation and image (syringe, stopper, and vial) that is compatible with preservatives and maintains the desired antimicrobial efficacy may not be straightforward.

The regulatory requirements assert that the antimicrobial efficacy of the formulation must satisfy the preservative efficacy test (PET) requirements of the target markets. The PET test consists of challenging the preparation, wherever possible in its final container, with prescribed inoculums of suitable microorganisms, storing the inoculated preparation at prescribed temperatures, and measuring the reduction in organism growth by colony count at specified intervals (European Pharmacopoeia (Ph. Eur.) 2009). The PET requirements of the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP) differ considerably, imposing an additional hurdle in developing a multidose formulation (Streefland et al. 2007; European Pharmacopoeia (Ph. Eur.) 2009; Akers and Defelippis 2000).

19.4 Process Development and QbD Approach

Once the TPP is defined and the CQAs are identified, the manufacturing process can be formally designed. This includes executing a series of studies specifically designed to facilitate the comprehensive identification of critical process parameters (CPP) that can affect the CQAs. This section focuses on the DP process parameters in vaccines development.

During a recent Parenteral Drug Association/FDA CMC Workshop on applying QbD concepts to vaccine development, it was concluded, in terms of regulatory expectations, that some well-understood unit operations are more amendable to QbD than others (e.g., lyophilization). Regulatory agencies encourage some application of elements of QbD, but because of the expectation that vaccine quality includes the overall consistency of the manufacturing process, manufacturers *should not necessarily* expect significant regulatory relief or license flexibility for filings that include elements of QbD (A-VAX 2012).

The current perception is that implementation of QbD represents an increased upfront investment of resources and external value may not be generated in the form

of regulatory flexibility. Thus, the initial application of QbD for vaccines should focus on the "internal" value of QbD with a particular focus on those areas where the enhanced approach will provide a specific benefit over the standard approach. The enhanced approach includes development of more customer focused products, risk assessments to prioritize development efforts/resources, more consistent manufacturing processes and analytical methods and better understanding of the process and product. Additional process understanding could be useful over the product lifecycle during anticipated postlicensure changes; e.g., equipment, raw material, process (scale-up), and site changes.

19.4.1 Process Design

The goal of a manufacturing design is to develop a process capable of routinely making a consistent, quality product in a cost-efficient manner. For vaccines, there are three areas that require particular focus; process control, low-cost structure, and process portability/scalability.

19.4.1.1 Process Control

As discussed in the sections above, the linkage between product quality attributes and clinical efficacy may not always be well defined for vaccine DPs. During scale-up from pilot scale to commercial scale, the impact of process changes must be monitored. The analytics are limited for complex molecular entities especially at low concentrations of the antigens and the combination of multiple antigens/adjuvants makes it difficult to understand the effect of process changes on the quality attributes. Thus, for vaccines, a stronger emphasis has been placed on the consistency of the manufacturing process itself as a measure of product quality (i.e., "the process is the product"). This is not aligned with a holistic QbD approach; however, this philosophy should continue to be challenged, especially for discrete unit operations as advancements are made in analytical and processing technologies. Acknowledgment of this expectation warrants the development of a process that is highly controlled, with an emphasis on advanced manufacturing technologies, feedback control, and process analytical technologies (PAT).

19.4.1.2 Low-Cost Structure

While not often discussed in the context of QbD, product cost continues to take on increasing importance in the pharmaceutical industry. In emerging markets and the developing world, the expectation is to maintain low cost of manufacturing while ensuring high quality for vaccines and other life-saving treatments. As the manufacturing process for vaccines constitutes a significant component of the overall

product cost structure, cost should be an important consideration during optimizing process design.

19.4.1.3 Portability and Scalability

Besides requiring a high degree of process control, "the process is the product" philosophy also necessitates that processes have consistent performance throughout clinical manufacturing and scale-up. To be successful in this endeavor, developers should select manufacturing technologies that are highly scalable and representative of commercial manufacturing operations. This proactive approach can greatly reduce risk during scale-up and avoid costly changes to manufacturing processes at critical times during the product lifecycle. For example, instead of starting development trials with material formulated in glass bottles, it would be better to scale-up at a pilot facility that uses portable stainless tanks (used for clinical trials) and eventually launching out of a facility that uses disposable bags. Implementing disposable bag technologies across the entire lifecycle of the product and maintaining consistent product contact surfaces throughout the manufacturing history reduces risk. In addition, these strategies may also position a commercial process for successful postlaunch transfers, which are becoming more prevalent as global access strategies increasingly require local in-country manufacturing.

When these three elements are combined with sound quality risk management principles and product understanding gained in earlier stages of development, manufacturing processes can be designed that will be positioned for successful progression through all stages of clinical manufacturing, scale-up, transfer, and commercial launch.

19.4.2 Process Development

As stated earlier, the implementation of QbD for vaccines will be most effective when it is focused on assessing which areas have the highest impact on product/process, through the use of risk assessments. For process development, two tools are commonly used for this purpose, depending on the stage of development.

19.4.2.1 Early Stage Risk Assessment

During the manufacturing design and development process, it is often valuable to map the impact that each proposed unit operation and process parameter will have on product quality attributes/CQAs. During process design, this can be instrumental in eliminating nonvalue added steps, keeping the process simple, robust, and as low cost as possible. In early development, this analysis can be fundamental to driving prioritization of development efforts and identifying where additional studies

Table 19.3 Example of risk analyses on process parameters that will impact product quality attributes/CQAs

	Critical Quality Attributes				
Unit Operations/Parameters	Potency	Purity	Recoverable Volume	Appearance	Sterility
Drug Substance					
Excipients and Other Raw Materials					
Compounding and Formulation					
Sterile Filtration					
Aseptic Filling					

Red = Significant impact on CQA

Yellow = Moderate impact on CQA

Green = Little/no impact on CQA

Gray = Unknown impact on CQA

may be required. In later stages of development, these analyses can serve as the precursor to highly detailed process risk assessments (failure mode-effects analysis, etc.) for the purposes of commercial process design, qualification, and licensing. A simple example of such risk analyses are shown in Table 19.3 for the purposes of illustration. Note that this can be extended further to include process parameters for each unit operation (duration, temperature, rates, etc.) for a more rigorous analysis, depending on the stage of development.

In this example (Table 19.3), general conclusions can be made about the overall risk profile of the process, as well as which attributes or process parameters are at the highest risk and should be examined through further development and control strategies. These analyses are also useful to identify unit operations where only limited information is available, suggesting either increased development efforts to better understand affects, or acceptance of limited development on those operations perhaps based on prior knowledge. Risk analyses such as these are especially critical early in development to help prioritize development efforts and focus analytical testing, depending on the process operations being studied.

19.4.2.2 Late Stage Risk Assessment—FMEA

As additional information is gained through focused development testing, manufacturing experience and clinical trials, the early stage risk assessment can be updated and eventually evolved into a full failure mode and effects analysis (FMEA). This method of risk assessment is common across the industry for all product types, including vaccines. FMEAs take into account not only the potential for impact (Severity, “S”) but also the likelihood of a failure to occur (Occurrence, “O”), and the ability to detect the failure (Detection, “D”). Numerical values can be assigned for each severity, occurrence, and detection based on predefined criteria. An overall

Table 19.4 Simplified example of an FMEA for a theoretical mixing process

Category	Sample Scoring Criteria									
	Process	Parameter	Range	Controls	Failure Modes	CQAs	Severity	Occurrence	Detectability	RPN
	Mixing	Mix Speed	150-300 RPM	Recipe Monthly calibration Batch record Operator training	Incorrect recipe Equipment failure Equipment calibration	Concentration Potency Purity	5	1	7	35
		Mix Time	20-40 minutes	Recipe Batch record Operator training	Raw material variability Operator error	Concentration Potency Purity	5	1	3	15
		Tank Temperature	0 - 10 °C	Recipe Monthly calibration Batch record Operator training Active tank cooling	Incorrect recipe Equipment failure Equipment calibration	Potency Purity	9	3	5	135
Severity	1 3 5 7 9 No CQA impact at 3X NOR → Significant CQA impact at 3X NOR									
Occurrence	1 3 5 7 9 Low frequency (1:1000) → High frequency (1:1)									
Detectability	1 3 5 7 9 Immediately detected → Not detected									

risk priority number (RPN) can then be obtained by multiplying the S, O, and D scores. This RPN number can serve as a means to prioritize further development efforts as well as to characterize "critical" process parameters that represent the highest risk to product quality and require the most control during manufacturing. This approach is consistent with FDA Guidance for Industry Appendix to Questions Q8, Q9, and Q10 (Jul 2012), which states that "process parameter criticality is linked to the parameter's effect on any CQA. It is based on the probability of occurrence and detestability and therefore can change as a result of risk management."

A highly simplified example of an FMEA for a theoretical mixing process is shown in Table 19.4. In this example, the tank temperature during mixing has a relatively high RPN, suggesting that it represents a particularly high risk to the process, and is a good candidate for further development and advanced control.

19.4.3 Design Space

Demonstration of a design space for a vaccine is similar to approaches that would be taken for other products. The output of the risk assessment is used to prioritize parameters for further evaluation. These parameters are tested within certain limits using statistically designed experiments to demonstrate relative impact and potential ranges of acceptable manufacturing. There are a few considerations of this approach that require particular focus for vaccines.

19.4.3.1 Analytical Variability and Statistical Power

Some bioanalytical methods for vaccines may have higher inherent variability than other modalities. This is especially true for cell-based potency assays that are nor-

mally associated with complicated molecules like a live-virus. In these cases, it is extremely important to employ proper statistical power to any experimental design to ensure that meaningful conclusions can be made about the study outcome. This assumes that there exists a solid understanding of analytical variability, either through assay qualification activities, measurement system analyses (MSA; Gage R&R), or other targeted characterization effort.

19.4.3.2 Ranges of Parameters for Analysis

With the increased scrutiny of the manufacturing process of vaccines, there is a potential for the utility of design space to be of an increased importance relative to other product types. To ensure that the design space has the maximum utility, special care should be taken when considering the ranges of parameters to be explored in supportive experiments. The common rule of thumb is to challenge three-times the normal operating range (NOR) in design space experiments, with the NOR equal to 2 standard deviations. For vaccines, there may be value in exploring further outside of this range to confirm robustness to demonstrate a measurable response in highly variable assays.

19.4.3.3 Selective Challenge of Parameters for Clinical or PPQ Manufacturing

In select cases where the analytical resolution for an attribute is particularly low or future manufacturing risks associated with a parameter/set of parameters are exceptionally high, it is possible that manufacturing flexibility will be restricted by the process used in clinical manufacturing and/or process performance qualification/validation. In these cases, a risk-based approach could be considered using a process during clinical manufacturing or process performance qualification that is not in the center of the process design space, but is still capable of delivering product of acceptable quality and safety. This will enable future flexibility and reduce the overall risk profile of the commercial process. Obviously, any such approach would be based on the highest level of consideration for patient safety, and product quality/efficacy.

19.4.4 Considerations for Unit Operations Specific to Vaccines

There are some similarities between the process development of vaccines and other biopharmaceuticals. For example, the process used in basic formulation and in fill/finish operations, such as freezing/thawing of the purified protein bulk, formulation/compounding (excipient addition), sterile filtration, filling, freeze-drying, and inspection that are commonly used in biopharmaceutical area can also be applied

to vaccine manufacture. However, with combination vaccines, the formulation processes can be much more challenging than biopharmaceuticals, including the potential for addition of >10 individual active components. The other chapters of this book and available literature references have illustrated how to apply QbD in these steps of manufacturing extensively (Kantor 2011; Jameeland and Khan 2009; Patro et al. 2002). The focus of this section is to discuss the process steps that are unique to vaccine production.

19.4.4.1 Order of Components Addition During Formulation

In developing a robust manufacturing process, the order in which components are added should be optimized. This is especially true in the case of adjuvants, as some of them may not be filterable and addition too early in the process can result in serious manufacturing and quality issues (i.e., filter plugging, or losses during filtration). In this case, the adjuvants should be added to the process after all other components have been sterile filtered. When vaccines are formulated along with aluminum salts directly, the order in which the antigens are added must be considered to ensure optimal adsorption. There are multiple methods of adsorption that can be explored. Three examples include monovalently adsorbed antigens to adjuvant (MBABs), adding the conjugate/antigen blend to the adjuvant, and finally, adding the adjuvant to a conjugate/antigen blend.

The pH of the process can also be an important consideration when defining the order of addition. In addition to protecting the vaccine antigens from damaging microenvironments of low/high pH during component addition, it can also be important for the adsorption of the antigen to an adjuvant. In processes where the pH is modified during the compounding step, special care should be taken to ensure that the vaccine antigens are compatible with pH ranges experienced across the manufacturing process, and that component addition is optimized to ensure the most efficient binding characteristics.

19.4.4.2 Adjuvant Sterilization

The standard means for sterilizing aluminum salts is by thermal exposure, most often autoclaving. As aluminum adjuvants are normally sterilized in large batches, and aluminum salts have a high heat capacity making it difficult for heat to penetrate, it can be challenging to achieve the proper temperature in the sterilization vessel. Thus, it is essential to ensure proper mixing so the heat is uniformly distributed, allowing the entire load to achieve sterilizing temperatures. Exposure to elevated heat and pressure may cause unacceptable changes to the adjuvant, i.e., deprotonation and dehydration, for both aluminum phosphate adjuvant and aluminum hydroxide adjuvant (Burrella et al. 1999). Care needs to be taken in the design of a sterilization cycle so that sterilizing conditions can be achieved without diminishing the quality of the product.

During the formulation process, aluminum adjuvants cannot be sterilized via filtration due to the particulate nature, the formulation process for addition of aluminum may need to be conducted in an aseptic manner. The final product containing antigens may not be amendable to terminal sterilization. This may require special consideration when designing the adjuvant container to ensure that it is compatible with modern close-system aseptic processes. In rare cases where adjuvants are sterile filtered, the properties of the adjuvants may create some challenges during filter selection and bacterial retention validation (Onraedt et al. 2010). For example, the particulate character of some adjuvants can cause premature plugging of filter membranes, and the low surface tension of many adjuvant solutions may contribute to reduced bacterial retention efficiency and potential breach in sterility (Onraedt et al. 2010). Thus, the risk of reduced bacterial retention needed to be considered very early in the process, i.e., when designing the filtration step in the process.

19.4.5 Process Development Case Study—Lyophilization

Lyophilization is a freeze-drying process that is used to increase the shelf life and improve the thermal stability. This technology is especially important for vaccines where complex molecules tend to be more prone to degradation and global distribution/access to regions with limited cold-chain capabilities require a higher degree of thermal stability. The applicability of using a QbD approach for the lyophilization process is generally well-accepted in the industry and by the agencies as compared to other as DP unit operations, as there is extensive industry experience in characterizing these processes (many of which are widely available in literature) along with demonstrated scale down models (Tang and Pikal 2004; Sundaram et al. 2010; Nailand and Searles 2008). Given its importance to vaccine manufacturing, and its favorable disposition for the application of QbD approaches, a fairly rigorous treatment of lyophilization process development is described below, with specific considerations for vaccines.

19.4.5.1 Product Characterization

Two additional CQA's for lyophilized products are the moisture content and the time it takes to reconstitute the product prior to delivery, both of which must be analyzed as a part of the product testing. The product should have a pharmaceutically elegant physical appearance without cake collapse or melt back. Although the cake appearance is not a CQA, it is a key attribute of the acceptability of the product. Specifications for the product moisture are typically defined by stability at the desired storage conditions/shelf-life. Specifications for reconstitution time are primarily defined by the product claim ("rapidly dissolving" claim requires reconstitution in <120 s), the customer (e.g., certain market segment requires reconstitu-

Table 19.5 Biophysical characterization attributes needed for lyophilization product and process development

Characterization attributes	General information
Solution glass transition temperature (T_g')	A function of formulation composition, important for considerations of freezing and drying temperature/pressure, freezing/annealing process, important for primary and secondary drying process design
Product collapse temperature (T_c)	T_c is usually a few degree higher than the T_g'
Dried product glass transition temperature (T_g)	A function of formulation and residual moisture, important for secondary drying ramp rates, temperature, pressure, endpoint, and final storage temperature definition
Eutectic temperature (T_{eu}) and melting temperature (T_m)	A function of formulation composition, important for considerations of freezing and drying temperature/pressure, freezing/annealing process, important for primary and secondary drying process design
Product crystallinity/morphology	A function of formulation composition and freezing/annealing process, can affect collapse temperature when crystallized during freezing, final product moisture when crystallized during storage, final product moisture and reconstitution times

tion in <10 min for pharmacy application), or market (e.g., better than or equal to competitor).

Development of a lyophilized product involves extensive biophysical characterization of the product which help determine the composition of the product as well as the lyophilization process cycle time. Table 19.5 lists the product attributes that are useful for lyophilization formulation and process development.

19.4.5.2 Process Description/Design Principles

A typical lyophilization process includes the following steps in Table 19.6 with an example in Fig. 19.1:

In general, a product should be frozen below its T_g' prior to drying, maintained below T_g' and/or T_c during primary drying, maintained below T_g during secondary drying. The drying conditions (temperature, pressure, and duration) should be designed to consistently deliver a product with acceptable appearance, residual moisture levels below limits required for product stability. In addition, the process should be designed within equipment capabilities for heat transfer, pressure control, vapor flow and condensation capabilities at the laboratory, pilot and commercial scales.

Table 19.6 Typical steps for a lyophilization process

Freezing	Reducing product temperature at near atmospheric pressure, separate crystalline water from formulation solutes, solidify remaining formulation solute matrix. Typical shelf temperature range: −40 to −50 °C
Annealing (optional)	Designed to control solute crystallization (if applicable) or homogenize/increase ice crystal size/pore size of cake by holding product between T_g' and eutectic or ice melt temperature for a defined period of time. Typical range: −30 to −10 °C
Primary drying	Crystalline ice is removed from the product at low pressure through sublimation process. Product temperature is controlled below T_g' and/or T_c by chamber pressure and shelf temperature. Typical Shelf temperature conditions: −30–0 °C, 50–200 mTorr, 15–100 + hours
Secondary drying	Residual bound water in partially dried solute matrix is removed by diffusion/desorption at low pressure, and a controlled increase in shelf temperature. Final product moisture is usually defined by combination of product temperature and duration of this step. Typical conditions: 50–200 mTorr, increase shelf temperature ramp at 0.1–0.5 °C/min to 20–40 °C, hold 5–10 h
Backfill/Capping	Return chamber to near atmospheric pressure using sterile, typically inert gas (nitrogen, argon, etc.). Backfill pressures below atmospheric conditions (e.g., ~10–12 psi) are commonly used to prevent displacement of the stopper during unloading and to aid in reconstitution

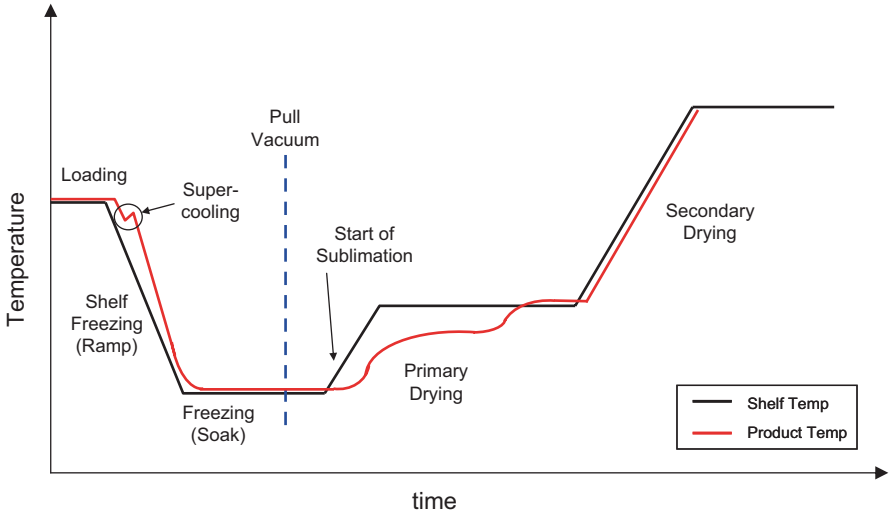

Fig. 19.1 Typical example of lyophilization process

19.4.5.3 Process Sensitivity Screening

In a preliminary risk assessment, the sensitivity of the product to each step of lyophilization should be studied. Considerations for these studies include:

Freeze Sensitivity During the freezing step, the active product will be exposed to ice/solute interfaces which can cause structural changes in large molecules and a highly concentrated solute environment that may impact stability. In some cases, the concentrated solute environment may also be associated with a shift in pH for crystalline buffer species that can also impact product stability. For this reason, crystalline buffers (high concentration of phosphates for example) may not be an optimal choice for pH sensitive products. The amount of time that the product is exposed to these aggressive conditions (between freezing point and T_g') is based on the method/rate of freezing. Studies to explore freeze/thaw sensitivity should include multiple temperatures and freezing rates (blast freezing versus shelf freezing).

Moisture Isotherms As stated previously, the final product moisture tends to be a function of product temperature and hold times in the secondary drying, as well as freezing and primary drying. Studies should be designed to define this correlation (e.g., lyophilizing product at various secondary drying temperatures and sampling over time).

DoEs to Screen Lyophilzation Process Parameters Although some process interactions are well documented in literature, product behavior during lyophilization is not always intuitive making *a priori* predictions of product sensitivity very difficult. In addition to the studies described above, partial factorial DoE designs powered to detect at least main effects and secondary interactions are recommended to evaluate the potential impact on product CQAs. Parameters that need to be explored in these studies should be guided by the risk assessment. These parameters may include: formulation composition/concentration of critical excipients, product fill volume, freezing method/rate/temperature, annealing temperature/time, primary drying temperature/ramp rate/pressure/duration and secondary drying temperature, ramp rate, and pressure/duration. Depending on the study size and the read out from the analytical methods used, these studies can be quite large (30–40 runs or more). As a result, such studies are usually executed at a laboratory scale. The outcomes of these studies help create a preliminary design space and/or identify areas for further development with higher resolution DoEs focusing on critical lyophilization parameters.

19.4.5.4 Equipment Characterization

The performance of a lyophilization process is highly dependent on equipment design and scale. The equipment design determines the capability of the equipment to control temperature/pressure, rates of heat transfer to the product, and rates of mass transfer of water vapor from the product. As most process development occurs in

laboratory scale equipment, it is important to understand and account for each of the differences during process development and scale-up. In general, the temperature and pressure control capabilities of lyophilization equipment are well understood and characterized by standard equipment qualification activities. It is important to incorporate the limitations of temperature/pressure control in pilot and commercial scale equipment into the process design, and to accommodate for the typical variability in pilot/commercial equipment in laboratory scale experiments.

The major equipment effect is heat transfer to the product. The primary means of transferring heat to and from the product during lyophilization is via the lyophilization shelf. Traditional lyophilization shelves are hollow, with channels arranged in a serpentine pattern through which a heat transfer fluid (usually a silicon-based oil) flows. Heat transfer via the shelf is controlled by the temperature and flow rate of the heat transfer fluid through the shelf system. Factors that influence heat transfer from the fluid to the product in this system include the shelf design (thickness and conductivity), the type of tray used to load the product (standard vs. perforated vs. bottomless). Heat transfer can also be impacted by the type of product container (vial vs. syringe), the design of the product container (such as thermal properties of the container material, wall thickness, and concavity of vial). In addition, available shelf space can also influence the heat transfer, i.e., peripheral vs. center location, contact of container with shelf. A secondary mechanism of heat transfer to the product is from other lyophilizer surfaces (mostly by radiation), specifically from the chamber walls, adjacent shelves, and vertical tray surfaces. The relative contribution of these combined sources of heat transfer can vary greatly by location in a single lyophilizer (center vs. edge location of a shelf, top shelf vs. bottom shelf, etc.) as well as between different pieces of equipment. For example, as shown in Fig. 19.2, the heat transfer may not be homogeneous in a small laboratory scale lyophilizer and can be as much as three times between the locations between the center and edges. There are various approaches to this characterization, which

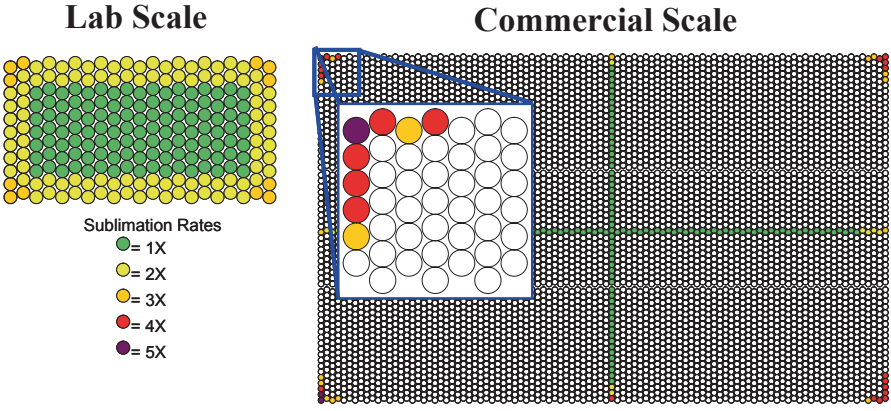

Fig. 19.2 Examples of sublimation rate difference at various locations in lab scale and commercial scale lyophilizers

include gravimetric assessment of sublimation at various locations and time points throughout a cycle. This lack of homogeneity can increase to five times or more in a commercial chamber with a similar process. Characterization of heat transfer and its variability is a critical part of developing a successful lyophilization process design, scale-up, and transfer.

In addition to heat transfer variability within/between lyophilization equipment, differences in mass transfer of water vapor from the product can also be very important. The sources of mass transfer resistance in a traditional lyophilization system are dried product cake pore structure/size, dried layer thickness/fill volume, which is dynamic throughout the cycle. Mass transfer resistance is also impacted by the product container neck design, container closure/stopper design/placement. Furthermore, the spacing between lyophilization shelves, geometry of flow path between shelves and condenser (shelf spacing, spool piece diameter/length), condenser design (internal vs. external, coil vs. place), and condenser temperature/efficiency also influence the mass transfer resistance. Similar to heat transfer in homogeneities, characterization of these mass transfer resistances are critical to successful lyophilization process design, scale-up, and transfer. Specific studies might include assessments of mass transfer resistance of the product at different freezing conditions, pressure gradients at different locations in the chamber for a representative cycle. In addition, it is also useful to have a definition of choke flow conditions at maximum sublimation rates, which is a result of flow restriction between chamber and condenser, well documented in literature. Further, specific studies might include assessment of the dynamic condenser capacity challenging the maximum amount of water than can be removed from the chamber over a defined period of time per surface area of condenser (Tang and Pikal 2004; Sundaram et al. 2010; Nailand and Searles 2008; Schneid and Gieseler 2011).

19.4.5.5 Modeling, Scale-up, Tech Transfer, and Validation

After obtaining an understanding of the product sensitivity and extensive characterization of the equipment across scales, the target manufacturing process and the design space can be developed. The design space is based on the vast process knowledge accumulated at the laboratory scale, expected performance at the commercial scale, and the various aspects of process performance that can be impacted by equipment changes. This can be most efficiently accomplished by application of another tool advocated by the QbD approach: using scaled-down models. Expectations for the application of scaled-down models are described in ICH Q8 and subsequently issued appendices on frequently asked questions for QbD implementation (Sundaram et al. 2010; Pikal 1985; Koganti et al. 2011; Fissore et al. 2011; Giordano et al. 2010; Kramer et al. 2009). For the most part, these models are parametric equations based on the steady state mass and heat transfer balances during primary drying, with the primary output being a prediction of product temperature and sublimation rate over time as a function of shelf temperature, chamber pressure,

container heat transfer coefficients, and dry cake mass transfer resistance/cake pore size estimates.

The heat transfer differences between equipment and scales could be accounted for mathematically, and the design space demonstrated at the laboratory scale can be "translated" to a corresponding commercial scale design space using the available inputs product and equipment characterization. Ideally, optimum process conditions could be identified by positioning the target process in the center of the translated design space, providing the basis for preliminary scale-up/engineering batches. In some cases, commercial scale batches may be required to gather modeling inputs (e.g., dry cake resistance, Kv, etc.). The success of scale-up has been greatly increased by maintaining consistent product quality across various pieces of equipment.

19.4.5.6 Control Strategy and Process Analytical Technologies (PAT)

As the lyophilization process is being designed and scaled up, continuous iterations of the risk assessment should be used to define a control strategy for the process. This control strategy might include elements of raw material control and screening, preventative maintenance for equipment, routine assessments of performance (leak rates, temperature/pressure control, etc.), operator training, and many others. In some cases, the use of PAT can also be considered to supplement a more traditional control strategy. QbD application here would be running a "challenge cycle" at the edge of the design space during development or process qualification for the purposes of confirming process robustness, or to assess the potential impact of process variability in support of process deviations. Many applications for PAT/process monitoring are available for lyophilization (Fig. 19.3), including:

Product Thermocouples Historically, thermocouples placed in product containers are used to confirm product temperature throughout processing and detect endpoints of certain stages of lyophilization. While it remains a valuable tool in development and scale-up, usage in routine manufacturing is becoming less common due to broad acknowledgment of limitations in the accuracy of data (sensitive to highly variable positioning of thermocouple in product container by human operator, possible impact of thermocouple on freezing behavior), potential impact on sterility (difficult to position thermocouple in product container using aseptic practices without "breaking first-air," only able to place thermocouples at locations near the edge of the shelf/tray limits a full representation of the entire lot/center locations), and increased usage of automated loading in modern facilities which prevent the manual placement of product thermocouples.

Headspace Concentration Monitoring To supplement/replace the use of thermocouples, a number of methods have been developed to detect the presence/concentration of water vapor in the lyophilizer headspace. These tools are valuable to detect the endpoint of the overall drying process (when completion of sublimation results in a decreased concentration of water vapor in the chamber headspace) but

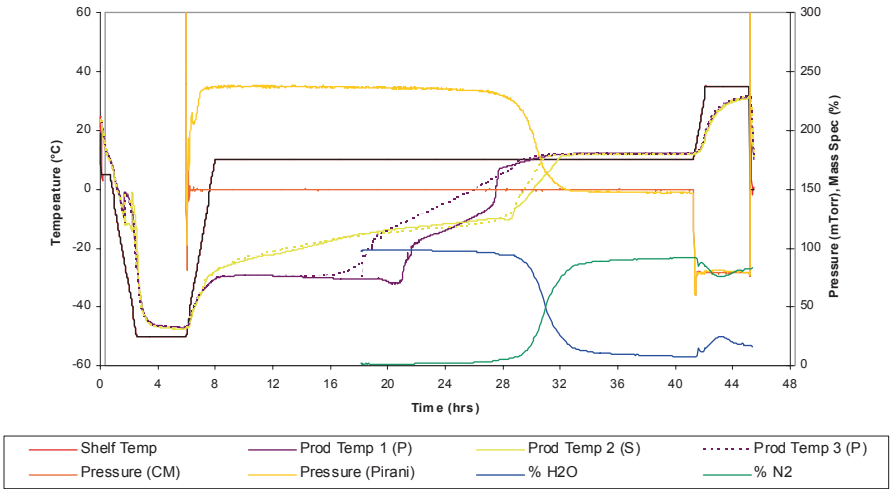

Fig. 19.3 Example of drying endpoint by various methods. Plot provided demonstrates Primary Drying Endpoint by multiple methods of detection. These include thermocouples (Prod Temp vs Shelf Temp), Pressure (CM vs. Pirani), and Head Space composition (%H2O vs. % N2)

have limitations in representing local effects within the chamber or drying behavior prior to process endpoints. Available options range from very simple to relatively sophisticated applications, including relative humidity probes, Pirani gauges (pressure measurement calibrated for a specific gas, usually nitrogen), and mass spectroscopy (monitoring relative levels of H_2O and N_2 or Ar)

Sublimation Monitoring Of increasing popularity are PAT tools capable of monitoring the overall sublimation rate in the system throughout the entire drying process. In addition to simply detecting endpoints, these tools are capable of representing process behavior during manufacturing. This adds a tremendous value during development, equipment characterization and process scale-up, and technology transfer. Available options again range from low-tech pressure rise tests, manometric temperature monitoring MTM (requires ability to quickly isolate lyophilization chamber from condenser and rapid acquisition of pressure increase in chamber) to sophisticated near-IR measurement systems mounted between the chamber and condenser (such as tunable-diode laser absorption spectroscopy) (Brulls et al. 2003).

Single Container Monitoring Less commonly used methods of monitoring individual product containers have also been explored for lyophilization process characterization, such as microbalance gravimetric methods (sublimation monitoring) and in-process NIR/FTIR (product moisture, conformation testing). While these can be highly valuable analytical tools in a laboratory setting, a number of limitations have prevented widespread use at the commercial scale.

19.5 Conclusions

The development of a vaccine is expensive and complex as it requires the products to be safe, efficacious with robust and scalable processes utilizing different technologies based on the biology and type of vaccine (examples are live viruses, recombinantly expressed proteins including microbially expressed VLPs, polysaccharides, polysaccharides conjugated to a carrier protein, inactivated virus or DNA). In addition to the technical challenges there are analytical challenges to well characterize the DP and monitor stability (i.e., multicomponent, lack of well-defined preclinical models that may not always correlate to human immunogenicity, high degree of variability in animal responses to correlate in vitro/in vivo potency).

Historically, the process of producing the vaccine itself has defined the product or that the "Process is the Product." The process of technology development for scale-up must be implemented by also taking into consideration the regulatory environment and oversight. While the application of full QbD currently may not be feasible, it can be used to develop a robust final product where applicable. QbD can be instrumental in improving process efficiency which will lead to reducing cost, reduce time to market, improve quality and consistency, increase flexibility, and lower operating costs structure needed for global. At this stage, it is unlikely that a full QbD approach can be implemented for filing in the near future, however specific applications and "hybrid" files are expected to be the norm in the future.

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Chapter 20
Automation and High-Throughput Technologies in Biopharmaceutical Drug Product Development with QbD Approaches

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20.1 Molecule Assessment (MA) and Engineering

Manufacturability, or the ability to sustain the target quality product profile in typical manufacturing related stresses, is an important factor in the selection of the best candidates moving into the early development phase. Molecule assessment (MA), also called manufacturability assessment, is an excellent tool to identify candidates that are more likely to fit manufacturing criteria, and eliminate those with the higher risk to fail. This procedure can lead to significant investment savings in the long run considering the ever increasing development and clinical costs. It can also help to verify the effectiveness of molecular engineering, often through site-directed mutagenesis, to enhance the stability of the fragile or less stable modalities against the manufacturing stresses.

20.2 Computational Methods

During molecular assessment it is still possible to reengineer a drug candidate to improve the properties that are important for development. Many adverse characteristics of candidates can be spotted in early screenings by sequence, modeling, and crystal structure analysis. Many chemical degradation pathways for proteins are sequence dependent. For example, in monoclonal antibodies, the most common modifications include C-terminal processing of lysine residues, (Santora et al. 1999) N-terminal pyroglutamate formation (Chelius et al. 2006), deamidation (Hsu et al. 1998), glycation (del la Guntin˜as et al. 2003), and oxidation (Junyan et al.

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2009). Some of those modifications could be predicted by sequence and structure analysis with different levels of complexity and reliability. So-called hot spot analysis can be applied to very early stages of candidate selection. In addition to chemical modifications, aggregation propensity of proteins has been studied in order to establish the correlation between protein aggregation and structural features affecting such physicochemical properties as hydrophobicity, unfolding temperature, charge distribution, and others. Different computational algorithms have been developed to predict aggregation propensity using sequence analysis. In many cases, the aggregation behavior of proteins appears to be dependent on the presence of selective short regions with high-aggregation propensity (Castillo et al. 2011). Many prediction algorithms have been developed during the past decade to perform analysis based on this assumption (Tsolis et al. 2013). Other prediction methods have also been described in many publications recently reviewed by Magliery et al. (Hamrang et al. 2013). Protein aggregation stability remains a difficult problem, solution of which requires not only a computational approach but also high-throughput experimental methods that could take advantage of large data sets. We will describe this in detail later in this chapter. Another critical property of therapeutic proteins is increased viscosity at high concentration. High viscosity can be detrimental to drug product manufacturing and delivery processes. Selection of candidates with low viscosity in the early stages of development is often difficult since there is not much protein available to reach the concentration of interest at which high viscosity is observable. There are various methods to measure viscosity in small volumes. Some of them are automated and high throughput (Jezek et al. 2011; He et al. 2010a). Recently there have been several attempts to establish the correlation between charge distribution on the surface of an antibody molecule and high viscosity (Yadav et al. 2012). Our ability to predict the consequences of even a single mutation is still very limited. Protein engineering based on sequence analysis and computerized prediction of degradation mechanisms is promising but a lot of work needs to be done to fully implement those prediction algorithms in a real drug development process.

20.3 Automation Technologies

There are many challenges faced when using MA, including limited or no knowledge of molecular properties, impact of degradation products on potency and safety, lack of toxicity data, and no clinical results. Often, the most challenging aspect is the fact that there is a very limited supply of poorly purified protein samples. Small amounts of material in the early stages of product development force the investigators to limit the scope of MA with a relatively small number of samples or assays. Automation and high-throughput technologies can significantly improve early development by implementation of methods capable of handling many samples and using small volumes in a short period of time. In addition to small volumes and short operating times, automated methods also can provide high accuracy and reproducibility of measurements to avoid human errors. When well maintained and

regularly calibrated, automated processes can significantly improve the results of MA (Taylor et al. 2002). Liquid handling systems manufactured by companies such as Tecan Group Ltd. (Männedorf, Switzerland), Eppendorf (Hamburg, Germany), and Hamilton (Reno, NV) can be used to prepare samples containing both multiple candidates and multiple formulations. The required sample volumes may be as low as 1 μL, which is extremely useful in MA studies when only a small amount of material is available. In addition to the ability to prepare many samples these systems can include various platforms for stress applications and measurements. Therefore, it is possible to create an automated continuous workflow starting from sample preparation and completed by the set of characterization results obtained by different assays. A large variety of automation friendly sample holders are available for quick physicochemical characterizations and formulation screenings (Majors 2005). The majority of these systems is 96-well plate based and require only a small amount of material. They also follow the Society for Biomolecular Screening (SBS) footprint (American National Standard Institute 2004) and are readily adaptable for Automation and High-Throughput Technologies AHT applications. One note of caution is that the plates used in screening can have a surface chemistry, geometry, and other properties very different from the final drug product containers. If it is not evaluated early, formulation screening or any other studies can be affected. These surface effects may be less pronounced in quick turnaround screening but they can be more significant in tests with extended holding periods such as long term stability studies.

A mini vial system, suitable for early-phase development was developed to address potential surface concerns, where: (1) The selected glass insert has the same chemical composition and surface characteristics of the commonly used borosilicate type 1 glass vial with stoppers coated with Fluro Tec®; (2) it follows the SBS footprint of a typical 96-well plate format, and is readily adaptable to liquid handlers for liquid transfer and plate duplication for multiple assays; (3) individual mini vials can be removed and inspected if desired. This 96-vial containment system is shown in Fig. 20.1 and the results of its comparison with the commonly used single vial platform are shown in Fig. 20.2. These comparative data show that the mini vial containment system yields comparable results to the single vial platform.

20.4 High-Throughput Methods of Characterization and Analytics

MA is the perfect application for high-throughput methods developed to characterize biopharmaceutical candidates. As previously mentioned, there are different degradation pathways during manufacturing, storage, and delivery of pharmaceuticals. Generally, three types of stabilities can be considered: chemical, conformational, and colloidal. Mapping of various candidates in coordinates of different types of degradations not only rank the candidates but also can determine the weakest point to address in development. It is hard to predict exactly what kind of degradation will be prevalent for a specific protein and what candidate will be most stable under

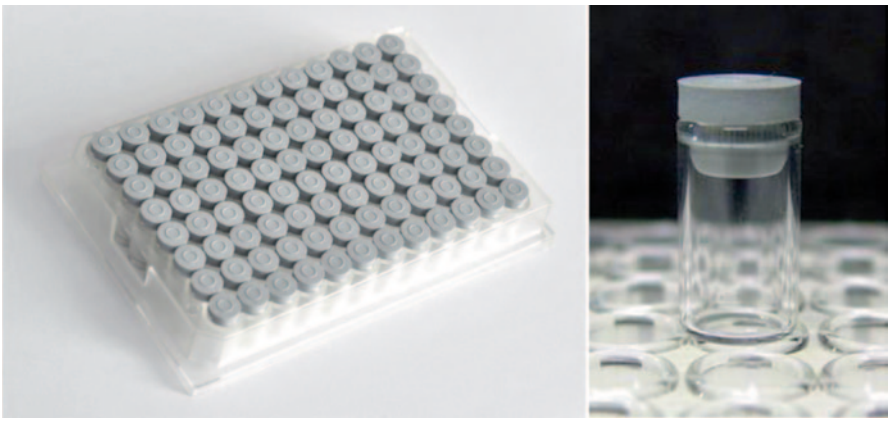


Fig. 20.1 *Left*: The mini vial containment system with SBS footprint and 96-well format are automation friendly and can be readily used in common liquid handling platforms. *Right*: An individual mini containment consisting of a borosilicate type 1 glass mini vial and a stopper made from butyl rubber with Fluro Tec® barrier film coating

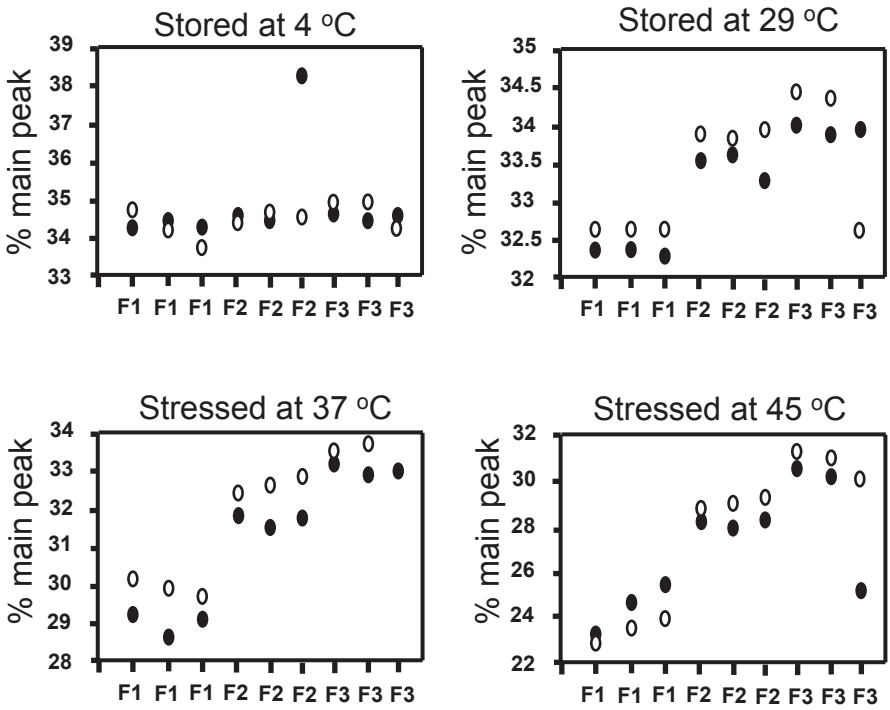


Fig. 20.2 Preservation of the CEX-HPLC main peak (primary glyco-isomer) of a monoclonal antibody in a formulation screening study, for formulation F1, F2, and F3, using the mini vial container system (*black circle*) matches well with the regular vials (*white circle*), except two outliers at 4 °C for F2 and 45 °C for F3. The utilization of this containment system allows more studies to be conducted using available materials with less concern regarding containment comparability. The system can be easily integrated into automated protocols of all widely used liquid handling platforms, which can significantly reduce sample handling and analytics-related resource requirements

manufacturing conditions. Most candidates are generally stable under short-term ambient conditions after purification. In many molecular assessment cases it is necessary to apply the relevant stress to distinguish the more stable candidates from less stable ones.

Some chemical modifications can be predicted based on amino acid sequence. Well characterized hot spots are easy to localize during protein engineering process and, if it is not possible to remove them, they can be controlled by formulation since the mechanism of degradation is generally known. Manufacturing, storage and delivery conditions are usually mild enough to maintain chemical stability of the most biopharmaceutical products. External factors can be relevant: low or high pH, the presence of active reagents such as peroxides, enzymes, and leachables. The exposure to light, heat, or container surface can also cause chemical reactions. The main problem in screening for chemical modifications is the lack of high-throughput sensitive methods. Many analytical methods are based on different types of chromatographic or electrophoretic separation: reversed phase, hydrophilic and hydrophobic interaction, ion exchange, electrophocusing, capillary electrophoresis, etc. (Ahrer and Jungbauer 2006). Chromatographic conditions could be very specific to the studied molecule and it normally requires some development time to obtain good resolution, sensitivity, and accuracy for the method. Recent advances in the developments of new resin material, smaller bead sizes, higher pressure pumps, and application of automated sample handling have allowed faster analysis time, without losing analytical quality, for many chromatography-based assays. One of the common degradations monitored during process optimization, formulation development, and stability studies is clipping of polypeptide chains. Typically, reversed phase high-performance liquid chromatography (RP-HPLC) can be used to quantify this type of product-related impurity; however, the run time for standard RP-HPLC is too long to be used in fast screening. A much shorter run time was achieved using an ultra-performance liquid chromatography (UPLC) system with a 1.7 μm phenyl column (Stackhouse et al. 2011). This RP-UPLC method allowed quantitation of molecular clipping in an IgG1 molecule and an acid induced aspartic acid/proline clip in an IgG2 molecule. The results from the UPLC method were comparable to those obtained with reduced capillary electrophoresis. Oxidation and other chemical modifications can also be detected, making this technique attractive for high-throughput characterization and formulation screens.

The gold standard for characterization of protein modifications has long been peptide mapping. Normally, this procedure, including both sample preparation and the measurements, can be very laborious and require a lot of time. A fully automated proteolytic digestion method has been developed to assist in stability studies, identity assays and quality control of therapeutic proteins (Chelius et al. 2008). The Tecan Evo 100 liquid handling system has been used to place antibody samples in a 96-well plate or in 0.5-mL Eppendorf tubes. The protein was then reduced and alkylated. The denaturing solution was replaced with a digestion buffer using a custom-designed 96-well size-exclusion plate for desalting. The samples were digested for 5 h with trypsin. The results of automatic digestion were compared to a manual digestion procedure. The completeness and reproducibility of digestion were verified by reversed-phase liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis of the digestion products. Peptide mapping can also be

performed by multiplexed capillary electrophoresis (Kang et al. 2000). Combination of charge and size separations was used in different channels and in a 96-capillary array. Fragments of digested proteins were readily resolved and analysis was completed within 45 min. In another example, direct infusion was used for characterization of breakdown products of monoclonal antibodies (Mazur et al.). The automated nano-ESI chip system, the TriVersa NanoMate, by Advion (Ithaca, NY) sufficiently eliminates the liquid chromatography step and can significantly accelerate mass spectrometry-based protein analysis. In most cases of drug product development, chemical degradation is not a major concern with protein solutions stored at low temperature, sometimes frozen or in lyophilized form. Even under conditions of manufacturing and purification most impurities are original posttranslational modification variants or host cell proteins and not the stress-induced chemical degradations.

The primary degradation pathways during manufacturing and storage processes are related to the conformational and colloidal stabilities of therapeutic proteins. The most immediate consequence of unstable structure or poor solubility is the formation of soluble aggregates and/or large particles. The problem of aggregation creates a significant barrier to product development. Recently, a notable amount of research has been dedicated to aggregation studies and rational design of safe and efficacious protein-based biopharmaceuticals using the QbD concept (Walsh 2010; del Val 2010; Rathore and Winkle 2009). It is important to create a proper design space for the factors affecting aggregation such as formulation or molecular properties. Protein aggregation is a complex process, which may involve different mechanisms, and various factors are responsible for aggregation propensity. Depending on the mechanism there are different sizes of aggregates ranging from simple dimers, soluble oligomers to large visible particles and even continuous phases such as liquid, gel, or solid precipitates. Each size range requires different analytical method for characterization. Several orthogonal methods to characterize and quantify protein aggregation are currently implemented in biopharmaceutical development (Zolls 2012; Mahler 2009) and many can be automated, modified, and developed to run in high-throughput format (Razinkov et al. 2013).

High-performance liquid chromatography (HPLC) is a common tool for protein characterization and determination of biopharmaceutical product quality. Recently, UPLC has been developed to match the resolution and sensitivity of conventional HPLC setups allowing high-throughput analysis of small volume samples. Under the constraints of time and material during the MA process, size exclusion chromatography (SEC) is widely used for fast evaluation of protein aggregation. Using UPLC methods along with new column resins, the run time for SEC can be reduced to 5 min without losing resolution between the monomer and dimer peaks of monoclonal antibodies. The SEC assay has been known for dissociation of reversible aggregates induced by a dilution and possible interaction with the column material. Also large aggregates are normally filtrated by the column. Dynamic light scattering (DLS) is well suited for the subvisible particles of sizes from 100 to 1000 nm. DLS measurements can be performed by a plate reader using as many as 384 wells per plate and as low as 20 μL per well. Analysis of a 96-well plate can be completed

in 2 h or less. The common problem is that DLS is a semiquantative method and the signal from large particles can easily overwhelm the signals from small particles. But this method is reliable in distinguishing the highly particulated samples from the homogeneous ones and can be used for the screening out the worst candidates. Another relatively new method to characterize subvisible biopharmaceutical aggregates is microflow imaging (MFI). MFI is a well established method to count particles and to sort them out according to morphological characteristics. It has been applied in the processes ranging from the detection of particles in formulations (Ludwig et al. 2011; Mach et al. 2011) to counting cells in bioreactors (Sitton and Srienc 2008). Unfortunately, it requires a relatively large volume of sample and significant time to run the experiment. MFI instruments are now available with an autosampler so that measurements can be simplified and automated. In the MA process MFI is used to evaluate the protein's propensity to form subvisible particles after applying various stresses such as mechanical agitation or pH changes.

Short-term exposure to high temperatures is used to explore conformational stability and, specifically, to determine the protein unfolding temperature. The ability to unfold at increased temperature might not be directly correlated to the long-term storage stability under low temperatures but it could be an important property indicating an energy barrier necessary to overcome the structural integrity of a protein. This barrier can be breached by not only temperature but also by chemical means such as low pH or the presence of salt in the formulation. The typical assay to determine the unfolding temperature of a protein is differential scanning calorimetry (DSC), a method which requires a significant amount of protein and takes a relatively long time to finish. DSC is not well suited for the early assessment of many candidates in multiple formulations. Several high-throughput methods have been developed to evaluate protein conformational stability. One method uses the extrinsic fluorescence of chemical probes that are sensitive to hydrophobicity. Under increasing temperature the protein starts to unfold exposing hydrophobic regions of the protein making them more accessible to the probe. Fluorescence increases as the protein unfolds. A typical experiment can be performed using a standard RT PCR instrument equipped with a 96-well plate holder, thermostat, and fluorescent detector appropriate to the fluorescent probe. Originally the method was applied to screening of protein-small molecule interactions and has been called differential scanning fluorimetry or the thermal shift assay because there is a shift in the unfolding temperature when a ligand binds to protein (Pantoliano et al. 2001). The method has been adapted to perform high-throughput screening of protein formulations (He et al. 2010b). In Fig. 20.3 the fluorescence traces of 96 antibody formulations are shown using the fluorescent probe Sypro Orange (Molecular Probes, Eugene, Oregon). There the temperature of unfolding or the temperature of hydrophobic exposure is determined by the minimum of the first derivative for the fluorescence versus temperature function.

Another method detects the temperature when proteins form aggregates by combining the gradual heating of the sample and simultaneous measurement of the light scattering signal. Custom made multi-well plates are used in the OPTIM 2 instrument manufactured by Avacta Analytical (Wetherby, UK) to screen 48 small volume

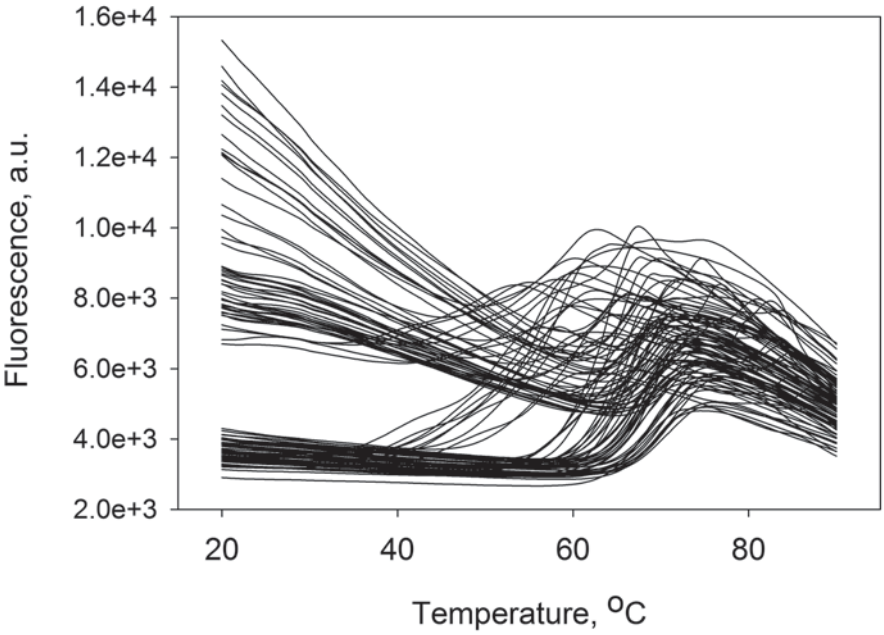


Fig. 20.3 Fluorescence signal of Sypro Orange dye from 96 formulations of monoclonal antibody during temperature scanning by DSF. *DSF* differential scanning fluorimetry

samples simultaneously for both melting temperature and aggregation temperature, although in many cases, those temperatures are close. Colloidal stability is a more difficult parameter to evaluate and predict than conformational and chemical stabilities. Colloidal instability of therapeutic proteins is often related to the phenomena of large particle formation, precipitation and liquid–liquid phase separation. The mechanism of protein self-association followed by aggregation or phase separation can be explained by the interaction between native molecules, without any conformational changes involved. Although, sometimes, it is hard to completely separate the cases of native and nonnative mechanisms. There are several ways to screen for colloidal stability and aggregation caused by colloidal interactions. Some of them are described in the above paragraphs devoted to the methods of aggregation measurements such as DLS or other assays for particle characterization. For example, in the work by Goldberg et al. (2011) a static light scattering method was applied and the colloidal stability was measured using a static light scattering plate reader, the StarGazer-384 (Harbinger Biotechnology and Engineering Corporation, Markham, Ontario, Canada). Twenty five microliters of prepared samples were added to a 384-well plate and heated at 70 °C. Protein aggregation was monitored by measuring the intensity of the scattered light with a CCD camera. The difficulty is not in the measurement of aggregation, particulation, or phase separation but in the prediction of protein colloidal stability under specific conditions of purification or after a long storage time at low or freezing temperatures. Mechanical agitation can be a predictor of colloidal stability and, at the same time, can simulate some conditions

of transportation (Fesinmeyer et al. 2009). Agitation induces protein exposure to the liquid/air interface and can result in conformational changes, including unfolding. Samples can also be agitated on multi-well plates to fit a high-throughput format. Another parameter that can be used to estimate self-association propensity is the second virial coefficient, which has been used to characterize solubility, aggregation, and crystallization of proteins (Valente et al. 2005). The measurements have been modified to run on DLS plate readers using 96-well plate. Instead of measuring the second virial coefficient, the method can determine the interaction parameter by using the dependence of diffusion coefficient on protein concentration (Saluja et al. 2010). Recently, a method based on a simple and old-fashioned precipitation technique, has been developed to predict long-term storage stability of monoclonal antibody formulations at low temperatures (Banks et al. 2012). The data from the stability studies of 11 months at 4 °C and 6 months at 29 °C were used to correlate with the ammonium sulfate concentration necessary to precipitate antibodies at different temperatures. A linear correlation was obtained to rank antibody formulations, containing different excipients, according to formulation ability to be soluble in the presence of ammonium sulfate. Ammonium sulfate precipitation has recently been used in high-throughput format for aggregation propensity screening of multiple classes of protein therapeutics (Yamniuk et al. 2013). Other precipitating agents such as polyethylene glycol (PEG) can also be used to test protein solubility. A multi-well plate and PEG-based precipitation method has been adapted to compare antibody preparations and to rank order buffer and pH conditions during formulation development (Gibson et al. 2011). The high-throughput PEG methodology was applied to the screening of different formulations to optimize protein solubility in terms of solution pH and buffer ions for both human and chimeric IgG1 monoclonal antibodies.

20.5 Formulation Development

Formulation development is arguably the most fundamental and critical step in drug product development because formulation is the only in situ protection for the majority of drug product critical attributes (PQA) from the point of drug product manufacture to the point of administration. Formulation development is the process of screening and selecting the most stabilizing composition and container to store and deliver the active component. This process is based on an understanding of the therapeutic molecule, its susceptibility to foreseeable or unforeseeable stress conditions during its production, distribution, and end use. A comprehensive formulation screen covers a large number of product attributes, including physical properties of the drug product, e.g., drug product appearance and viscosity, and the physical and chemical instabilities induced by manufacturing, distribution, and end use, e.g., vibration, drop-and-shock, radiation during safety check up, shelf storage temperature, etc. In the QbD process all those attributes can be considered as output parameters which are dependent on external stress and internal formulation factors. Various formulation studies are designed to establish the correlations between the desired drug product characteristics and all possible factors involved in the lifetime

of the drug before its delivery to a patient. A typical protein formulation consists of buffer for pH control, excipients against denaturation or for cryo- or lyoprotection, surfactants against interfacial degradations, viscosity reducers, and preservatives for multi-dose formulas. The FDA's *Q8(R2) Pharmaceutical Development* regulation document requires a rationale behind the selection of the best formulation. In addition to prior development, production knowledge, molecular assessment, and clinical experiences, a large number of experiments and testing are needed to fulfill that FDA requirement. Automation and high-throughput technology can significantly accelerate formulation development by performing all necessary experiments faster and with a smaller amount of material. Ideally, formulation components should be screened for the quality target product profile (QTTP) determined as a prospective summary of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product (according to ICH Q8(R2)). While it is desirable to conduct multivariate experiments, all at once, to truly reveal the interactive nature of drug product components, it is not practical due to the size of the formulation matrix and the required resources and material. In practice, formulation screening is often conducted in a scaled-back approach with fewer but carefully selected concurrent variables including formulation components, their concentrations, and the stress conditions employed. The choice of these variables is based on prior knowledge and development experiences. The principles of DOE and statistical analysis have been applied widely to formulation development. The major advantage of using DOE to develop formulations is that it allows all potential factors to be evaluated simultaneously, systematically, and quickly. The effect of each formulation factor on each response and the interactions between factors can be evaluated and statistically significant factors can be identified. Once the significant factors have been identified, the optimal formulation can be defined by using proper levels of all factors and the correspondent responses. In one DOE application 81 antibody formulations were screened measuring two responses, thermal stability and viscosity to optimize them in highly concentrated protein formulations (He et al. 2011a). As mentioned previously in the MA section, high-viscosity values at high-protein concentration and low-thermal stability can be a problem for drug product development. Some factors can improve the thermostability while increasing viscosity and vice versa. For example, the presence of salt is a significant factor in decreasing viscosity, but at the cost of decreased conformational stability. In this study two high-throughput biophysical methods were used to measure those critical parameters encountered in formulation development. Thermostability was determined by differential scanning fluorimetry (DSF) and the viscosity of the protein solution was measured by a DLS-based method using a 384-well plate and a dynamic light scattering plate reader. The temperature of hydrophobic exposure T_h under unfolding conditions was used as the thermostability parameter. Based on DOE and experimental results prediction models were constructed for T_h and viscosity to establish a statistically significant correlation with the formulation factors. Contour plots of protein concentration versus pH for six different combinations of categorical factors are shown in Fig. 20.4. The low limit for T_h and high limit for viscosity were set at 50 °C (blue line) and 6 cP (red line), respectively, to show the

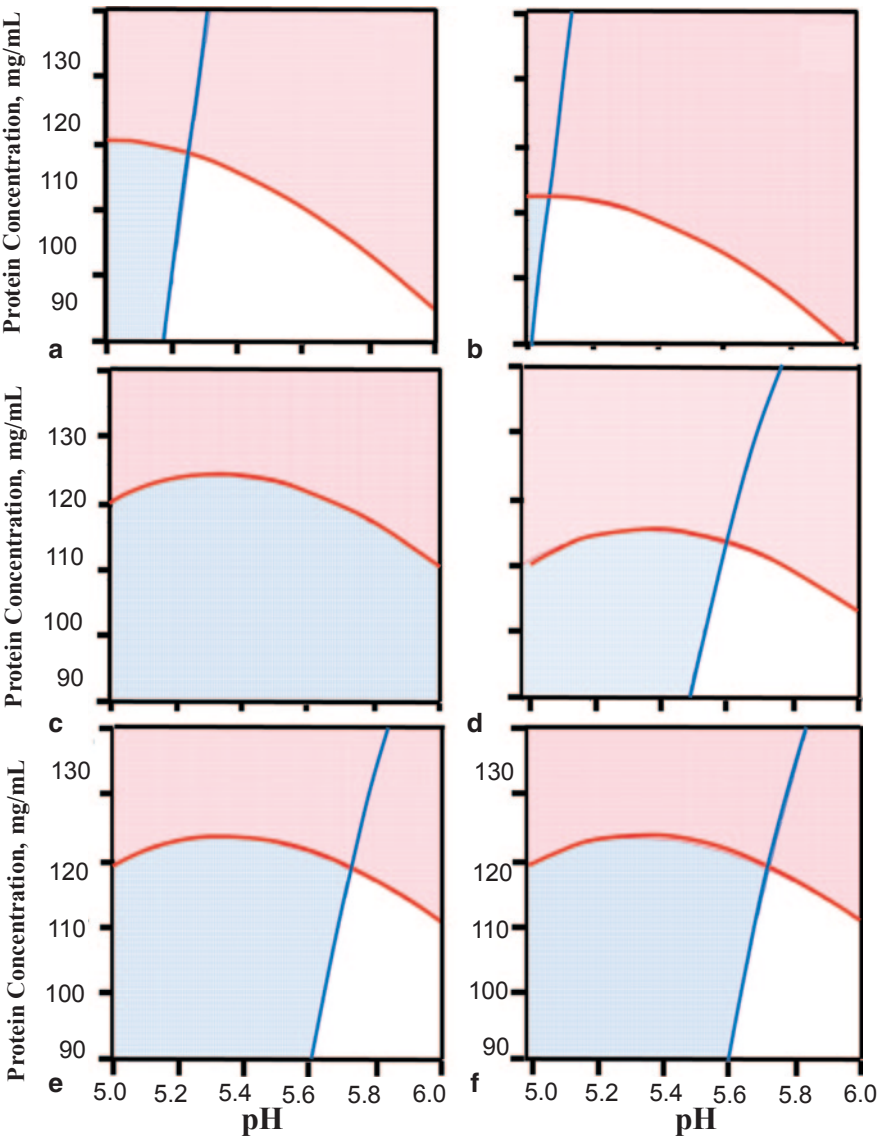


Fig. 20.4 Contour profiles derived from prediction formulas for T_h and viscosity values. The area in white is shown as acceptable at particular limits for T_h and viscosity values. The low T_h value limit is 50 °C and the high-viscosity value is 6 cP. Area with acceptable pH and protein concentrations is shown for the following formulation: **a** no ions + no excipients, **b** no ions + sucrose, **c** Ca^{2+} ions + no excipients, **d** Ca^{2+} ions + sucrose, **e** Mg^{2+} ions + no excipients, and **f** Mg^{2+} ions + sucrose. T_h temperature of hydrophobic exposure. (Reprinted from (He et al. 2011a) with permission from John Wiley and Sons)

borders for formulation space defined by thermal stability and viscosity. The areas of unwanted values are shown in blue and red correspondently. The area of desired stability and viscosity under defined formulation conditions is shown in white. According to plots addition of sucrose expands the optimal formulation space to the lower pH values (Fig. 20.4b), if compared with the formulations without excipients and ions (Fig. 20.4a) while the addition of Ca^{2+} ions, which decreases viscosity, eliminates any possibilities for formulation with T_h higher than 50 °C (Fig. 20.4c). Addition of sucrose to antibody formulation with Ca^{2+} ions returns the optimal formulation conditions at pH > 5.5 and protein concentration below 115–105 mg/mL (Fig. 20.4d). The similar effects are observed on the samples with Mg^{2+} ions (Fig. 20.4e, f). Protein thermostability and solution viscosity were selected in this study as output parameters to demonstrate the utility of DOE as a means of assessing multiple input factors. Once the significance of each factor is identified and the desired ranges of output parameters are set, the prediction model can be used to map the formulation space for further development.

Another example of a DOE application is presented in the case of formulation screening for thermal and colloidal stabilities (He et al. 2011b). These two types of stabilities are important parameters in protein characterization and can be affected by formulation factors. An ideal formulation should provide both thermal stability and colloidal stability. However, similar to the previous example, some factors may have opposite effects on protein conformation and propensity of native self-association. In the study, high-throughput screening for two protein stabilities was performed by DSF, to determine T_h, thermal stability, and by differential light scattering (DLS), to determine the diffusion interaction parameter, *kD*, colloidal stability. To determine the range of optimal stability values the antibody propensity to aggregate was evaluated by two types of stresses: increased temperature and mechanical agitation. Only 28 formulations were characterized because the method for particle analysis used in this study, micro flow imaging (MFI), is not high throughput. Based on prediction model building, correlations of both T_h and *kD* on significant formulation factors were obtained. The data obtained from the stress studies were used to determine the critical values for the stability parameters. It was found that for formulations with T_h values lower than 54 °C the aggregation level is higher

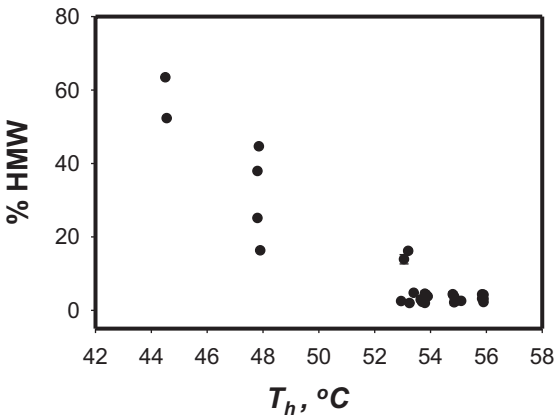


Fig. 20.5 The percentage of high-molecular weight species (*HMWS*) after mechanical stress versus T_h values. (Reprinted from (He et al. 2011b) with permission from John Wiley and Sons)

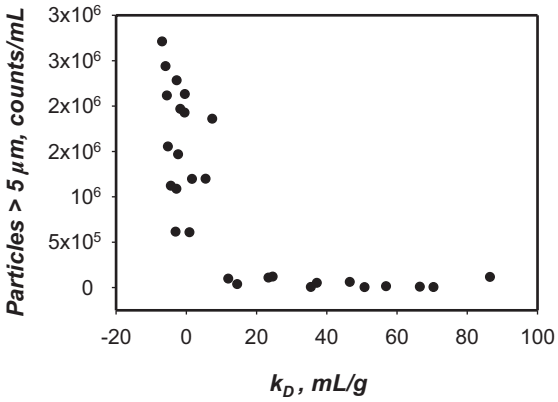


Fig. 20.6 The number of particles per milliliter after mechanical stress versus *kD* values. (Reprinted from (He et al. 2011b) with permission from John Wiley and Sons)

than 5% (see Fig. 20.5) after high temperature incubation. Colloidal stability was dramatically decreased when *kD* values dropped below 7 mL/g and the number of particles increased more than 100 times (see Fig. 20.6) after mechanical agitation.

At a T_h of 54 °C, the desired formulation conditions are limited to a narrow range of pH values and low salt concentrations (Fig. 20.7a). There is no allowed combination of pH value and salt concentration that satisfies the conditions, when the T_h is higher than 54.5 °C, for formulations without addition of sucrose at the same *kD* criteria. The presence of sucrose (see Fig. 20.7b) results in more available formulation conditions in the high pH range and at salt concentrations below 50 mM.

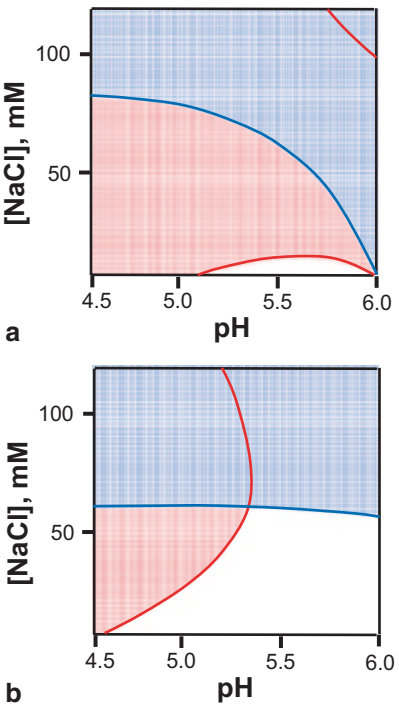


Fig. 20.7 Contour profiles derived from prediction formulas for T_h and *kD* values. The area in white represents the acceptable values of pH and salt concentrations at the particular limits for T_h and *kD*. The area with T_h < 54 °C is shown in *red*. The area with *kD* < 7.0 mL/g is shown in *blue*. Area with acceptable pH and salt concentrations is shown for the formulations without excipients (**a**) and with sucrose (**b**). (Reprinted from (He et al. 2011b) with permission from John Wiley and Sons)

The prediction of thermal and colloidal stability screenings should be carefully evaluated for each specific molecule being studied. The selection of the optimal formulation (buffer, pH, excipients, etc.) is dependent upon many parameters, not only on thermal and colloidal stability. The techniques described here can be widely used in formulation investigations where a fast screening of various samples is necessary. In these case studies, AHT allowed a full factorial design to be used in the screening of all tested formulation conditions. An example of a fractional factorial experimental design was shown by Awotwe-Otoo et al. (2012) in the study of lyophilized murine IgG3 monoclonal antibody. Preliminary results were obtained to eliminate the especially unstable buffer systems for the mAb product. Then DOE was applied to screen the effects of buffer type, pH, and different excipients on the glass transition temperature (T_g), protein concentration (A280), level of aggregation, unfolding temperature (T_m) of the lyophilized product, and particle size of the reconstituted product.

A Box–Behnken experimental design was applied to study the main effects of factors and their interactions on characterization parameters. In Fig. 20.8 the results of such an analysis is shown in the form of Pareto charts where statistically significant effects of formulation factors and interactions are ranked according to regression coefficients of correlation model. Using correlation models, response surface profiles were plotted to show the dependences of particle size, 280 nm absorbance and melting temperature on pH, NaCl concentration and Polysorbate 20 concentration (Fig. 20.9).

AHT has been used in formulation screening of numerous biopharmaceuticals (Roessner and Scherrers 2012; Gibson et al. 2011; Ahmad and Dalby 2010; Capelle et al. 2009; Johnson et al. 2009; Bajaj et al. 2007), including monoclonal antibodies (Bhambhani et al. 2012; Li et al. 2011; Gibson et al. 2011) and vaccines (Walter et al. 2012; Ausar et al. 2011) with much improved efficiency in material and sample handling, and analytical testing. High-throughput methods can be readily implemented using robotic liquid handlers that can conduct most of liquid solution manipulations usually involved for such studies. As mentioned in a previous section, the plate-based SBS footprint containment systems should be carefully selected with some additional discussion. For formulation physical property screens that are analyzed immediately, a large variety of automation friendly 96-well microtiter plates are commercially available. For studies involving in-plate optical detections using either UV-Vis or fluorescence spectroscopy, optical properties, such as optical transparency, wavelength cutoff, and well to well interference, should be considered. A note of caution is that some plates are made with a special surface treatment, such as high- or low-protein binding, which can have significant impact on the study if not evaluated. In formulation screening for protein physical and chemical instabilities, characteristics of the containment system must be carefully considered as it can significantly impact the outcome of the studies. Surface characteristics, such as protein binding property, can affect protein concentration or lead to denaturation. The potential impact of plate leachables and extractables should be considered, especially for studies conducted at accelerated or stressed conditions at elevated temperatures.

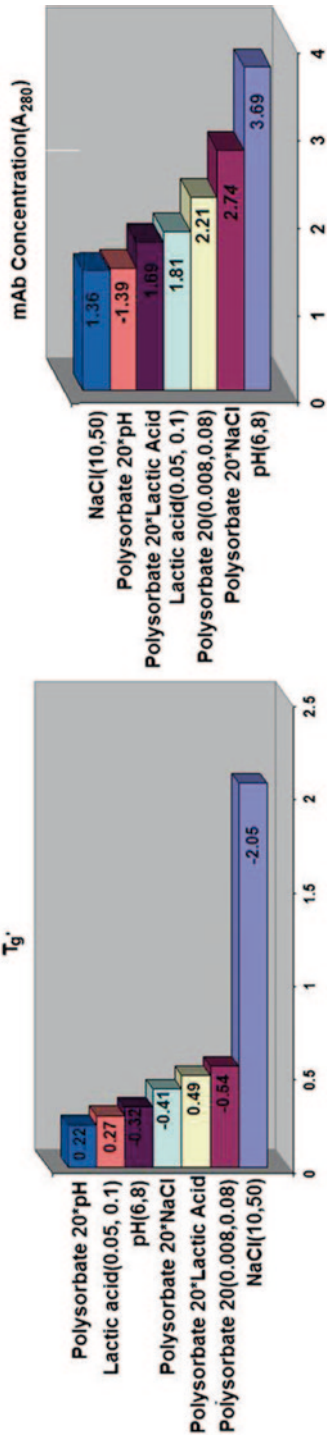


Fig. 20.8 Pareto charts showing the effects of formulation variables on the responses for the fractional factorial experimental design. (Reprinted from (Awotwe-Otoo et al. 2012) with permission from Elsevier)

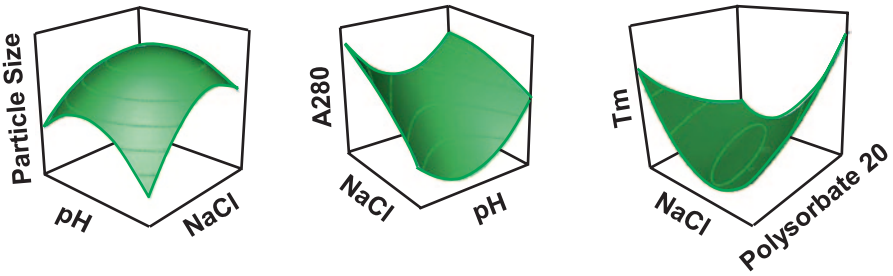
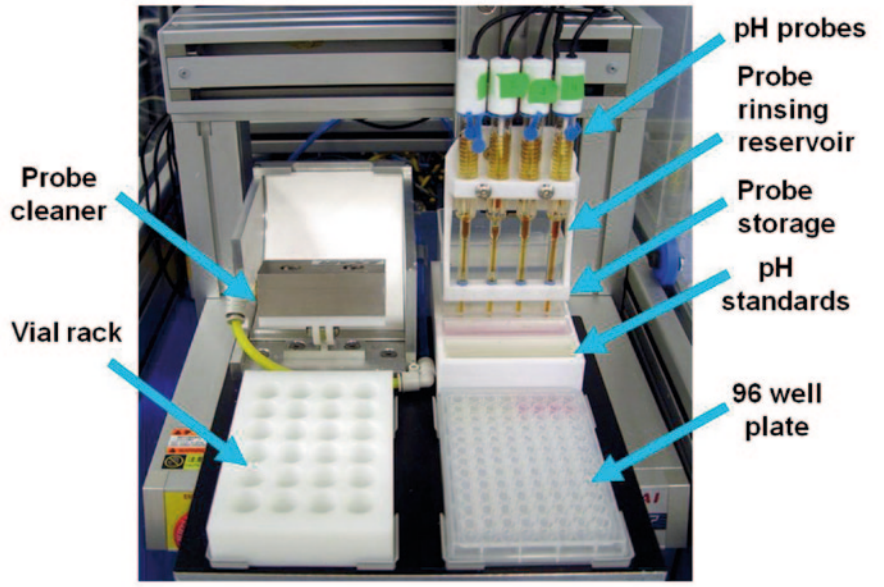


Fig. 20.9 3D Response surface profile showing the presence of curvature in the effects of pH, NaCl, and Polysorbate 20 on the particle size, concentration, and unfolding transition temperature (T_m) of the formulations. (Reprinted from (Awotwe-Otoo et al. 2012) with permission from Elsevier)

Once the leading commercial formulation candidates are identified, the top candidate is usually selected based on performance of scale-down studies by subjecting them to a series of small scale simulated and scalable real world stresses covering all major unit operations such as bulk drug product processing, distribution, hold times, room temperature, light intensity, etc. The top priority in conducting the scale-down study is to accurately reflect the stress at production scale. Protein instability can be very scale-dependent due to heat and mass transfer dependency, such as bulk drug product freeze and thaw (Colandene 2010). Bulk drug product processing studies are usually limited and extensive AHT support on material handling is relatively small. In contrast, solutions to shorten assay turnaround time is constantly pursued throughout the DPD process including formulation development phase, where AHT has wide applications for studying aspects of DP PQAs, such as pH as shown in Fig. 20.10, protein concentration measurement (Fig. 20.11), spectroscopic analysis (Chollangi et al. 2014), purity assays (Hiratsuka and Yokoyama 2009), and subvisible particle and visible particle analysis using instruments with autosamplers. Additionally, automated sample treatments can replace laborious manual steps in analytical laboratories for both increased efficiency and reduced variability, such as peptide-mapping sample desalting post reduction and alkylation (Fig. 20.12).

20.6 Drug Product Commercial Process Development (DP CDP) and Characterization

DP CPD is the phase late in the development of a biotherapeutic, where all aspects of drug product development are brought together to create a commercial process that is both robust and controllable. DP CPD forms its framework from a collection of prior knowledge of the same class molecules or platform experiences, experiences from earlier development such as first-in-human (FIH) process, as well as specific requirements on SKUs (per QTPP), batch size, and yield. These earlier



Average	StDev	%RSD		A	B	C	D	E	F	G	H
5.23	0.01	0.25	1	5.23	5.22	5.23	5.25	5.21	5.22	5.22	5.24
5.24	0.01	0.28	2	5.24	5.24	5.25	5.26	5.22	5.22	5.25	5.25
5.25	0.01	0.27	3	5.24	5.25	5.26	5.26	5.23	5.22	5.25	5.25
5.25	0.01	0.22	4	5.25	5.25	5.26	5.26	5.23	5.23	5.25	5.25
5.25	0.01	0.23	5	5.24	5.25	5.26	5.26	5.23	5.23	5.25	5.25
5.25	0.01	0.22	6	5.25	5.25	5.26	5.26	5.23	5.23	5.25	5.25
5.23	0.01	0.25	7	5.23	5.24	5.23	5.25	5.21	5.22	5.22	5.24
5.24	0.01	0.26	8	5.24	5.24	5.25	5.26	5.22	5.22	5.24	5.24
5.24	0.02	0.29	9	5.24	5.24	5.26	5.26	5.22	5.22	5.24	5.24
5.23	0.01	0.25	10	5.23	5.22	5.23	5.25	5.21	5.22	5.22	5.24
5.23	0.01	0.25	11	5.23	5.24	5.23	5.25	5.21	5.22	5.22	5.24
5.25	0.01	0.22	12	5.25	5.25	5.26	5.26	5.23	5.23	5.25	5.25

Fig. 20.10 Automated pH measurement and measurement consistency. Measurement results are independent of both probe and well location. Throughput increase is five folds and it requires no analyst intervention during the measurement

phases of drug development, including MA, formulation development, and commercial formulation development (CFD), have been discussed in detail in earlier sections of this chapter.

A typical biopharmaceutical production process follows a well-defined workflow, i.e., UF/DF if DP has a different formulation than DS, bulk drug product processing (freeze and thaw, mixing, bio-burden filtration, stainless steel container hold, and sterile filtration), filling, lyophilization (if needed), inspection, labeling, packaging, distribution, shelf life study, reconstitution (if lyophilized), and dilution into an IV bag and infusion if the drug is to be administered intravenously. Although there are few incentives to significantly alter the existing process from a regulatory familiarity point of view, opportunities do exist for AHT applications to significantly improve production efficiency, utilization of processing equipment, and more

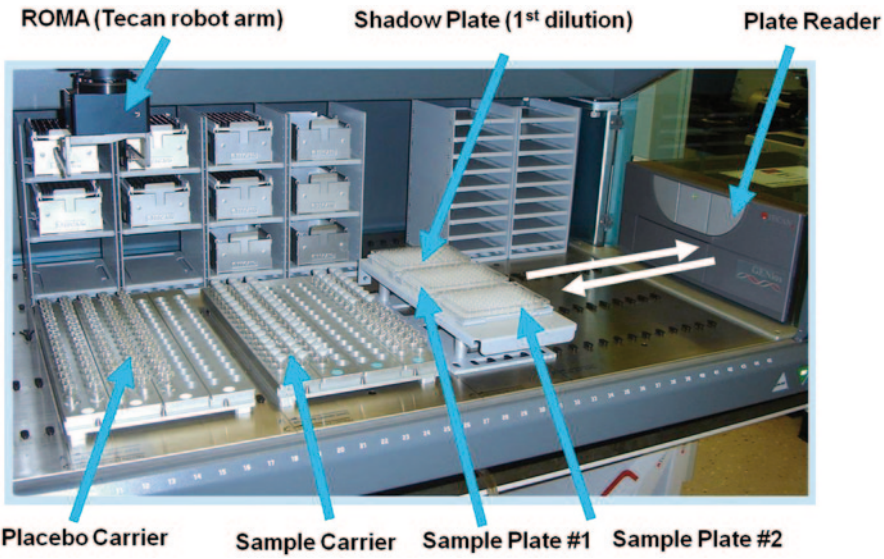


Fig. 20.11 Concentration measurement platform

importantly, improvements in product quality, driven by both cost of goods considerations and in the presence of constantly heightened regulatory requirements.

AHT can be applied to DP CPD in two different aspects, as a tool to assist in process development or as a part of the actual process itself. AHT can assist in the development of the process by providing support in material and sample handling and also in analytical testing in process development activities. DP CPD is also the entry point for any process enhancing technologies to be adapted into the DP production process. Most biopharmaceutical companies forgo major independent process equipment development and fabrication as they are not their business focus. Advancements in DP production technology, realized as improvements in efficiency and product quality, have been primarily driven by collaborations between the drug manufacturers and companies that specialize in process technology and equipment. Over the past few decades, significant advancements have been achieved in nearly all DP production unit operations, including filling operations and visual inspection where equipment with the capability of processing hundreds of units per minute without compromising the filling accuracy and product quality. Establishing the DP CPD process and the target process parameters requires a great deal of effort in identifying the required unit operation, running scale down processes, generating in-process and stability samples, and the testing of these samples.

An important part of DP CPD is the characterization of the process, sometimes referred to as drug product process characterization (DPPC). The purpose of this is to better understand the process and to establish the robustness of the process. This is an opportunity to apply AHT to allow detailed mapping of the drug product production process design and to establish in-process control and monitoring strategies. An automated, high-throughput system for this purpose should ideally consist

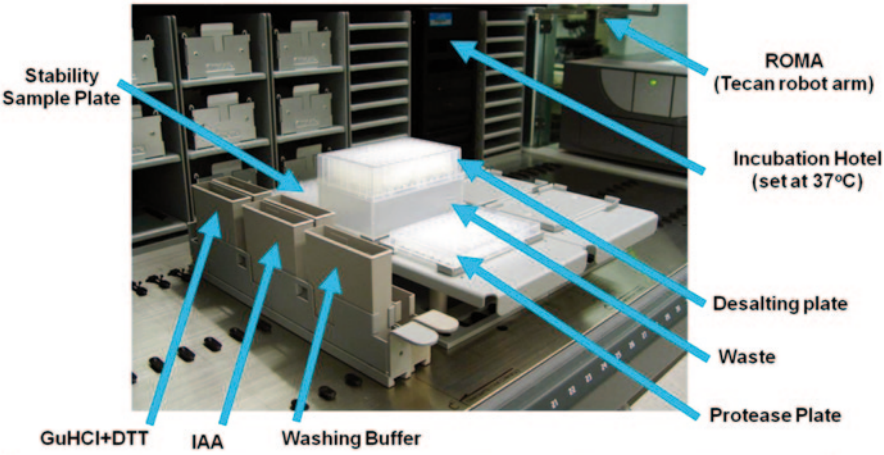


Plate Type	*BSA A280, initial*	*BSA A280, final*	*BSA, recovery %*	*Peptide cocktail A280 initial*	*Peptide cocktail A280 final*	*Peptide cocktail recovery %*
Corning 3364	0.567	0.566	**100**	0.682	0.682	**100**
Corning 3371	0.567	0.569	**100**	0.682	0.675	**98.9**
Corning 3355	0.567	0.570	**100**	0.682	0.688	**100**
Corning 3635	0.567	0.530	**93.8**	0.682	0.630	**92.3**
Corning 3686	0.567	0.522	**92.0**	0.682	0.645	**94.5**

Fig. 20.12 Automated peptide mapping digestion based on a liquid handler. Protein recovery study in selection of 96-well plates

of an integrated platform that can handle both vials and syringes and contain the necessary analytical instruments to carry out the required assays. One such system, available commercially, is the Core Module 3 (CM3) (Freeslate, Inc.), a configurable system for preparing, processing, and testing biological samples and is an ideal system for performing formulation studies. This system is highly customizable but generally contains components that facilitate sample prep and measurement of pH, viscosity, particle count, turbidity, color, and protein concentration and aggregation.

Aggregation and particulation is an especially vexing problem often encountered during drug product development and it is important to have the capability to measure aggregates and particles ranging in size from simple dimers to higher oligomers and particles of increasing size covering the size ranges of submicron (50 nm to 1 μm), subvisible (generally 1 to 125 μm), and ultimately visible (> 125 μm). Analytical

instruments have been developed in recent years to measure these aggregates and particles and some of them have been automated to allow the assay of many samples that may be part of a large study. Dimers and oligomers can routinely be measured by SEC a technique that covers the size range of approximately 1–50 nm. Assays have been developed in the past few years that are very fast, typically less than 10 min, and are automated in the sense that high-volume autosamplers can be employed to make the injections onto an SEC column and suitable chromatography data systems can be used to rapidly collect and analyze the chromatographic data. One such system is the Waters Acquity UPLC system with the sample organizer shown in Fig. 20.13. SEC columns packed with sub 2 μm beads allow higher speeds with greater sensitivity and improved resolution compared with more traditional SEC columns leading to higher throughputs.

For the measurement of visible particles (> 125 μm) the most common technique is a simple visual inspection. This approach is very subjective and is dependent upon the skill and training of the inspector and, furthermore, can be monotonous

Fig. 20.13 Waters Acquity UPLC system for automated SEC analysis

and time consuming. Fortunately, these types of inspections have been automated using a camera-based system instead of the human eye. One such system developed and used at Amgen, is the Particle Vision system. This system uses a robotic arm to grasp and swirl the sample to suspend any particles that may have settled out and then a high-resolution camera to measure the particles.

For particles in the subvisible range (2–125 μm) the technique of light obscuration has been used for many years and is the most commonly used method. Light obscuration is simply the blockage of light by a particle and can be readily measured. Instruments for this application are commercially available and, for the biotherapeutic industry, the most common instrument is the HIAC, shown in Fig. 20.14. While the HIAC instruments currently do not offer automation, custom systems using the HIAC and autosamplers have been designed and built by individual companies (Fig. 20.14a). A second approach to the measurement of particles in the subvisible size range is by imaging the particles. The technique of microflow imaging captures images of particles suspended in a liquid as the liquid passes through a flow cell. Each particle is imaged and in addition to counting, the particles can also be analyzed by shape and transparency. Automated instruments are commercially available where an autosampler is used to introduce the samples into the instrument and can also conduct samples flushing and cleaning of the flow cell between samples allowing for unattended operation. One such series of automated instruments is the MFI 5000 series (ProteinSimple) shown in Fig. 20.14b.

The low micron and submicron range of particles has been difficult to measure because of a lack of instrumentation that reliably works in that size range. A recently introduced instrument, the Archimedes (Malvern), uses the technique of resonant

Fig. 20.14 Instruments used in the measurement of particles in protein solutions. **a** Light obscuration instrument, the HIAC 9703+ (Beckman Coulter), for subvisible particles, **b** Automated Microflow Imaging instrument, the MFI 5000 series (ProteinSimple), for subvisible particles, **c** Resonant mass measurement instrument, the Archimedes (Malvern) for submicron particles

mass measurement to detect and count particles in the size range of 50 nm–5 μm. Currently this instrument is not automated but it is probable that an autosampler can be used to automate sample introduction at some point in the near future. Multiple techniques for particle analysis are now available, covering a wide range of particle sizes, from submicron to visible particles (approximately 50 nm to hundreds of μm). Unfortunately, automation of many of these techniques is still in its infancy with a lack of commercially available options and automation often being a customized solution for the needs of individual customers.

Transportation studies are an important part of drug product development. The stresses that drug product may encounter during shipping and transportation from one site to another include temperature excursions, shaking, and dropping from various heights and may lead to undesirable consequences such as protein aggregation. These stresses can be mimicked in a controlled laboratory environment and some of them are amenable to automation and high-throughput testing. Most of the commercially available equipment is not designed for use with biopharmaceuticals but custom designed systems using commercially available robotic arms are being used in the industry and these provide the automation necessary to perform repetitive motions involving multiple samples. One such robotic arm is shown in Fig. 20.15.

Another application example of AHT in process development is the adaptation of dynamic thaw of drug substance stored in polycarbonate carboys over the traditional static thaw during bulk drug product processing steps, which resulted in not only a shortened process time but also improved product quality in comparison to the static thawing process.

AHT can be readily adapted to platform approaches in formulation development. Platform approaches categorize development efforts by either therapeutic areas or molecular types, e.g., native protein, fusion protein, or monoclonal antibody, with improved utilization of historical knowledge and development experiences. A platform is usually composed of large number of standardized methodologies and protocols covering material handling, sample preparation, and analytical handling that significantly increase the impact of AHT applications. Considering modular structure of many components, AHT can be flexible to screen different types of molecules and formulation conditions beyond existing formulation platform. When the formulation of a pharmaceutical product are optimized by a systematic approach using DOE, manufacturing scale-up and process validation can be very efficient.

20.7 Process Analytical Technology (PAT)

Biopharmaceutical development relies on product quality testing. PAT brings an important aspect of AHT applications to QbD approaches. By applying AHT elements, PAT enables monitoring and control of DP PQAs in real time, unlike standard laboratory testing. PAT enables real-time intervention and course-correction during execution of a given unit operation thereby improving flexibility in manufacturing

Fig. 20.15 Robotic arm used for automated drop shock testing (Dynamic Automation)

operations. A large number of PAT applications have been developed and implemented in biopharmaceutical development and production in such areas as process performance parameters, physical properties (Shah et al. 2007), and molecular DP PQAs including physical and chemical instabilities of the therapeutic molecule. The application of PAT, using both automation and high-throughput technologies, has greatly enhanced both the efficiency and the effectiveness of process development and production (American National Standard Institute 2004). It is desirable to develop and implement PAT to cover as many CQAs as possible. The challenges are often on the interfacing aspect between the process and the assay (e.g., mobile phase compatibility) and the potential environmental impact on the production environment. PAT offers the benefits of reducing lot disposition cycle time, better control of the process leading to reduced patient risk, and more efficient assays such as single multi-attribute methods (e.g., mass spectrometry).

The focus of this section is on the application of AHT to assist PAT in the development process with the goal of adapting it to the production environment. A closer look at a typical drug product production process helps to identify the application

of PAT and support from AHT. The application of AHT for PAT during these unit operations is discussed together with some design concepts of novel in-process testing methods.

Implementation of AHT for PAT can be explored by separating the assays into three categories: in-line, on-line, and at-line. In-line PAT is measurement without removing a sample from the process stream; on-line refers to samples which are diverted from the manufacturing process; and at-line means samples are removed, isolated, and analyzed at the development or production site.

20.8 In-line PATs

In-line PAT provides the best of what PAT offers, real time information. The impact of in-line PAT varies with the selection of the point of detection. For example, monitoring the compounding process inside the tank not only provides real time information about the mixing process but also allows corrective actions to be taken; whereas, monitoring at the transfer line provides the end result where process intervention is too late. pH can be measured in-line, both in-vessel (2a in Fig. 20.16) and in transfer lines (2b) using glass-membrane pH probes, which can replace off-line drug product pH release testing. At the transfer line, pH can be measured using off-the-shelf plumbing joints. The in-vessel measurement can be implemented by positioning the probe into the top of the vessel. The point of detection can be adjusted

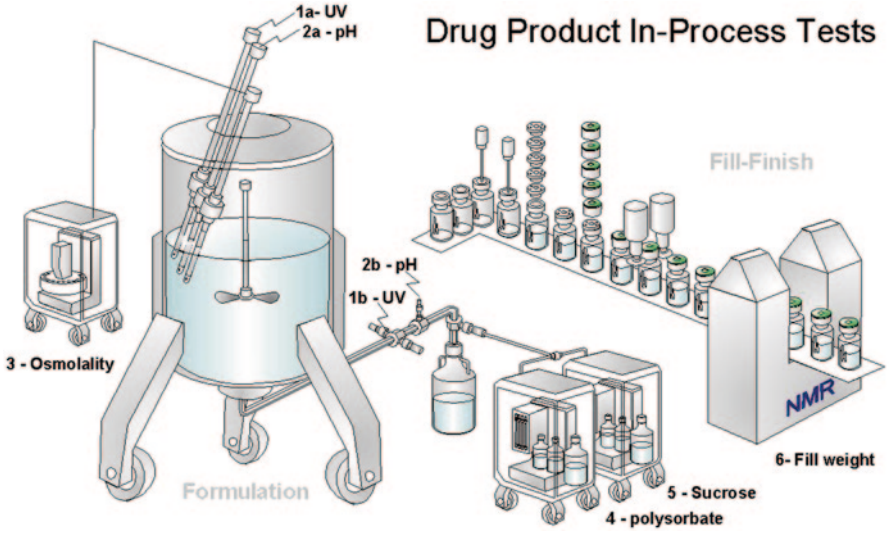


Fig. 20.16 A typical drug product production process and the proposed in-process tests. Scope is limited to drug product PQA tests. Equipment process conditions, such as solution temperature in jacketed vessels and mixer RPM, and environmental conditions, such as room temperature, relative humidity are out of scope

by the use of an extension tube. Both the probe and the extension tube are commercially available and are GMP compliant. For GMP and non-GMP drug product development and manufacturing, pH/multi-parameter transmitters can be used for pH monitoring and trend analysis. A similar example includes in-line aggregation measurement using light scattering probes or Raman spectroscopy (Mungikar and Kamat 2010).

Protein concentration can be monitored using online concentration measurements both in the compounding vessel (Fig. 20.16-1a) and across the transfer line (Fig. 20.16-1b) by measuring UV absorbance at 280 nm. The transfer line test can be conducted using a dual wavelength UV absorption sensor, with the potential to replace drug product release testing. To date, there is no commercially available in-vessel UV absorbance probe. Therefore, a custom-designed optical unit is needed to allow in-vessel testing. A design concept is shown in Fig. 20.17.

While the working principle is straightforward, the design considerations are geared toward minimizing the impact on the compounding process and maintaining sterility. To reduce solution stagnancy between the probe arms, the top-to-bottom edges of these arms can be sharpened to allow better flow-through for easier solution equilibration of the bulk. Such modifications can be more important when the path length is further reduced for highly concentrated drug product solutions as shown in Fig. 20.18. In-vessel testing (1a) can potentially be used to monitor the drug compounding process by providing real-time concentration values at the point of detection. A multi-point arrangement can help to map out the product mixing profile within the product compounding vessel but this is more likely to be limited to characterization and development studies.

Protein content consistency of finished drug product is another area for PAT application. Fill weight can be monitored by weighing the finished product with the assumption that the other drug product components, e.g., vial, stopper, and seals, are of consistent weight. More accurate protein content can only be measured by extracting the drug product solution. In-line, noncontact check weighing (NCCW) equipment, based on time-domain nuclear magnetic resonance (NMR), has demonstrated its potential to provide a measure of liquid content (Kamath 2006). At a speed of up to 400 vials/min, this system is able to provide 100% in-line protein content check. This technology allows development of protein-specific measurement for drug product vial content determination based on the intensity of chemical shifts for protein specific elements. Development work needs to address potential interference from excipients at the targeted protein concentration range, and side-by-side comparison with manual weight checking methods.

Another example is visual inspection of the nude container, e.g., vial, prefilled syringe, and cartridges, postfill but prior to labeling. Filled and sealed drug product can be checked for both content as well as the DP PQAs such as particle, color and turbidity, and the cosmetics of the finished product including defects on the container and seals. Automated visual inspection would significantly increase line throughput and reduce operator fatigue and human errors.

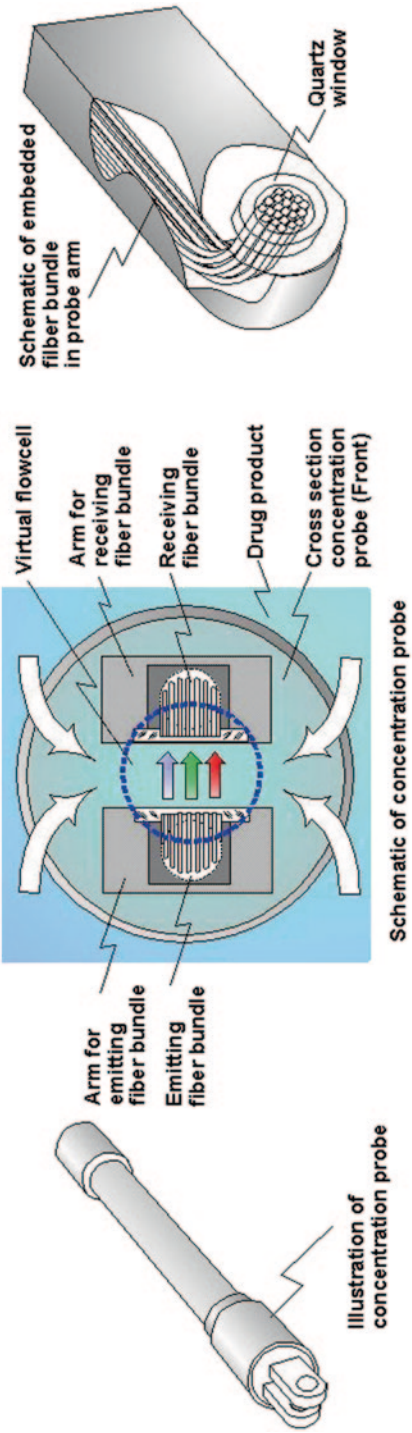


Fig. 20.17 Design concept of an in-vessel UV absorption probe. The proposed probe is illustrated on the *left*, explained with a cross section view in the *middle*, and the arranged fiber bundle for transferring either emission or absorption light on the *right*

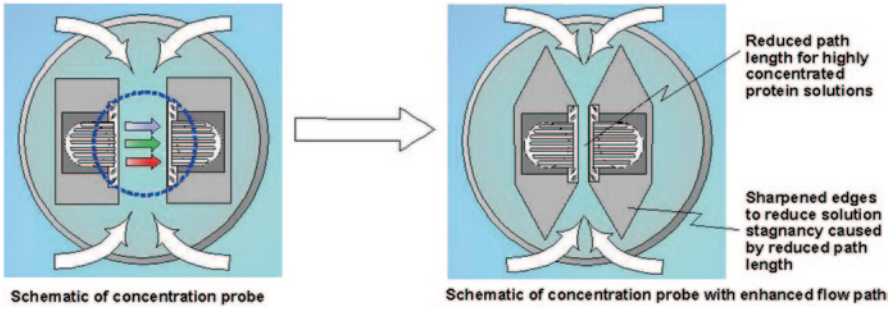


Fig. 20.18 Design concept of an in-vessel UV absorption probe with design feature to allow improved UV detection for concentrated samples

20.9 On-line PAT

Some analytics require the sample to be extracted from production streams before analysis. Sample extraction impedes the overall analytical efficiency, but expands the scope and capability of the adaptable analytics. The two most popular sample extraction methods are sample loop and probe based. A sample loop diverts samples from the production stream which allows a continuous sample feed with time savings. Probe-based extraction involves using a physical arm to remove samples. Compared to in-line PAT, extra caution is needed to minimize the risk of product contamination due to exposure of drug product to the environment where the analytical equipment resides. Different from in-line PAT where testing results become available instantaneously, turnaround time for on-line and at-line PATs includes: (1) the time to extract the sample and (2) the time to get the sample ready for analysis. One often needs to factor in the added time for assay and data processing due to the complex nature of some of the analytics. The total time is important to know during its justification and planning, especially if it is potentially used to guide corrective actions during the drug production process. One example is the monitoring of solution osmolality. Currently, there is no commercially available osmolality analyzer to conduct on-line measurements. Osmolality can be measured using two types of commercially available instruments, based on freezing point depression or vapor pressure change. A design concept for an on-line osmolality measurement by interfacing an osmometer with an automated sample loading module is illustrated in Fig. 20.19. Development work is needed to assess the feasibility and the robustness of the loading mechanism. The performance of the freezing point depression instrument can be compromised in highly viscous samples by the detection mechanism, while the vapor pressure change instrument is not suitable for volatile solutes.

HPLC is widely used to monitor compositional and purity-related CQAs, including biologics, formulation components, or process impurities. HPLC methods are easily adaptable to on-line monitoring using the Dionex DX-800, for example, and shown in Fig. 20.20. This system is self-contained for extended unattended operation.

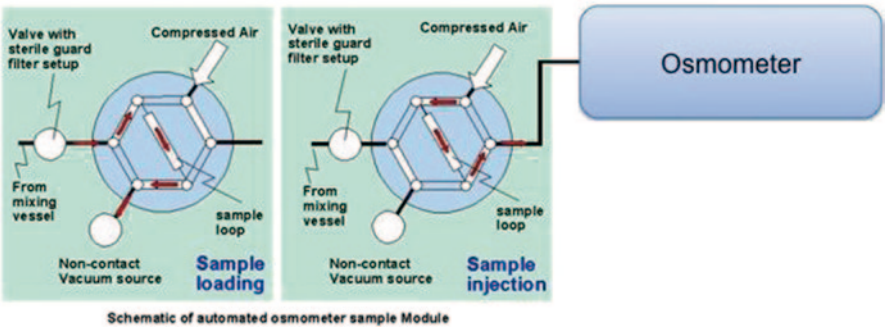


Fig. 20.19 Design concept of an inline automated osmometer. This setup uses a loading valve to collect sample from the mixing vessel periodically for osmolality measurement

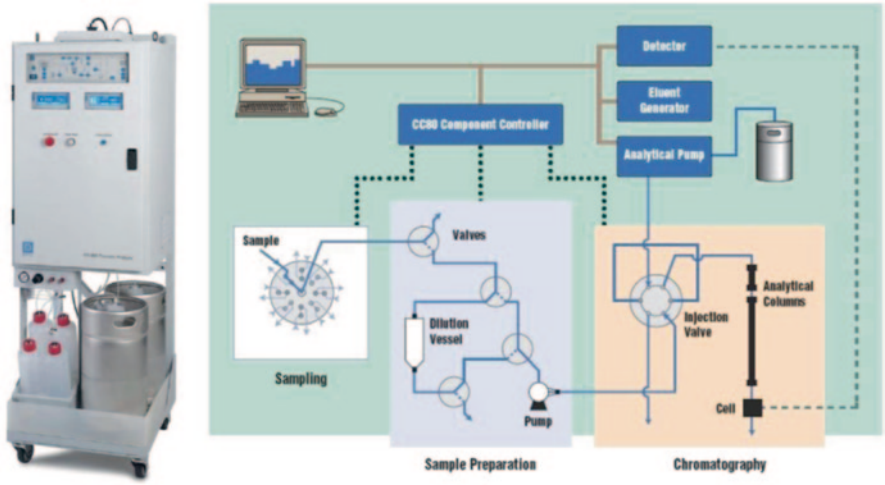


Fig. 20.20 Dionex DX-800 Process HPLC system. The system is composed of a sample injection module, sample preparation module and a HPLC module. (Reproduced from the on line Dionex DX-800 Process Analyzer User Guide, Nov 2003, with permission from Thermo Fisher Scientific Inc)

20.10 At-line PAT

At-line PAT, where samples to be analyzed are removed from the production process prior to analysis, offers an opportunity to utilize more sophisticated analytical techniques. HPLC (or UPLC), capillary electrophoresis, and even mass spectrometry all have high-resolving power and the analyses can usually be completed in minutes making them suitable techniques for at-line PAT. These techniques are usually applied for the assessment of chemical stability of the protein drug.

Mass spectrometry, a powerful analytical tool widely utilized throughout DPD cycles including molecular assessment, formulation, and drug product development, is the method of choice for monitoring chemical stabilities. Mass spectrometry has been used as a PAT tool to monitor bioreactor head-space gases (e.g., oxygen and carbon dioxide) to provide real-time monitoring to calculate a host of related process indicators, such as oxygen uptake rate (OUR), carbon dioxide evolution rate (CER), respiration quotient (RQ), and oxygen mass transfer coefficient ($k_L a$). These are simple gas molecules that are easy to sample and analyze. Sampling for in-tank or in-process liquid state drug product can be more challenging than head-space gas analysis and often requires extended sample treatment as discussed below. For the purpose of monitoring chemical modifications on the protein drug product, mass spectrometry is usually carried out on peptide fragments of the protein (the so-called bottom–up approach) where the protein is treated with a protease, most often trypsin, to hydrolyze the protein at specific sites to generate a set of peptides that represents most of the sequence of the protein. These tryptic peptides are then analyzed by the technique of LCMS or LCMS/MS where the peptides are separated by reversed-phase HPLC and analyzed by mass spectrometry. An LCMS/MS experiment takes the analysis one step further and fragments the peptide ions within the mass spectrometer using, most often, a process known as collisionally induced dissociation (CID) to form product ions of the original peptide. These product ions can be used to localize a chemical modification at a single amino acid. The disadvantage of an LCMS approach is the time required to separate the peptides prior to analysis, often exceeding an hour and rendering this approach unsuitable for PAT. However, methods are being developed to eliminate chromatography and instead the peptide mixture is introduced into the mass spectrometer by direct infusion. Such a method requires a high-resolution instrument and relies on separation of the peptide ions in the gas phase within the instrument based on their mass to charge ratio. These methods will allow a complete data set of all the tryptic peptides to be collected in one minute or less. Of course, sample prep, including denaturation, reduction, and alkylation, before trypsin digestion usually takes too much time for PAT but methods are also being developed to accelerate sample prep to allow a bottom–up mass spec approach to be feasible for PAT applications. An alternative mass spectrometry approach is the top–down, where intact molecules or, in some cases, a chemically reduced molecule are analyzed directly by mass spectrometry without any prior proteolysis. With the development of high-resolution mass spectrometers this is now a possibility even for proteins of molecular weight greater than 100,000.

For PAT applications, quick turnaround time is key, so a top–down approach where no extensive sample treatments are needed, may be more desirable, but this approach requires a high-resolution mass spectrometer and its application in PAT may be hindered by complexity, cost, and space. The mass spectrometer is a highly sophisticated and precise instrument that requires frequent tuning and calibration, which makes validation difficult for its use in the GMP environment. Furthermore, mass spectrometers require a vacuum pump with exhaust that is both a heat source and a potential contamination source for a production environment. For PAT, the most important aspect is to ensure that the sample is truly representative of the

targeted process. At the final stage of production, the drug product can be highly concentrated and is not always homogeneous, so the location of sampling and method of sampling are crucial. Highly sensitive analytical instrumentation like the HPLC and mass spectrometer, require only minute amounts of material necessitating a series of dilutions and, in some cases, a solvent exchange step to prepare the sample for a certain solvent environment suitable for analysis. Steps such as these can significantly increase the sample processing time.

As discussed in previous sections, high-throughput analytics are widely used during the formulation characterization phase, and this automation of analytics is desirable not only to increase the throughput but also to relieve the analyst from performing routine and monotonous operations and minimize the operator dependency of the assay. In DPPC and PAT, turnaround time rather than throughput is the key attribute for its potential to be integrated into the production process. Ample time for corrective actions requires fast-responding analytics, which can benefit from automation for quick sample extraction, preparation, and assay, while throughput is secondary.

20.11 Real-Time Multivariate Statistical Process Monitoring of Fill and Finish Operations

There are many variables measured during the course of a production batch either offline or online and at different frequencies depending on the measurement system used. As discussed in earlier sections, with the advent of PAT and more data available it is important to efficiently monitor and diagnose deviations from the in-control space for troubleshooting, correcting, and process improvement purposes. Multivariate modeling and real-time statistical process monitoring (MacGregor and Kourti 1995; Undey et al. 2003) is the one of the solutions that has been successfully applied in chemical industry. Those applications have been also successfully extended to pharmaceutical and biopharmaceutical cases (Albert and Kinley 2001; Undey et al. 2010). For the typical process there is the number of executed batches (I), with the number of measured variables (J) at the certain time interval (K), forming a three way data array (X) as depicted in Fig. 20.21. Developing data-driven multivariate process models that define process variability has been shown beneficial in proactively monitoring the process consistency, performance, and in troubleshooting purposes. Typical process performance (as contained within data array X from the process variables shown in Fig. 20.21) can be modeled using multivariate techniques such as principal components analysis (PCA) and partial least squares (PLS). These models are used real-time monitoring of a new batch progress whereas batch level models are used at the end of the batch for across batch trends also known as “batch fingerprinting.” Details and mathematical formulation of the modeling in PCA and PLS for batch processes (when it is performed for batch processes they are usually referred to as Multiway PCA or PLS models) can be found in the literature (Undey et al. 2003; Wold et al. 1998).

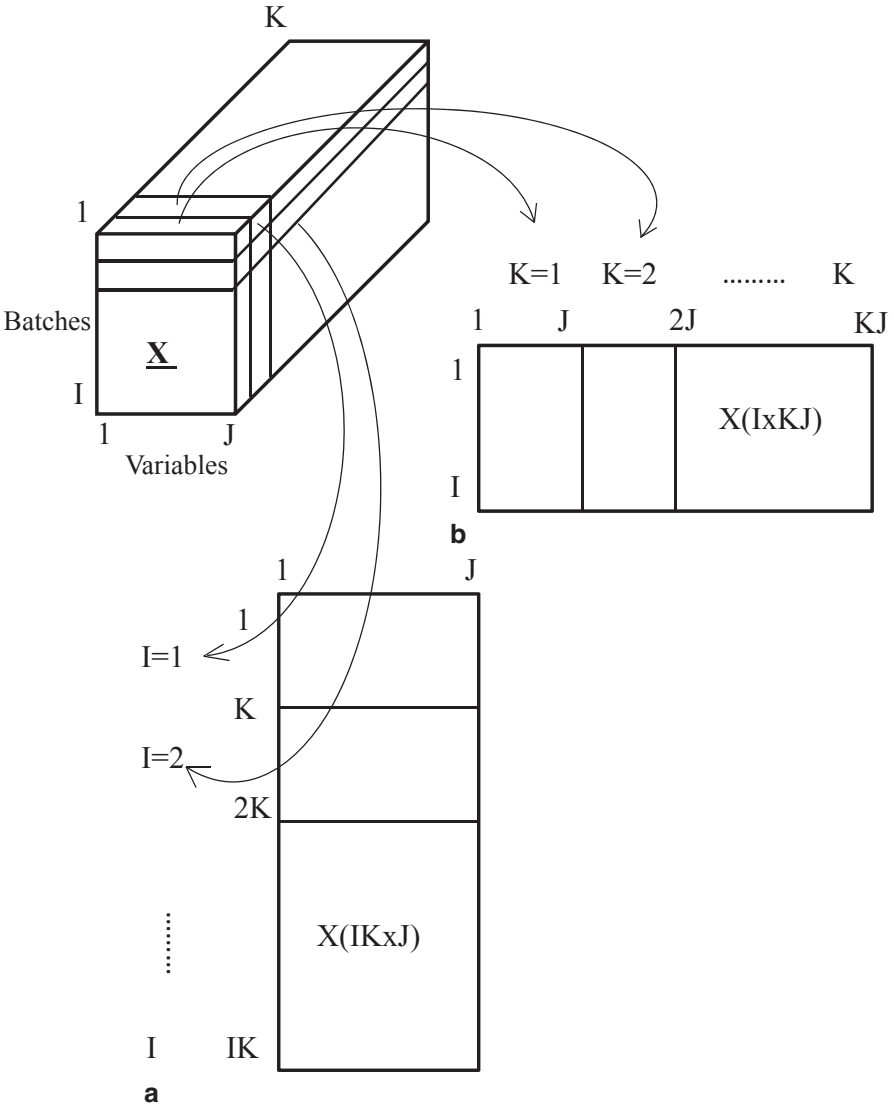


Fig. 20.21 Unfolding of three-way batch data array **a** Observation level, preserves variable direction, **b** Batch level, preserves batch direction

In one example, statistics-based concepts have been applied to a fill and finish line of vial filling to establish a real-time monitoring approach for comprehensive control of many variables simultaneously. In this example, 12 different needle positions are monitored for dosing time, temperature, and net weight as depicted in Fig. 20.22a. Multivariate charts were used to detect weak signals from many variables measured during the filling process. Multiple needles were detected using the

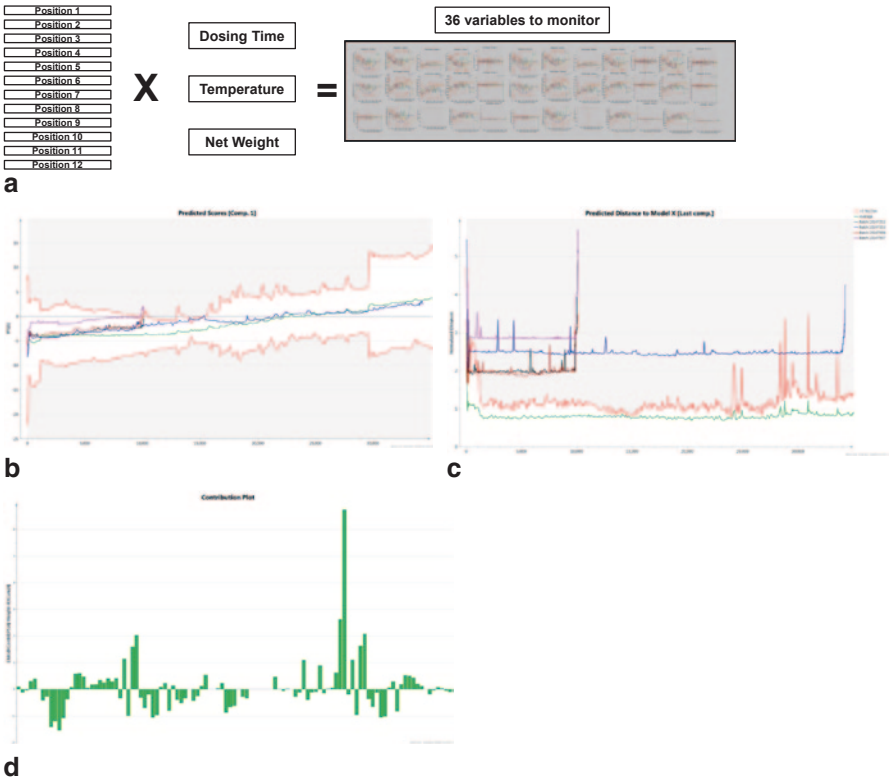


Fig. 20.22 Parameterization and multivariate charts for vial filling. **a** Typical parameters measured at fill and finish line of vial filling **b** Scores chart for batches that are consistent with historical performance (*green line* is the average trajectory and *red lines* represent +/− 3 standard deviation around it), **c** normalized distance plot across number of primary containers filled for the detecting batches with issues with 95 and 99% confidence limits (shown in *green* and *red* colors, respectively), **d** contribution plot identifying the issues by inspecting the highest variable contributions to the inflated normalized distance statistic, some of the needles were problematic causing incorrect fill weight

normalized distance chart (Fig. 20.22c) on the fill line not following (i.e., above the 95 and 99% confidence limits, shown in green and red colors, respectively) expected operational performance as shown in Fig. 20.22b as a score trajectory chart (of the first latent variable) across number of primary containers filled. After evaluation of the variable contributions plot in Fig. 20.22d, we diagnosed potential issues with certain needle fill positions and conducting an inspection of fill line, a leak in one needle manifold was identified and some needles were found bent. Multivariate analysis helped diagnosing the issue from many different variables offering unique opportunities to fill and finish lines.

20.12 Summary

The implementation of AHT often requires a substantial capital investment. The decision to implement AHT should be based on criticality, adaptability, cost effectiveness, and regulatory impact. A preliminary technical framework and cost analysis can justify the investment by assessing the projected productivity increase against the impacts on the risks, and the investment. A platform approach in formulation development provides a straightforward integration for AHT technologies with abundant standardized methodologies and protocols. AHT formats can adapt to different DPD conditions including molecule modalities and manufacturing processes. If PAT is considered for clinical or commercial production use, all equipment or process robustness and readiness validation and any potential regulatory hurdles must be fully evaluated.

AHT should not affect the essence of the original process. Examples include screening formulation candidates, conducting bulk drug product freeze-and-thaw study using much smaller container, implementing a fill-and-finish process without considering production related impacts, generating sample sets insufficient to address statistically low-occurrence instabilities, and implementing a PAT measurements that might interfere with an existing process.

During AHT implementation planning, it is important to map out the short-term and long-term goals, conduct throughput and utilization analysis with scheduling considerations, and identify the bottlenecks where the greatest impact can be achieved if staged approaches are needed. AHT has successfully demonstrated productivity impact in many industries and may ease the resource constraints in biopharmaceutical DPD.

Successful implementation of QbD principles in DPD relies on (1) information about molecule-independent and molecule-specific CQAs from historical knowledge and experiences; (2) an established design space for CQAs to provide guidance and to ensure product quality; (3) sufficient control strategies and methodologies. AHT can greatly improve our ability to apply QbD principles by enhancing efficiency, throughput, operational consistency, and supplying real time data. At early stage DPD, AHT can expedite the learning process and quickly capture knowledge to better understand the molecule itself, its functionality for intended use, and the formulations to maintain its stability and safety profile. At late stages of DPD, close to the manufacturing environment, the focus shifts from exploratory AHT and sample handling toward PAT aspects. With quick turnaround, real-time process monitoring and reduced operator-related inconsistencies, the application of AHT can significantly strengthen understanding and control during QbD implementation in DPD.

AHT can address the unique needs at different stages of DPD. At the MA stage, the target is to select the optimal drug candidate by screening manufacturing and storage conditions. The accurate and precise liquid handling is important to decrease the consumption of scarcely available material and to reduce operator error. At the formulation screening stage, the high-throughput sample handling and automated analytics significantly increase efficiency of identifying the most stable formulations. The same benefits can be observed for formulation robustness studies

as well. Scalability prevails once DPD enters process characterization stage. There the focus of AHT is primarily on analytical sample handling, implementation of high-throughput techniques and automation of laborious sample preparation procedures. AHT applications in the production stage are mainly for development of PAT for faster turnaround or real time monitoring of drug product CQAs. This chapter demonstrated that AHT can greatly enhance efficiency and throughput in development to better identify CQAs, construct operational space, establish an informed risk-management system, and facilitate the integration of PAT in drug product development and production. In summary, AHT significantly improves both the capability and the capacity of QbD approaches.

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Chapter 21
Critical Quality Attributes, Specifications, and Control Strategy

Timothy Schofield, David Robbins and Guillermo Miró-Quesada

21.1 Introduction

Quality by design (QbD) is a systematic risk-based approach that uses scientific understanding of the product and the manufacturing process. This approach ensures that the manufacturing process is well controlled to consistently deliver product meeting the quality requirements for the intended patient population. To achieve this, critical quality attributes (CQAs) are identified using risk assessment tools and information on potential impact on safety and efficacy. Acceptable ranges are then set which provide quality targets for process characterization studies. These studies will determine the multivariate effects of process parameters on product quality. Based on this process understanding, critical process parameters (CPPs) are identified. The CPPs and their multivariate acceptable ranges become part of the definition of a design space, which more broadly consists of "the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality" (ICH Q8(R2) 2009). Once the process has been characterized and defined in this way, the ability of the process to control each CQA can be assessed in order to determine whether additional analytical testing controls are required to ensure control of product quality. These provide for the definition of an integrated control strategy, including both process controls and analytical testing (including release limits). An overall quality attribute risk assessment can then be conducted to confirm the robustness of the proposed control strategy to ensure the quality, safety, and efficacy

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of the drug that is provided to patients. Additional consideration is given to the assessment of comparability to support manufacturing and analytical changes, either as part of or in addition to the control strategy.

21.2 Determination of CQAs

ICH defines a CQA as "…a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality" (ICH Q8(R2) 2009). Determination of CQAs is an iterative process beginning with a listing of the attributes of a product which may impact patient's safety and efficacy, and followed by an evaluation of the attribute through a CQA risk assessment. That assessment culminates in the process and product control strategy, a comprehensive collection of process and analytical controls which help ensure quality to the patient. This section will outline the process and tools used for CQA determination.

21.2.1 Quality Attributes of Biopharmaceuticals

Determination of CQAs of a biopharmaceutical begins with an inventory of properties of the product. Those properties or quality attributes will be product specific and generally based upon knowledge of the physical, chemical, and biological characteristics of the product. An example of a list of quality attributes for a monoclonal antibody product is illustrated in Table 21.1. The quality attributes are divided into various categories to simplify further assessment.

Over the course of development, the quality attributes of a product are evaluated to establish criticality to quality. Criticality to quality relates to potential impact on patient's safety and efficacy. In some cases a quality attribute may be designated a CQA because of its known impact on the patient (e.g., potency and bioburden). Others will require further investigation throughout the course of development to establish their impact. That investigation will often take the form of an assessment of the potential impact of an attribute on bioactivity, PK/PD, immunogenicity, or safety. If an attribute is determined to have a potential impact on one or more of these clinical categories it may become a CQA. It is important to identify CQAs so that those product characteristics having an impact on product quality can be studied and controlled through selection of an appropriate manufacturing process and definition of a control strategy.

It is important to not only determine the direct impact of a quality attribute on the safety and efficacy of the product but also to consider indirect impact(s) through known or suspected interaction(s) or correlations with other quality attribute(s). For example, it is suggested in the literature that glycation of lysine residues may result in an increase in the rate of formation of soluble aggregates (Banks et al. 2009). Therefore, glycation could potentially have an indirect and more severe impact on safety and efficacy through an increased level of soluble aggregates than through the

Table 21.1 Examples of quality attributes for a monoclonal antibody drug product

Quality attribute categories					
General properties	Product variants	Bioactivity attributes	Contaminants	Process-related impurities	Excipient levels
Clarity and color Subvisible and visible particles pH Osmolality Protein Concentration Extractable volume Container closure integrity ***For lyophilized products:*** *Cake appearance* *Cake moisture* *Solubility of reconstituted product*	Aggregates Fragments Charge isoforms Deamidation Oxidation Glycation Glycosylation: Fucosylation Galactosylation High mannose Sialylation Nonglycosylated forms	Effector Function Potency	Sterility Endotoxin Virus	DNA HCP Insulin	Surfactant (e.g., Polysorbate) Sugar (e.g., Trehalose) Buffers and other salts

effect of glycation alone. Similarly, excipient concentrations and general properties of the drug product (DP; e.g., pH) can have a large impact on the rate of formation of various product variants during storage, such as aggregation, fragmentation, and deamidation (Wang et al. 2007).

Another consideration in quality attribute assessment is the route of administration. For example, the potential impacts of a particular quality attribute on immunogenicity or PK might differ with subcutaneous administration compared to intravenous. It is possible that an attribute might be a CQA in one case but not the other. However, managing a control strategy for a product with multiple commercial product presentations could become very complicated if a different set of CQAs is applied for each case. It may be simpler to take a conservative approach and develop a single universal list of CQAs that covers all routes of administration under consideration for the product.

21.2.2 CQA Risk Assessment

A CQA risk assessment is conducted throughout development to establish a pathway for analytical, process, and formulation development and to create the com-

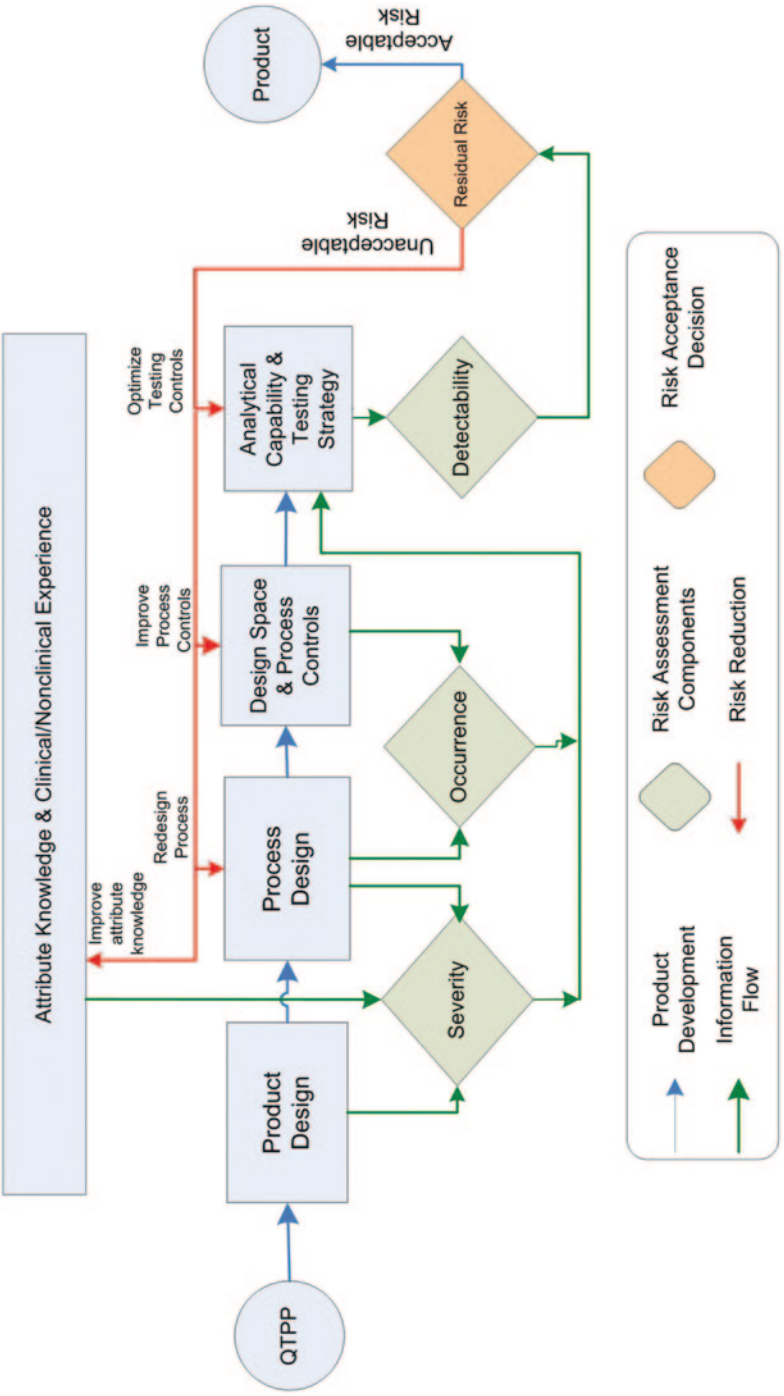

Fig. 21.1 High-level strategy for quality attribute risk assessment

Table 21.2 Definitions of severity, occurrence, and detectability

	IEC-60812 definition	Definition adapted for quality attribute risk assessment
Severity	"… an estimate of how strongly the effects of the failure will affect the system or the user …"	Impact on patient's safety and efficacy when dosed with product quality attribute outside of its appropriate requirements
Occurrence	"… probability of occurrence of a failure mode …"	Likelihood that a quality attribute will be outside of its appropriate requirements
Detectability	"… an estimate of the chance to identify and eliminate the failure before the system or customer is affected …"	A measure of the ability to identify whether a quality attribute is outside of its appropriate requirements prior to patient dosing

mercial product control strategy. The key elements of this approach are described in this section and outlined in Fig. 21.1.

Early in development it is recommended to define a quality target product profile (QTPP) to guide development and provide for an early understanding of potential CQAs and quality targets. A QTPP should consist of a prospective summary of the quality characteristics of a DP that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the DP (ICH Q8(R2) 2009). The definition of the QTPP considers multiple factors, such as the indication, patient population, route of administration, dosage form, bioavailability, strength, and stability. The *product design* step in Fig. 21.1 allows for any sequence liabilities of the molecule that could impact quality attributes to be addressed through optimal molecule design.

Each of the relevant attributes is subjected to a failure mode effects analysis-like (FMEA) risk assessment over the course of development (Fig. 21.1). The use of a FMEA approach to quality attribute risk assessment is not dictated by the regulatory agencies or guidances, but is commonly used in the industry and is provided here as an example. The International Electrotechnical Commission standard IEC-60812a is a useful reference for the FMEA approach, and provides general definitions of severity, occurrence, and detectability (detection). These can be applied to the CQA risk assessment using the definitions provided in Table 21.2.

Note that this particular application of the FMEA approach is focused on the quality attributes themselves and the potential for harm to the patient if a quality attribute fails to meet its requirements. It is not to be confused with more traditional FMEAs often used in industry which focus on manufacturing failures. While such traditional assessments focused on manufacturing failure modes can potentially be useful tools in QbD approaches for assuring reliability of the manufacturing process, they are not part of the approach described here that is focused on potential for harm to the patient.

The first assessment in the quality attribute risk assessment is *severity* that takes into account how each quality attribute impacts safety and/or efficacy. Categories of clinical impact which may be assessed are bioactivity, PK/PD, safety, and immunogenicity. The severity assessment can be based on the knowledge linking the quality attribute to clinical performance and the certainty in that knowledge. The sources of knowledge are divided according to prior knowledge (literature and in-house knowledge) and product specific knowledge obtained from laboratory (protein characterization), as well as nonclinical and clinical experience. A detailed discussion of quality attribute severity assessment is in Sect. 21.2.3.

The quality attribute severity assessment is a progressive exercise during various stages of product development. During early stages of product development (e.g., preclinical), severity scores will be defined based on limited product knowledge. As the product moves through clinical development (Phase 1, Phase 2, and Phase 3), new information may be gained which may result in a change in severity score. Changes in the QTPP, including route of administration, dosage, and patient population may also have an impact on the severity score. The severity score, and consequently, the CQA identification are used to guide the development of the control strategy in support of commercial process validation and the marketing application. At this later stage of development, control of quality can rely more heavily on robust process controls (i.e., resulting in lower *occurrence*) with the potential to reduce reliance on testing (which is associated with *detectability*) to ensure quality.

Occurrence is primarily based on process capability, which represents the ability of the manufacturing process (or formulation) to maintain the quality attributes within their requirements. Process capability arises from both the design of the process (process sequence, linkages, robustness, redundancies, etc.), as well as the ability of the process controls to maintain the parameters within the design space. *Detectability* reflects the suitability of analytical methods and sampling plan for testing at the appropriate process stage(s) as well as the fitness-for-use of those methods.

Residual risk is used to assess the ability of the *occurrence* and *detectability* to ensure sufficient control of each quality attribute, taking into consideration its severity (Fig. 21.1). If the residual risk assessed for a given quality attribute is too high, then one or more of the following risk mitigation approaches can be undertaken, to provide for a more robust control strategy and/or demonstrate reduced risk based on improved product or process knowledge:

- Process control can be tightened by operating process parameter(s) within narrower range(s), or by imposing additional process controls.
- New data may be obtained that provide additional assurance of process capability and the effectiveness of process controls already in place.
- Redundant and/or tighter analytical controls, or improved analytical methods, may be introduced.
- The process can be optimized or redesigned to improve process capability.
- Further knowledge can be gained about the attribute to decrease the uncertainty or better characterize the impact, leading to a lower residual risk due to a reduced severity score.

Figures 21.2 through 21.4 illustrate the assessment of residual risk using the FMEA approach. In this example, severity, occurrence, and detectability considerations

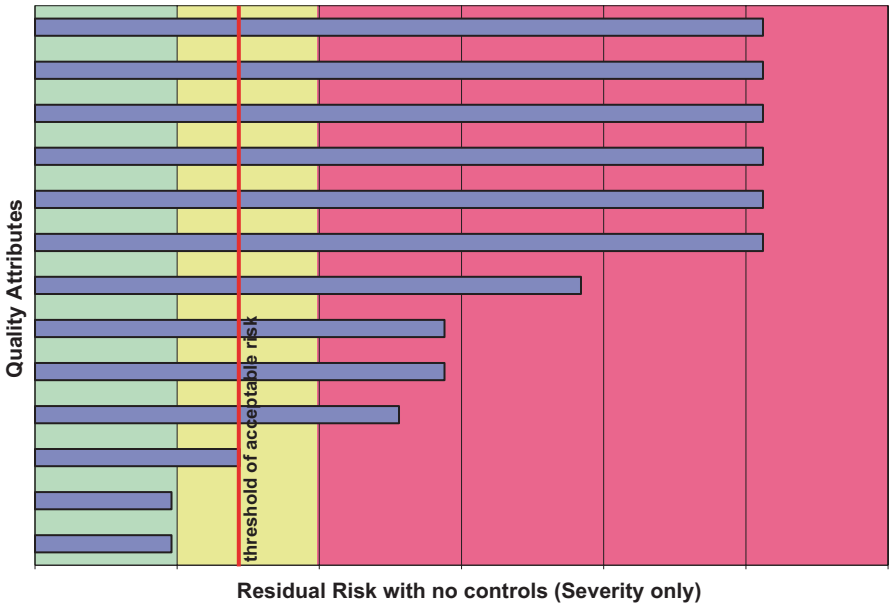

Fig. 21.2 Preliminary assessment of potential risk to patient (severity only). Process capability and control strategy not taken into account

are added sequentially for simplicity. Over the course of actual process and product development, these assessments are typically done in a more holistic, iterative fashion as depicted in Fig. 21.1. While the attributes are not identified in these examples, they are based on actual data for a monoclonal antibody product and therefore represent a realistic scenario of what can be expected in biopharmaceutical development.

Figure 21.2 shows an initial assessment of risk that takes only the severity of each of the quality attributes into account (not yet considering mitigation of risk through process and testing controls). The green zone in the diagram represents acceptable risk and the red zone unacceptable risk. All of the attributes exceeding the threshold of acceptable risk for severity (vertical red line in Fig. 21.2) are considered CQAs, because they must be controlled within an appropriate limit, range, or distribution if the desired product quality is to be ensured. (The red line is placed within a yellow zone to illustrate that it is often useful to think of criticality as a continuum rather than a sharply defined binary classification.) Without assurance of such controls they might present unacceptable risk to the patient. Control of these CQAs must therefore be the primary focus for product and process design, for optimization of analytical methods, and ultimately for the development of the control strategy for the commercial manufacturing process.

As development proceeds the manufacturing process is optimized for control of CQAs, process understanding is obtained through manufacturing experience and development studies, and the impacts of CPPs and their interactions on quality attributes are elucidated. These combine to make it possible to assess process capability

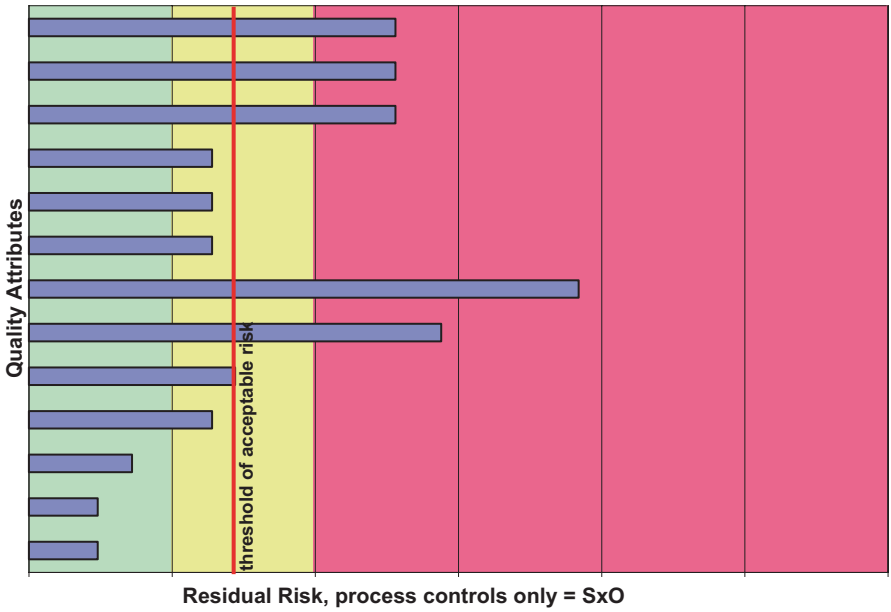

Fig. 21.3 Assessment of risk to patient after taking into account process capability and process controls (S × O). Release limits and other analytical testing controls are not taken into account

(occurrence "O"). Figure 21.3 shows an assessment of residual risk to the patient that accounts for both severity and occurrence (S × O); this can be thought of as the risk that the manufacturing process would produce product of unacceptable quality in the absence of further assurance through analytical testing. A comparison of Figs. 21.2 and 21.3 shows that process controls reduce the risk for many of the attributes. Indeed for several CQAs with the highest possible severity scores (Fig. 21.2), process controls alone are demonstrated to ensure minimal risk to the patient so that routine release testing or other routine analytical testing may not be required for control of those attributes.

Those attributes whose S × O scores in Fig. 21.3 exceed the threshold of acceptable risk represent the CQAs for which the residual risk is high after accounting for process capability. Exceeding the threshold in Fig. 21.3 is an indicator that release limits or other testing controls may be needed to ensure sufficient control of the attribute to provide a high level of confidence of low risk to the patient. This may be true for attributes that are expected to be stability limiting (e.g., aggregation in many products), or for which the understanding of process capability may be limited (e.g., particles for some products).

Once the proposal for release limits and other testing controls for the commercial product has been developed, both to address the concerns mentioned above as well as other considerations (e.g., testing mandated by compendial or regulatory guidance), the quality attribute risk assessment is completed with the inclusion of detectability (D) assessment based on the analytical control

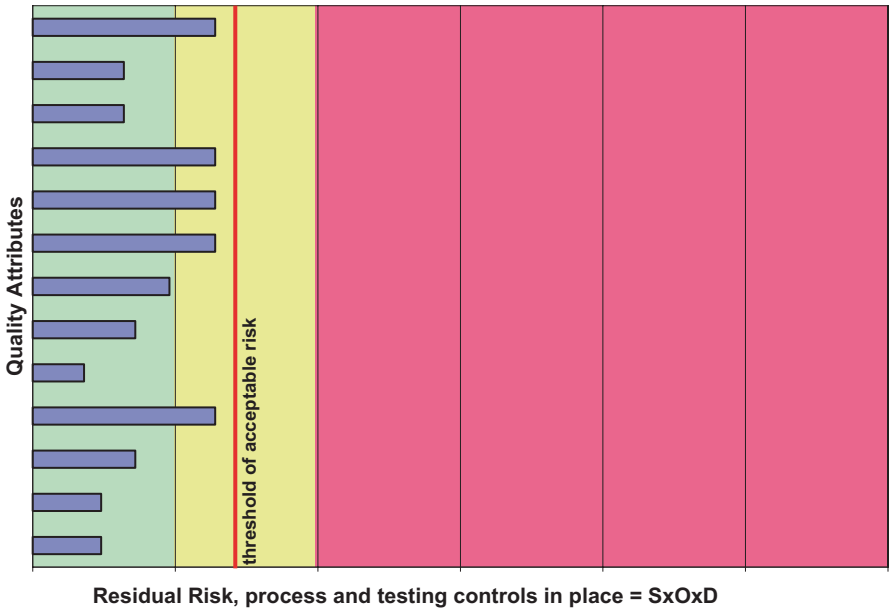

Fig. 21.4 Assessment of residual risk to patient after taking into account both process and analytical controls, based on the proposed commercial control strategy (S × O × D)

strategy. Figure 21.4 shows the final residual risk S × O × D, which takes into account all elements of the integrated control strategy, including both process and testing controls. Comparison to Fig. 21.3 shows that the residual risks for many of the attributes have been reduced through the inclusion of release limits or other testing controls such as in-process tests. All attributes finally fall below the threshold of acceptable risk, demonstrating that risks to the patient associated with these attributes are minimal under the proposed control strategy and specifications for the process and product.

Risk assessments such as these may also provide useful tools for lifecycle management of the product and process. At the time of marketing application, limited knowledge of the potential patient impact and of process capability may result in conservative estimates of severity and occurrence. As experience grows post-licensure, more knowledge may be gained regarding the severity of an attribute based on new in-house data or published literature. Process capabilities and variability are also likely to become better understood through long-term manufacturing experience and continued process verification. An updated quality attribute risk assessment taking all this into account can provide more accurate estimates of severity and occurrence. In some cases, these updated assessments may reveal effective control even in the absence of testing for a particular attribute. In such a case, the risk assessment provides a systematic instrument to justify proposals to drop analytical controls that are no longer needed, or other refinements to the control strategy.

21.2.3 Quality Attribute Severity Assessment

The tools used to assess components of risk throughout product development vary from company to company. Ideally quantitative or semi-quantitative bases for assessing and communicating risk are favored over nonquantitative methods. Like all measurement systems, however, quantitative risk scoring should be both accurate (i.e., specific to the outcome it is measuring) and precise (i.e., can be reproduced by a competent panel of measurers). Ideally potential patient impact should be mapped from the levels of a quality attribute to precisely defined outcomes such as the probabilities of specific impacts related to safety and efficacy. This ideal is seldom if ever met, however, due to the complexities of the inter-relationships between quality attributes and specific outcomes, and the diversity of individual patient outcomes. This is particularly true for complex molecules such as monoclonal antibodies and other therapeutic proteins, with some inherent heterogeneity for which clinical impact is often not well characterized.

The following example of a severity assessment approach utilizes a numerical scoring system to represent the potential Impact of an attribute on safety and efficacy, based on a variety of information sources. Since not all sources of information are equally certain, the scoring system accounts for the uncertainty in the impact assessment by elevating severity scores to reflect the potential for a more severe impact than what is indicated by the available data. For purposes of illustration, impact and uncertainty may be determined as follows.

Impact The impact of a quality attribute on safety and efficacy is determined based on the attribute's potential to cause harm to the patient. An example is provided in Table 21.3, which presents five distinct levels of potential patient harm.

Risk scoring of a quality attribute should be multivariate, to reflect the multiple ways by which variability in a quality attribute could potentially affect a patient. Thus, in this example, each quality attribute is assessed with respect to four impact categories, bioactivity, PK/PD, safety, and immunogenicity. The levels of potential patient harm are defined in Table 21.4.

To take the most conservative approach, the highest impact score among the four impact categories should be taken as the final severity score for that attribute.

Table 21.3 Levels of potential patient harm (impact)

Level	Definition
Very high	Life-threatening or irreversible impact (irreversible disease progression, other irreversible effects)
High	Nonlife threatening and reversible impact (reversible disease progression, other reversible effects)
Medium	Tolerable, manageable and transient impact
Low	Small but detectable impact
None	Negligible or no impact

Table 21.4 Definition of impact levels (potential patient harm) for each impact category

Level of potential patient harm	Impact categories			
	Bioactivity	PK/PD	Safety	Immunogenicity
Very high	Impact on bioactivity with potential life-threatening effects or irreversible disease progression[a]	Impact on PK/PD with potential life-threatening effects or irreversible disease progression due to loss of efficacy[a]	Irreversible or life-threatening AE	Immunogenic response observed with potential life-threatening effects or irreversible disease progression
High	Impact on bioactivity with nonlife-threatening loss of efficacy and reversible or no disease progression	Change in PK/PD with nonlife-threatening loss of efficacy and reversible or no disease progression	Reversible and nonlife-threatening AE, including those that result in stopping treatment or requiring hospitalization	Immunogenic response observed with nonlife-threatening effects and confers limits on safety or efficacy
Medium	Tolerable impact on bioactivity within the limits required for efficacy	Tolerable impact on PK/PD within the limits required for efficacy	Manageable and transient AE that does not require hospitalization; minimal intervention required	Immunogenic response observed with tolerable effects on safety or efficacy
Low	Small but detectable change in bioactivity	Small but detectable change in PK/PD	Minor and transient AE; asymptomatic or mild symptoms; intervention not required	Immunogenic response observed with minimal in vivo effects
None	Nondetectable impact on bioactivity	Non-detectable impact on PK/PD	No AE detected	Immunogenic response not observed

[a] Loss of efficacy can result in life-threatening effects or irreversible disease progression depending on indication

AE adverse event, *PD* pharmacodynamics, *PK* pharmacokinetics

Uncertainty When using various sources of information to establish a link between a quality attribute and the in vivo performance of the product, the source, the relevance, and the amount of information available should be accounted for in the severity scoring system. Considerations include the following:

- Data for the product being assessed or a related product.
- Experimental models used to study the impact; that is, in vitro or nonclinical models.
- Data from a literature source or generated in-house.

Table 21.5 provides an example of definitions for a three-level uncertainty scoring system.

Table 21.5 Definition of uncertainty levels for observed impacts

Uncertainty level	Uncertainty of observed impact
High	Impact of attribute is based on relevant scientific literature
Medium	Impact of attribute is predicted from laboratory or nonclinical studies with this molecule, or data from laboratory, nonclinical or clinical studies with related molecule in a relevant model/patient population
Low	Impact of attribute is established from clinical studies with this molecule

Table 21.6 Severity scoring system for assigned impacts and uncertainties

		Uncertainty		
		Low	**Medium**	**High**
Impact	**Very High**	32	32	32
	High	16	24	32
	Medium	8	12	18
	Low	4	6	9
	None	1	2	3

red = CQA; green = less-critical quality attribute

Severity Scoring and Determination of potential CQAs Based on the impact and uncertainty analysis, a severity score can be assigned to each quality attribute. An example of a severity scoring system is shown in Table 21.6, depicting progressively increasing severity with increasing impact and uncertainty levels. The following are some of the key features of this scoring system:

- The highest severity score of 32 is assigned for an attribute that has an impact level of "very high," regardless of the degree of uncertainty. This is because a life-threatening impact is the worst possible potential harm to a patient, and the severity will not decrease as a result of increased certainty.
- At impact levels of "high," "medium," "low," or "none," the severity score increases with increasing degree of uncertainty.

The severity scoring system is used to assess the criticality of the attribute. In this example, a threshold score of ≥ 9 is selected as a threshold to classify quality attributes as potentially critical. This choice of threshold ensures that an attribute with estimated low impact, but with high uncertainty around this impact assessment, will be conservatively classified as a CQA. However, an attribute for which there is high confidence than any patient impacts are tolerable (medium impact, low uncertainty) would not be classified as critical.

Interactions between attributes should be considered in assigning the severity score. This often means that two interacting attributes will receive the higher of the scores from either attribute. For example, the level of a formulation excipient that is added to control product aggregation during storage might receive the same severity scoring as the aggregate impurity itself.

The resulting severity scores for quality attributes are used to identify the attributes that pose high potential harm to the patients so they can be identified and their impact mitigated. Mitigation is achieved in part through development of an effective manufacturing process design and control strategy, but may also involve further studies and data analyses designed to increase product and/or process knowledge (Fig. 21.1). For example, a lower severity score (and thus lower overall risk) may result from improved understanding (i.e., reduced uncertainty) based on results from additional nonclinical and in vitro studies, or from a greater body of clinical experience.

21.3 Development of a Commercial Control Strategy

ICH defines control strategy as "A planned set of controls, derived from current product and process understanding that assures process performance and product quality. The controls can include parameters and attributes related to drug substance (DS) and DP materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control" (ICH Q10 2008). The commercial control strategy for a biopharmaceutical includes analytical, process and procedural components which together help ensure that final product is fit for its intended purpose. ICH Q8(R2 2009) provides the following guidance on these components: "At a minimum, those aspects of DSs, excipients, container closure systems, and manufacturing processes that are critical to product quality should be determined and control strategies justified. Critical formulation attributes and process parameters are generally identified through an assessment of the extent to which their variation can have impact on the quality of the drug product." The guidance also states that, at a minimum, these controls should include control of the CPPs and material attributes.

The FDA guidance on process validation (FDA Guidance for Industry 2011) notes that in an approach to process validation that employs risk-based decision-making throughout the lifecycle, criticality of attributes and parameters should be considered as a continuum rather than a binary state, and that all attributes and parameters should be reevaluated as new information becomes available.

This section describes the major components of the commercial biopharmaceutical control strategy. Those components are informed by the CQA risk assessment (Sect. 21.2.2).

21.3.1 Control of Material Inputs (Raw Materials and Components)

Control of raw materials, such as excipients, and components, such as container closure systems, should not be overlooked in developing a control strategy. In many cases there may be little impact of variability in these materials on product quality, and simple controls may be sufficient, for example, testing and releasing excipients to the standards of compendial monographs (e.g., USP, NF, or Ph.Eur.) However, monograph standards should be considered to represent a minimum requirement, and some products may be more sensitive to impurities. There is always a potential for raw material quality to affect product CQAs, which can be evaluated in systematic studies.

Products that are packaged and stored in prefilled glass syringes provide an example of a component that may require more elaborate controls supported by laboratory studies in order to assure product quality. Syringes typically contain residual levels of tungsten used in their manufacture, as well as low levels of silicone oil used as a lubricant. Silicone oil (Thirumangalathu et al. 2009) and tungsten (Jiang et al. 2009) have been demonstrated to be particularly impactful to stability of protein therapeutics, especially with respect to particle formation. Different protein molecules can vary in their sensitivity to these substances, so product-specific requirements on maximum tungsten and silicone oil levels in syringes are often determined, based on multivariate experimental studies of these impacts. In the case of silicone oil, a lower limit will often need to be set to ensure functionality of the syringe over the product shelf-life. If the syringe manufacturer's release limits are too broad to meet the requirements to ensure quality of the DP, it may be necessary for the biopharmaceutical manufacturer to set tighter, product-specific specifications that have been determined to be appropriate for a particular application. In such cases, it is important to evaluate the likelihood of failing these tighter specifications, in order to assure uninterrupted supply of drug to the market. It may be necessary to collaborate with the syringe manufacturer to develop custom syringes to ensure suitability for the particular product application.

21.3.2 Process Controls and Process Capability

Process control is exercised in part through the identification of CPPs and establishment of operating limits on those parameters. Both are informed by the requirements on CQAs and the relationships between parameters and attributes. A CPP is defined as "a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality" (ICH Q8(R2) 2009).

CPPs are typically defined by first applying prior knowledge, development experience, and risk assessment tools to evaluate which parameters might be expected to affect product quality. Experimental process characterization studies are then

conducted at small scale to elucidate the functional relationships that link the process parameters to CQAs. These studies are typically designed based on an understanding of the acceptable ranges for the CQAs and the capability of the manufacturing facility to control the process parameters. Multivariate design of experiments (DOE) approaches such as factorial designs or response surface designs are most frequently used because of their efficiency and capability of determining interactions between process parameters. However, models derived from DOE studies are typically empirical. Mechanistic models should be taken advantage of in designing process characterization studies whenever possible. Process characterization studies provide an opportunity to evaluate impacts of process parameters on process performance (e.g., yield, processing times) as well as on quality.

Once the functional relationships between process parameters and CQAs have been determined, CPPs can be defined using systematic risk assessment tools. Regulatory agencies and guidances do not dictate exactly which risk assessment approaches should be used for determining CPPs, but these approaches and the resulting CPPs are subject to regulatory review as part of the marketing application.

As discussed in Sect. 21.2.2 on CQA risk assessment, the knowledge gained from manufacturing experience, and from process characterization and other development studies enables the assessment of process capability to control each of the CQAs. Returning to the FMEA-based risk assessment approach described in that section, an occurrence or "O" score can be determined as a measure of the capability of the process to assure meeting the previously determined quality requirements for each of the CQAs. Considerations for assessing process capability should include assessment of controllability of each of the CPPs. Both the magnitude of the expected impact on each CQA and the ease of control of the CPP within the operating range that is required to ensure quality are considered. This is illustrated in Table 21.7. CPPs cannot be assumed to be the only source of process variability that can influence product quality. Other sources of variability (known and unknown) must be considered based on (for example) prior process experience at various scales, or experience with other similar products. This variability needs to be assessed in the context of how closely the level of the attribute approaches its acceptable limit. This can be done in a manner akin to a *Process Capability Index*, *Cpk* (Montgomery 2013). This is reflected in the bottom row of the table that assesses the overall variability of the process.

In this example, it can be seen that CQA #1 is affected by a number of CPPs all of which are well controlled. Thus there is little risk of going out of the acceptable range of the CQA. Experience with the process may also show that CQA #1 levels in the product always fall well within the acceptable range. Therefore process capability is excellent for this attribute, and the FMEA occurrence score "O" for CQA #1 will be low. In contrast, CQA #2 would receive a high "O" score based on overall process capability, despite the fact that no CPPs were identified. This might be the case for an attribute with very restrictive requirements or specifications, even though it is only minimally impacted by individual process parameters. As a third example, CQA #3 might receive a medium "O" score as there is one CPP (#1) that is difficult to control, perhaps because of a very tight range for that parameter; but overall process control of this CQA is robust.

Table 21.7 Evaluation of process capability based on CPP understanding

	CQA #1	CQA #2	CQA #3	••••••	CQA #N
CPP #1	No impact	No impact	Control difficult	••••••	Easy to control
CPP #2	Easy to control	No impact	No impact	••••••	No impact
CPP #3	Easy to control	No impact	No impact	••••••	Control difficult
• • •	• • •	• • •	• • •	• •••••• •	⋮
CPP #N	Easy to control	No impact	No impact	••••••	No impact
Overall Process Capability	Always well within acceptable limits for CQA	CQA near upper limit in some lots	Always well within acceptable limits for CQA	••••••	CQA near upper limit in some lots

21.3.3 In-Process Testing and Facility/environmental Controls

In-process tests and controls in biopharmaceutical DP manufacturing are often focused on controls to assure microbial control and sterility, for example, pre-filtration bioburden testing and filter integrity tests. For the same reason stringent facility and procedural controls are required, such as environmental and personnel monitoring.

21.3.4 Analytical Control Strategy

As described in Sect. 21.3.1 an analytical control strategy comprised in part of shelf-life and/or release limits may be necessary to reduce the residual risk to a patient. Both release and shelf-life limits may be used to control an attribute which changes over the shelf-life of a DS or a DP. Release limits alone are used when an attribute is subject only to manufacturing variability. A framework for the analytical control strategy is illustrated in the following series of figures (Fig. 21.5). The illustration depicts control of a CQA which is forecast to decrease over shelf-life (e.g., potency). Panel A shows minimum and maximum requirements for the attribute throughout product shelf-life. Requirements represent the true level or levels which must be maintained to ensure product quality. Panel B shows the minimum release limit, calculated from the estimated loss throughout a commercially desired shelf-life as well as the uncertainties associated with the estimate and release assay mea-

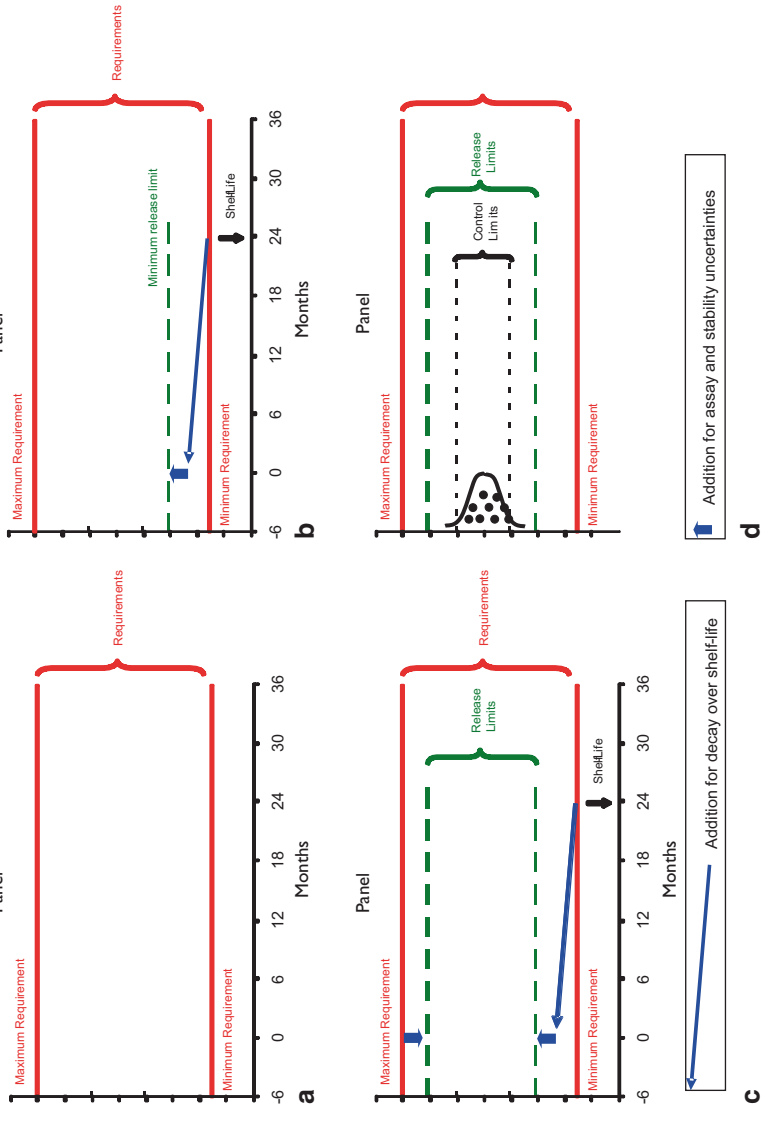

Fig. 21.5 Framework for the analytical control strategy

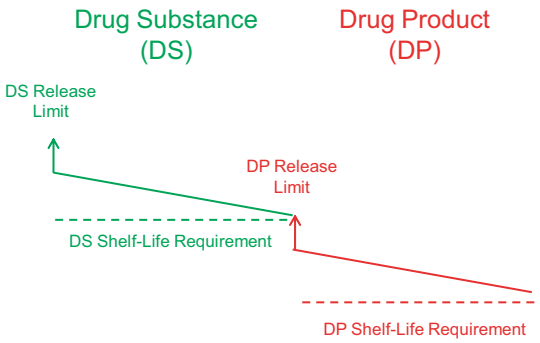

Fig. 21.6 Analytical control of DS and DP for an attribute which decreases over shelf-life (*sloped lines* represent degradation over shelf-life; *arrows* represent assay and degradation uncertainties; *dashed lines* are minimum requirements). *DS* drug substance, *DP* drug product

surement variability. A maximum release limit is likewise calculated accounting for release assay uncertainty in Panel C. It is important to note that the maximum release limit is administered only at the time of release of the DS or the DP. Panel D illustrates long-term manufacturing variability which is managed through control (alert) limits. Such limits might be included as part of a continued process verification (CPV) program, as the final stage of process validation (FDA Guidance for Industry 2011). The figure shows control limits which fall well within the release limits, signifying acceptable process capability in the attribute.

This basic framework constitutes analytical control of either DS or DP. A holistic view toward control of both is illustrated in Fig. 21.6. Here DS is controlled through a DS release limit that ensures that DP will meet the DP release limit when DS is formulated and filled.

Individual elements of the analytical control strategy are discussed in more detail in the Sect. 21.3.4.1 through 21.3.4.5.

21.3.4.1 Requirements

Requirements are the foundation for risk-based development of the analytical control strategy. The requirements on CQAs are the true limits that the product is controlled within. Unlike release limits these are not test limits. They are the true levels that an attribute must meet to ensure quality. A risk-based approach to analytical control may use these requirements to establish release limits and an approach for commercial stability of the product. These limits manage risk through acknowledgement of the uncertainty in the release measurement and stability predictions.

21.3.4.2 Release Limits

ICH defines specifications as "a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described" (ICH Q6B 1999). It goes on to say "The setting of specifications for drug substance and drug product is part of an overall control strategy

which includes control of raw materials and excipients, in-process testing, process evaluation or validation, adherence to Good Manufacturing Practices, stability testing, and testing for consistency of lots." Thus specifications are used to manage product quality and are only one part of the overall control strategy.

ICH goes on to define release limits as follows. "The concept of release limits vs. shelf-life limits may be applied where justified. This concept pertains to the establishment of limits which are tighter for the release than for the shelf-life of the drug substance or drug product. Examples where this may be applicable include potency and degradation products." An appropriate basis for distinguishing release and shelf-life limits is to include the estimated change in a stability indicating attribute together with the variability associated with that estimate, as well as release assay variability.

When the release limit is not met the lot is called out of specification (OOS) and is quarantined from release. The decision to release the lot is based upon a risk assessment made by the quality function of the company. Guidance is given in the FDA *Guidance for Industry 2006, Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production.*

As discussed above, excipient levels and other formulation properties can be CQAs through their impacts on other CQAs. Setting release limits for the former attributes should therefore depend in part on the levels shown to provide sufficient control over the latter attributes. For example, both surfactant concentration and formulation pH can affect rates of particle formation in liquid DP, but the degree to which this is true can be highly product-dependent. Therefore product specific studies are recommended to elucidate these relationships. These studies may be multivariate or univariate; the appropriate study design may be chosen based on risk assessments that leverage understanding from earlier stages of development for the product.

21.3.4.3 Control Limits

Control limits (alert limits) are typically based on product performance and used to monitor a manufacturing process for potential shifts and trends in a quality attribute. These are forecast from manufacturing modeling or calculated from manufacturing data using statistical process control (SPC) methods such as Shewhart limits or tolerance limits. Those limits may be used to earmark an attribute of an individual lot as being atypical, or may earmark a manufacturing event with rules which detect a trend or a shift across a series of lots. This approach could be formally incorporated into a continued process verification (CPV) program as a part of a company's process validation strategy.

Control limits and the rules for acting upon the limits are typically a part of the manufacturer's quality system. Unlike release and stability specifications control limits are used to manage the manufacturing process. When the numerical requirement or rule is not met the lot is called out of trend (OOT) and a manufacturing investigation is undertaken. Results of the investigation may reveal a systemic

problem with either the manufacturing process or an assay. A risk assessment may show that there is no impact to product quality or corrective action may be taken to bring the process back into control. If it is determined that there is no impact to product quality the control limits may be updated or the rules amended to address the nature of the trend. The conduct of the investigation together with corrective action should be part of the manufacturer's quality system and subject to inspection by a competent regulatory authority. In the meantime the lot may be released by the quality function.

21.3.4.4 Process Capability

Adequate process capability is essential for long term supply of drug to the market. Process and product characterization studies are utilized during development to verify process capability due to normal variation in the process parameters. However, long-term process performance includes routine events which may result in changes in process capability. Long term process performance in a CQA is depicted in Fig. 21.7. Panel A shows the distribution of clinical development lots which have been manufactured at set points of the manufacturing process parameters. Release limits (dashed lines) are typically calculated from these data using statistical methods. Process and formulation experiments are subsequently performed under parameter conditions which can be maintained under routine manufacturing conditions. The resulting distribution of experimental results is illustrated in Panel B. Remaining panels show product performance after qualification of a new working reference standard (Panel C), after a planned process change (Panel D) and due to the introduction of a raw material from an alternative vendor (Panel E). Setting limits based on a few clinical development lots does not acknowledge the product lifecycle, creating a vulnerability to commercial supply of a much needed drug. Limits should be set which balance clinical experience with development lots and the realities of lifecycle management.

21.3.4.5 Comparability

One approach to managing the impacts of routine process and analytical changes is comparability. Process and analytical changes are inevitable in the lifecycle of a biopharmaceutical product. Change control is necessary to bridge process or analytical performance across the change. Those changes include but are not limited to:

- Change from development scale to full-scale manufacturing
- Transfer to a manufacturing facility
- Change or addition of a process step
- Introduction of a new raw material/component or a new vendor of an existing raw material/component
- Method transfer

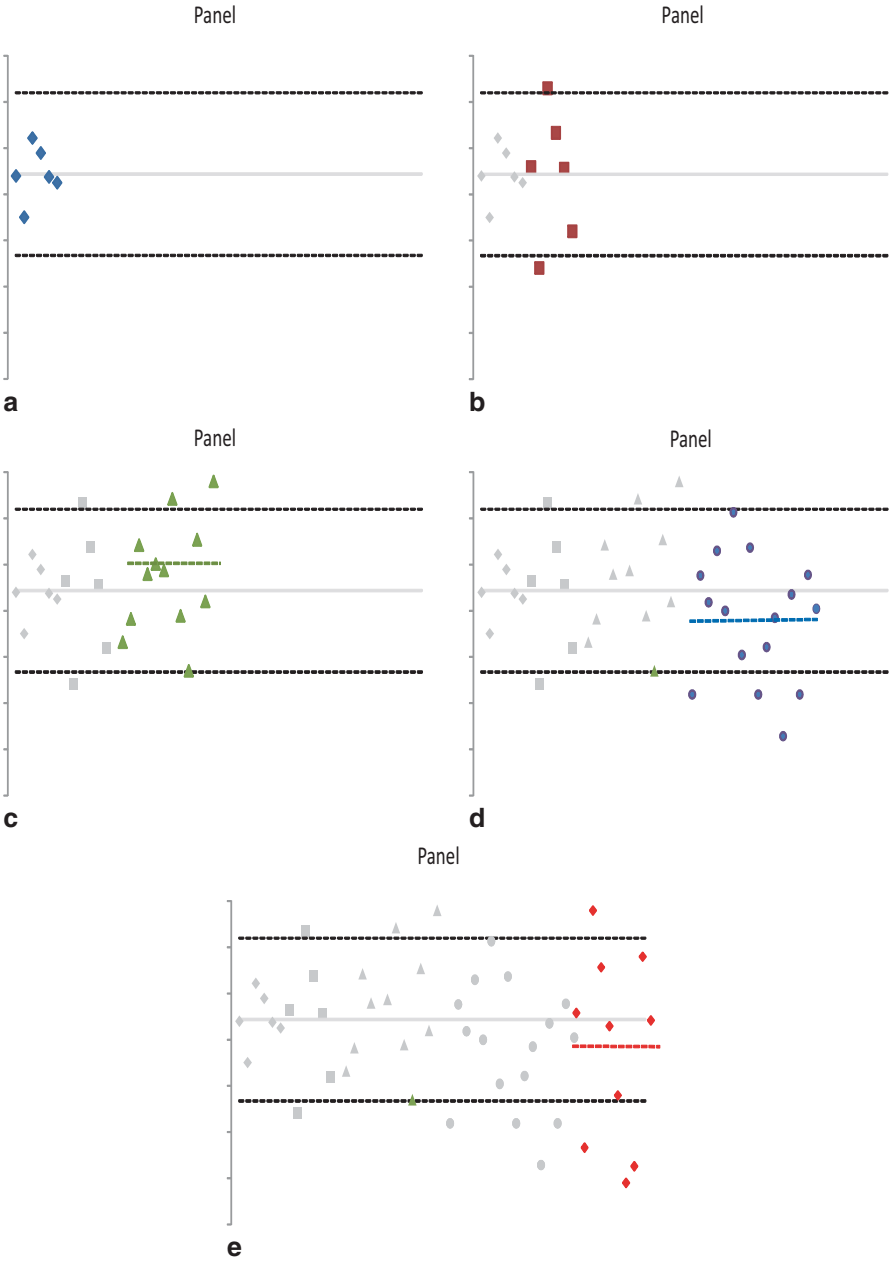

Fig. 21.7 Long-term process performance: Panel A, development lots; Panel B, characterization experience; Panel C, standard qualification; Panel D, process change; Panel E, new raw material vendor

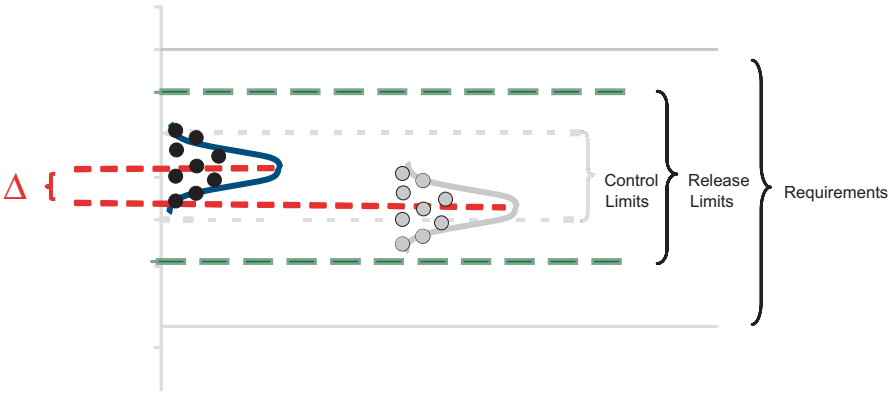

Fig. 21.8 Illustration of the determination of an equivalence margin for comparing distributions after a process or analytical change

- Qualification/calibration of a new reference standard
- Introduction of a new vendor for a critical reagent of an assay

Change is necessary and some changes are predicted to result in equal or better outcomes. Change management which is intended to improve product quality or process consistency may be carried out differently from change management that assesses the impact of a change which has unknown or uncertain impact. The approach taken should be accompanied by a well-defined risk assessment approach. That risk assessment may result in a comparability study, a study intended to assess the hypothesis that the change has resulted in no meaningful impact to the performance of the manufacturing process or the assay. Specifically, the product or an assay can be considered comparable if performance is similar before and after a change.

Risk based methods such as equivalence testing can be used to establish that materials made by one process are "equivalent" to materials made by another process, or that changes such as method transfer or introduction of a new working reference standard have not resulted in a meaningful shift in apparent process performance.

A key component of equivalence testing is an equivalence margin (Δ). That margin can be established as a difference in an attribute that will result in continued satisfactory process capability. This is illustrated in Fig. 21.8 in the case of the scenario depicted in Panel D of Fig. 21.5.

The distribution yielding the measurements before the process or analytical change is shown as a normal curve on the left side of the graph. That distribution can shift downward until it begins to impinge on the lower release limit predicting a higher likelihood of an OOS result. This shift (Δ) in the means for the two distributions is the difference which is predicted to result in continued satisfactory process capability. This approach changes the paradigm from demonstrating "no difference" in process performance to demonstrating that the difference does not have product impact. It should also be emphasized that the shift still falls well within the release limits, ensuring that manufactured product still meets its requirements throughout shelf-life.

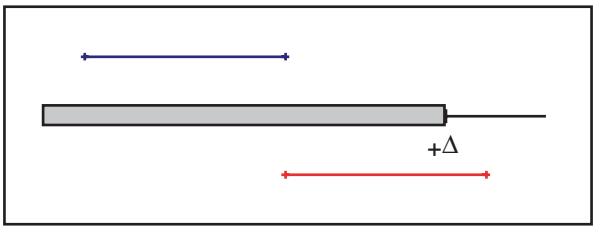

Fig. 21.9 Illustration using the 90% confidence interval to demonstrate equivalence. The *blue* interval falls within the equivalence boundary signifying equivalence; the *red* interval falls partially outside and, thus, equivalence cannot be concluded

A formal equivalence approach proceeds in determining the sample sizes (numbers of lots and/or the number of independent assay determinations) required to minimize the risks of concluding that the processes are not equivalent when they are, and of concluding they are equivalent when they are not. In some cases due to time or production constraints the approach will use data from a fixed number of lots. In this case the risk of failing to conclude equivalence may be calculated rather than the sample size as a basis for proceeding.

The data resulting from testing of the materials is analyzed using a two one-sided test (TOST). This is illustrated in Fig. 21.9.

A 90% confidence interval on the difference in means for the two processes (or some other comparison) is calculated. The processes are declared equivalent if the confidence interval falls within the equivalence margin ($\pm\Delta$). It is concluded that there is insufficient evidence to declare equivalence if the interval falls partially or wholly outside the range. This does not mean that the processes are not equivalent. This conclusion may be due to a failure in the assumptions associated with the equivalence method (e.g., the variabilities utilized to calculate the sample size or the study risks are greater than expected); or the fixed sample sizes were too small to establish equivalence (i.e., the width of the confidence interval is too wide to fall within the equivalence margin due to too few of lots because of time or factory constraints).

A conclusion that there is insufficient evidence to declare equivalence may be used to illustrate one of the advantages of the equivalence approach. The equivalence approach rewards work; that is, the risks of drawing the wrong conclusion are minimized by collecting data on more lots or performing more independent runs of an assay. Other approaches such as tracking and trending penalize work. An approach which uses ranges to show equivalence of individual lots has greater risk of failing with increases in the number of lots evaluated, and is further flawed in potentially missing a meaningful shift in the process.

The equivalence approach has other benefits in addition to controlling the risk of drawing the wrong conclusion from the comparability study and rewarding rather than penalizing work.

- The equivalence approach is conservative. In statistically controlling the study risks the true difference between processes is likely to be considerably smaller than the equivalence margin.
- Similar to the paradigm of interim analysis in clinical trials, if, when the study concludes there is insufficient evidence to declare equivalence, the laboratory has the opportunity to include more lots to decrease the study risks. The equivalence approach might be coupled with CPV to manage the risks of associated with a change.

Approaches taken for comparability assessment can also be simple extensions of routine change control (e.g., tracking and trending of CPPs and CQAs for the product, controls for an assay) and/or may be carried out as a separately designed study. A designed study employs additional characterization components such as nonroutine assays in the case of a process change or nonroutine analytical performance measures in the case of a change in an assay. ICH Q5E 2004 provides guidance on comparability assessment principles to be followed for changes in manufacturing processes. As a separately designed study comparability should be managed through a study protocol. The protocol may include: (1) a risk assessment mapping the change to quality attributes or assay performance measures, which may be impacted by the change; (2) a study design including materials, the potentially impacted quality attributes and their tests, and statistical considerations regarding the amount and strategy of testing; (3) prescribed acceptance criteria on study results; (4) a data analysis plan. Acceptance criteria should be determined on the basis of impact of a resulting change on quality to the patient. Historical data may be used to facilitate study design or to evaluate the risks associated with drawing the wrong conclusion from the study.

When the comparability protocol for a proposed change is included in the marketing application it becomes a part of the product license along with other measures of control. The comparability protocol is reviewed by the competent regulatory authority to ascertain that implementation of the plan will likely result in continued quality to the customer. Appropriate application of the approved plan should subsequently be subject to a reduced level of review and approval by the competent authority.

While not in the scope of this chapter the assessment of corrective action, the result of a manufacturing or analytical investigation, may be carried out using principles similar to those used for comparability. The study may show that the outcome of the corrective action has resulted in comparable performance after its introduction.

21.4 Discussion

This chapter has provided the reader with a high-level overview of three essential components of the control of a biopharmaceutical product: (1) the process of identifying CQAs; (2) elements of the process, formulation, and analytical control of commercial product; (3) the associated concept of a comparability study, which may

be used to help manage changes to the manufacturing and analytical processes, and thereby facilitate management of the quality of the product. The concepts presented in this chapter are neither required nor the only principles employed by industry and regulators. These serve in some cases as a framework for development principles and commercial control, and in other cases as approaches to these principles. Many approaches are utilized throughout the pharmaceutical industry, and some of these have been accepted by regulatory authorities and become standard practice. The authors and contributors to the chapter believe that a thoughtful approach to QbD is the foundation of a more progressive approach to delivering quality medicines to the patients in need.

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Chapter 22
Multivariate Analysis for Process Understanding, Monitoring, Control, and Optimization of Lyophilization Processes

Theodora Kourti

22.1 Introduction

The introduction of the Quality by Design (QbD) framework has changed the approach to process understanding and control in the pharmaceutical and biopharmaceutical industry. Process modeling is an integral part of the QbD framework. Models can be derived to assist in process development, process understanding, design space determination, on-going process verification (multivariate statistical process control models), as well as process control (Feedback or Feedforward), thereby making modeling a part of the product lifecycle (Kourti 2010). The International Conference of Harmonization (ICH 2011), recognizing the importance of modeling has issued points to consider when describing a model in a regulatory submission. The models may be mechanistic based on first principles, or empirical based on appropriate data, or hybrid. Models are available to deal with batch and continuous operations. Lyophilization is currently performed in batch operations. Batch processes are dynamic, nonlinear, and of finite duration, and the process variables are both auto- and cross-correlated; these characteristics should be considered when choosing the appropriate approaches to model batch operations.

Batch unit operations may be described with first principles models when the chemical, biochemical, and physical processes that are taking place in the batch vessel are well understood; examples involving first principles modeling are the attempts to build the design space for the primary drying of the pharmaceutical freeze drying process (Fissore et al. 2011; Koganti et al. 2011) and use of modeling to develop soft sensors for monitoring (Bosca and Fissore 2013). When appropriate data are available, empirical (data driven) models can also be developed to address certain problems. For example, empirical models can be used to analyze available historical data from past batch runs for process understanding and troubleshooting. Furthermore, empirical models may be used for monitoring to establish that the

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process is in a state of statistical process control. For process monitoring purposes, the batch is checked against expected behavior; this may happen in real time as the batch evolves, or in a "post-run analysis" immediately after the batch has been completed. Different types of models are required for each objective, and in the case of data-based models different types of data are used to derive these models. Therefore, under the very general expression of "multivariate models in a QbD framework," there are many problems that can be addressed with a corresponding large number of methods available to address each problem. The objective of this chapter is to give practitioners the main principles and fundamentals in order to assist them to select appropriate multivariate models depending on the objectives of the analysis in a lyophilization process.

22.2 Nature of Data for Batch and Semibatch Processes

Data collected from batch processes come from a variety of sources and cover a range of different formats; such data are process variable trajectories (e.g., measurements obtained at frequent intervals for the duration of the freezing, primary drying, and secondary drying from variables like shelf temperature, chamber pressure, condenser temperature, as shown in Fig. 22.1), analyzer spectral data (e.g., mass spectrometer for residual gas analysis) and measurements from other hard or soft sensors (Jameel and Kessler 2011), batch product quality data (e.g., residual

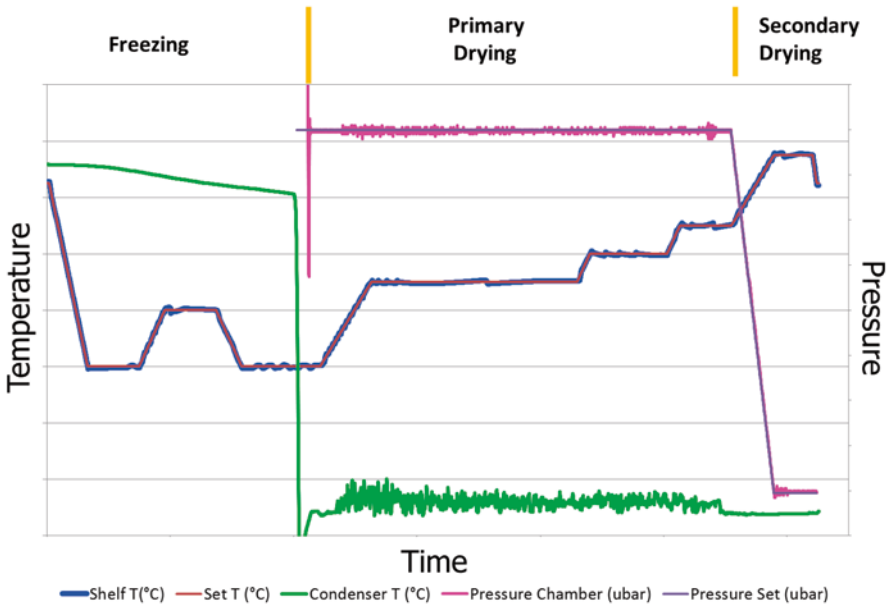

Fig. 22.1 Measurements of variables vs. time—trajectories in a lyophilization process

moisture content, reconstitution time, shelf life); other data such as information on formulation parameters and component preparation may also be included. As a result very large datasets are accumulated. These datasets or, subsets of them, may be used in different ways to build models to analyze process and product performance behavior. Multivariate models can be built for the lyophilization process to address the following objectives (different types of data and different types of models are required to address each one of these problems):

- Relate spectral data to residual gas concentration
- Process understanding/troubleshooting, using variable trajectory data from many batches
- Infer at the end of a batch run the final product quality from process conditions during production (e.g., without using lab tests)
- Get an estimate of the final quality at a given time during the run from process measurements and decide on midcourse correction of variable trajectories to control final quality
- Establish an overall "process signature" and monitor it during the batch, to determine that the batch progresses in a similar fashion with previous typical batches of good quality
- Detect unusual/abnormal behavior in the process, equipment, or product (either in real-time or post-run analysis). Consider appropriate modeling and control limits to detect faults (variable weights, choice of components, type of model)
- Establish operational knowledge and build appropriate models that can be used for product transfer and scale-up
- Explore feed forward control possibilities
- Establish best operating conditions to satisfy certain criteria (quality, cost, safety, environmental requirements, etc.)
- The structures of the data that are typically available are described below:

Trajectories: Process variables are being recorded for the duration of freezing, primary drying, and secondary drying at different time intervals (shelf temperature, product temperature, condenser temperature, chamber pressure, pressure into the pumps, etc.). Using the trajectory information, introduces a complex data structure that brings very rich information about the auto- and cross-correlation of variables for the duration of the batch and leads to detailed understanding of the process.

Summary data: Sometimes, despite the fact that full trajectories are recorded, only summary data like the minimum, maximum values, rates, total time for an operation, etc., are reported for the full run or for different phases of the run. Examples of summary data are average condenser temperature, times of the different steps, slopes of some variable trajectories, etc. With summary data the detailed information on the trajectories is lost; however, by capturing key characteristics one might be able to use this information for certain types of simpler models in order to address certain problems with less modeling effort.

Other Data related to the Batch Process: Product quality properties ($\boldsymbol{y}_{it}$) which typically are measured at the end of the batch process at time t. When utilizing these data for modeling, one should keep in mind that these properties (e.g., residual

moisture content, reconstitution time, shelf life, etc.) are a function of the process conditions at time t, but also a function of the process conditions that prevail several lags before, and in most cases a function of the conditions prevailing during the entire batch and of course the formulation.

Other data related to a batch process may be formulation parameters (composition, excipient ratios, etc.), information on equipment preparation as well as suites and operators involved. Data may also be collected from analyzers in the form of spectral data.

22.3 Latent Variable Modeling for Two-Way Matrices

Latent variable based methods are most suitable for modeling data retrieved from process databases that consist of measurements on large numbers of variables that are highly correlated. Latent variables exploit the main characteristic of these correlated variables that the effective dimension of the space in which they move is very small. Models related to two-way matrices are discussed first. Examples of two-way matrices are: a ($n \times k$) matrix **X** consisting of measurements from n batches on k summary data from process variables (minimum temperature, maximum temperature, rate, duration); the corresponding ($n \times m$) matrix of product quality data **Y**; a ($n \times r$) matrix **Z** with information on r formulation parameters.

For a dataset consisting of a ($n \times k$) matrix of process variable measurements **X** and a corresponding ($n \times m$) matrix of product quality data **Y**, for linear spaces, latent variable models have the following common framework (Burnham et al. 1996):

$$\mathrm{X} = \mathrm{T}\,\mathrm{P}^{\mathrm{T}} + \mathrm{E} \tag{22.1}$$

$$\mathrm{Y} = \mathrm{T}\,\mathrm{Q}^{\mathrm{T}} + \mathrm{F} \tag{22.2}$$

where **E** and **F** are error terms, **T** is an ($n \times A$) matrix of latent variable scores, and **P** ($k \times A$) and **Q** ($m \times A$) are loading matrices that show how the latent variables are related to the original **X** and **Y** variables. The dimension, A, of the latent variable space is often quite small and it is determined by cross-validation or some other procedure. (Jackson 1991; Wold 1978).

Latent variable models assume that the data spaces (**X**, **Y**) are effectively of very-low dimension (i.e., nonfull rank) and are observed with error. The dimension of the problem is reduced by these models through a projection of the high-dimensional **X** and **Y** spaces onto the low-dimensional latent variable space **T**, which contains most of the important information. By working in this low-dimensional space of the latent variables (t_1, t_2, ... t_A), the problems of process analysis, monitoring, and optimization are greatly simplified. There are several latent variable methods. Principal component analysis (PCA) models only a single space (**X** or **Y**) by finding the latent variables that explain the maximum variance. Principal components

(PC) can then be used in regression (PCR). Projection to latent structures or partial least squares (PLS) maximizes the covariance of **X** and **Y** (i.e., the variance of **X** and **Y** explained, plus correlation between **X** and **Y**). There are other methods, as for example, reduced rank regression (RRR), canonical variate analysis (CVA), that are discussed in detail elsewhere (Burnham et al. 1996).

The choice of method depends on the objectives of the problem; however, all of them lead to a great reduction in the dimension of the problem. PCR and PLS model the variation in the **X** space as well as in the **Y** space. This point is crucial in most of the applications related to process understanding, process monitoring, and process control as well as for the problem of treating missing data. The properties of PCA and PLS are discussed briefly in the following sections as well as their use for historical data analysis, troubleshooting, and statistical process control.

22.3.1 Principal Component Analysis (PCA)

For a sample of mean centered and scaled measurements with n observations on k variables, **X**, the PC are derived as linear combinations $\mathbf{t}_i = \mathbf{X}\mathbf{p}_i$ in such a way that, subject to $|\mathbf{p}_i| = 1$, the first PC has the maximum variance, the second PC has the next greatest variance and is subject to the condition that it is uncorrelated with (orthogonal to) the first PC, etc. Up to k PCs are similarly defined. The sample PC loading vectors $\mathbf{p}_i$ are the eigenvectors of the covariance matrix of **X** (in practice, for mean centered data the covariance matrix is estimated by $(n-1)^{-1}\,\mathbf{X}^T\mathbf{X}$). The corresponding eigenvalues give the variance of the PCs (i.e., var $(\mathbf{t}_i) = \lambda_i$). In practice, one rarely needs to compute all k eigenvectors, since most of the predictable variability in the data is captured in the first few PCs. By retaining only the first A PCs, the **X** matrix is approximated by Eq. 22.1.

22.3.2 Partial Least Square (PLS)

PLS can extract latent variables that explain the high variation in the process data, **X**, which is most predictive of the product quality data, **Y**. In the most common version of PLS, the first PLS latent variable $\mathbf{t}_1 = \mathbf{X}\mathbf{w}_1$ is the linear combination of the x-variables that maximizes the covariance between $\mathbf{t}_1$ and the **Y** space. The first PLS weight vector $\mathbf{w}_1$ is the first eigenvector of the sample covariance matrix $\mathbf{X}^T\mathbf{Y}\mathbf{Y}^T\mathbf{X}$. Once the scores for the first component have been computed, the columns of **X** are regressed on $\mathbf{t}_1$ to give a regression vector, $\mathbf{p}_1 = \mathbf{X}\mathbf{t}_1/\mathbf{t}_1{}^T\mathbf{t}_1$; the **X** matrix is then deflated (the **X** values predicted by the model formed by $\mathbf{p}_1$, $\mathbf{t}_1$, and $\mathbf{w}_1$ are subtracted from the original **X** values) to give residuals $\mathbf{X}_2 = \mathbf{X} - \mathbf{t}_1\mathbf{p}_1{}^T$. **Q** are the loadings in the **Y** space. In one of the PLS algorithms, $\mathbf{q}_1$ is obtained by regressing $\mathbf{t}_1$ on **Y,** then **Y** is deflated $\mathbf{Y}_2 = \mathbf{Y} - \mathbf{t}_1\mathbf{q}_1{}^T$. The second latent variable is then computed from the residuals as $\mathbf{t}_2 = \mathbf{X}_2\mathbf{w}_2$, where $\mathbf{w}_2$ is the first eigenvector of $\mathbf{X}_2{}^T\mathbf{Y}_2\mathbf{Y}_2{}^T\mathbf{X}_2$, and so on.

The new latent vectors or scores ($\mathbf{t}_1$, $\mathbf{t}_2$,…) and the weight vectors ($\mathbf{w}_1$, $\mathbf{w}_2$,…) are orthogonal. The final models for **X** and **Y** are given by Eqs. (22.1) and (22.2).

22.3.3 Lyophilization Application Examples

The models described above are applicable to two-dimensional arrays **X** and **Y**, and there have been several reports from applications to lyophilization examples.

1. In one type of applications, **X** contains data from formulation and **Y** data from the corresponding quality. PLS can be used to relate **X** and **Y**.

a. An example of application of PLS is a study of lyophilized protein formulations of progenipoietin (ProGP), a potential protein therapeutic agent, to determine the relative importance of certain formulation variables that affect long-term storage stability of this therapeutic protein (Katayama et al. 2004). Using PLS, a retrospective analysis was conducted of 18 formulations of progenipoietin. The relative importance of composition, pH, maintenance of protein structure (as determined by infrared (IR) spectroscopy), and thermochemical properties of the glassy state (as measured by differential scanning calorimetry) were evaluated. Various stability endpoints were assessed and validated models constructed for each using the PLS method. Retention of parent protein and the appearance of degradation products could be adequately modeled using PLS.

2. The **X** matrix may contain spectral data and **Y** the corresponding property we wish to monitor; in that case PLS can be used to predict this property (be it residual gas composition, moisture, etc.) from spectral data.

   a. An example of the use of PLS to determine moisture from NIR can be found in a study to develop and optimize a fast, inexpensive, noninvasive, and nondestructive method for determination of moisture content in lyophilized mannitol, based on an NIR microspectrometer instead of a conventional NIR spectrometer. (Muzzio et al. 2011).
   b. An earlier work that uses principle components together with NIR for in situ monitoring of the lyophilization process is that of Brülls et al. (2003).
   c. Bai et al. (2005) used PLS to compare NIR and Fourier transform infrared (FTIR) spectroscopy for monitoring structural changes in lyophilized protein formulations.
   d. Maltesen et al. (2011) used multivariate analysis to evaluate the residual phenol content in spray-dried and freeze-dried insulin formulations by Fourier transform infrared (FTIR) spectroscopy and near infrared (NIR) spectroscopy. PCA and PLS projections were used to analyze spectral data.
   e. Excipient selection is critically affecting the processing and the stability of a lyophilized product. Grohganz et al. (2010) applied NIR spectroscopy to investigate freeze-dried samples containing varying ratios of the commonly used excipients mannitol and sucrose. They utilized PCA to project the NIR

spectra of the freeze-dried samples and to investigate the clusters formed. They concluded that NIR can analyze versatile freeze-dried samples and classify these according to composition, water content, and solid-state properties.

f. Yip et al. (2012) presented a method, called Main and Interactions of Individual Principal Components Regression (MIPCR) that was used to model NIR data and they claimed to have significantly increased predictive ability of moisture content compared to a traditional PLS approach. It will be interesting to see the performance of the method in other examples.

22.3.4 Using Latent Variable Methods for Historical Data Analysis and Troubleshooting

Latent variable methods can be used for troubleshooting. By plotting the latent variables (t_1, t_2,…, t_A) against each other, the behavior of the original data set [be it formulation or summary process data (**X**), or quality data (**Y**)] can be observed on the projection space. By examining the behavior in the projection spaces, outlier and cluster detection becomes easy, and one can observe batches with typical behavior and unusual batches, as shown in Fig. 22.2. An interpretation of the process movements in this reduced space can be obtained by examining the loading vectors ($\mathbf{p}_1$, $\mathbf{p}_2$,…, $\mathbf{p}_A$) in PCA or the weights ($\mathbf{w}_1$, $\mathbf{w}_2$,…, $\mathbf{w}_A$) in the case of PLS, and the contribution plots.

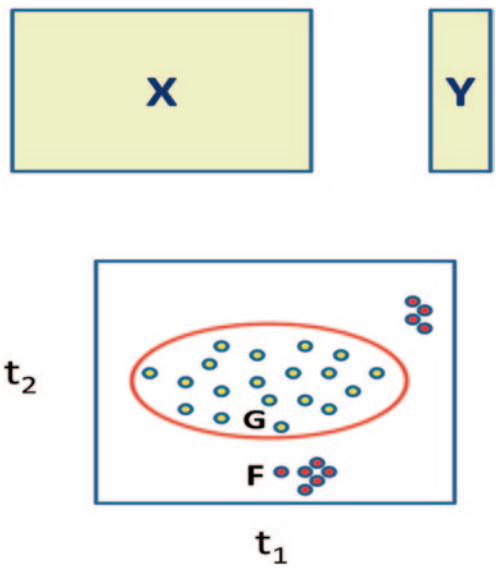

Fig. 22.2 Latent variable methods can be used to relate **X** to **Y**. A powerful characteristic of these methods is that they can model the data in the **X** space and they can be used for troubleshooting, by projecting the data into low-dimensional spaces

Contributions to the Score Deviations: A variable contribution plot indicates how each variable involved in the calculation of that score contributes to it. For example, for process data **X**, the contribution of each variable of the original data set to the score of component q is given by:

$$c_j = p_{q,j}(x_j - \bar{x}_j) \text{ for PCA and } c_j = w_{q,j}(x_j - \bar{x}_j) \text{ for PLS between } X \text{ and } Y \qquad (22.3)$$

where c_j is the contribution of the jth variable at the given observation, $p_{q,j}$ is the loading and $w_{q,j}$ is the weight of this variable to the score of the PC q and $\bar{x}_j$ is its mean value (which is zero form mean centered data).

As an example consider Fig. 22.2 which illustrates that some clusters of points were observed on a t_1 vs. t_2 plot. The use of contribution plots may help to investigate which variables contribute to the difference between a good batch (point G) and a failed batch (F). So Eq. (22.3) would give the contribution of variable j to the move of the score values between two observations (say, G and F) for component *2* is calculated as $p_{j2} \times (x_j, \text{G} - x_j, \text{F})$ for PCA and $w_{j2} \times (x_j, \text{G} - x_j, \text{F})$ for PLS between **X** and **Y**, where p_{j2} is the loading of variable j on component *2* and w_{j2} is the weight of variable j on component *2*.

22.3.5 Using Latent Variable Methods for Statistical Process Control

From routine operation we can establish acceptable limits of good process behavior. On a t_1 vs. t_2 plane, such limits will take the form of an ellipse, as shown in Fig. 22.2. When the process is in statistical control, the points will be within the ellipse. If there is a problem in the process, the points will plot out of the ellipse.

To monitor the process in real time, however, it would have become cumbersome to have to plot all combinations of PCs. A statistic (Hotelling's T^2) can be calculated and the overall variability of the main events of the system can be monitored with a single chart. The Hotelling's T^2 for scores is calculated as:

$$T_A^2 = \sum_{i=1}^{A} \frac{t_i^2}{\lambda_i} = \sum_{i=1}^{A} \frac{t_i^2}{s_{t_i}^2} \qquad (22.4)$$

where $s_{t_i}^2$ is the estimated variance of the corresponding latent variable t_i. This chart, calculated by using the first A important PC's, essentially checks if a new observation vector of measurements on k process variables projects on the hyperplane within the limits determined by the reference data.

As mentioned above, the A PCs explain the main variability of the system. The variability that cannot be explained forms the residuals (squared prediction error, SPE). In some software packages the term distance to the model (DModX) is used.

This residual variability is also monitored and a control limit for typical operation is being established. By monitoring the residuals we test that the unexplained disturbances of the system remain similar to the ones observed when we derived the model. It is therefore important to check the validity of the model by checking the type of disturbances affecting the system. When the residual variability is out of limit, it is usually an indication that a new set of disturbances have entered the system; it is necessary to identify the reason for the deviation and it may become necessary to change the model.

SPE_X is calculated as:

$$SPE_X = \sum_{i=1}^{k} (x_{new,i} - \hat{x}_{new,i})^2 \tag{22.5}$$

where $\hat{\boldsymbol{x}}_{\boldsymbol{new}}$ is computed from the reference PLS or PCA model. Notice that SPE_X is the sum over the squared elements of a row in matrix **E** in Eq. (22.1). This latter plot will detect the occurrence of any new events that cause the process to move away from the hyperplane defined by the reference model.

The above nomenclature applies if the scores were determined from a PCA on the **X** matrix or a PLS between **X** and **Y**. It should be emphasized that the models built for process monitoring model only common-cause variation and not causal variation. The philosophy applied in developing multivariate SPC procedures based on projection methods, is the same as that used for the univariate SPC charts. An appropriate reference set is chosen which defines the typical operating conditions for a particular process. Future values are compared against this set. A PCA or PLS model is built based on data collected from periods of plant operation when performance was good. Periods containing variations due to special events are omitted at this stage. The choice and quality of this reference set is critical to the successful application of the procedure.

The main concepts behind the development and use of these multivariate SPC charts based on latent variables were laid out in early 1990s. (Kourti and MacGegor 1995). The calculation of limits for the control charts (Hotelling's T^2 and SPE_X) is discussed in Kourti and MacGegor (1995) and Kourti (2009).

These two charts (T^2 and SPE $_X$) are two complementary indices, as in Fig. 22.3; together they give a picture of the wellness of the system under investigation at a glance. As long as the points are within their respective limits everything is in order. Once a point is detected out of limit, then *contribution plots* can be utilized that give us a list of all the variables that mainly contribute to the out-of-limit point, and hence allow us to diagnose the problem immediately. Contribution plots can be derived for out-of-limit points in both charts. Notice that when there are only two PCs the limit shown on the Hotelling's T^2 chart corresponds to the ellipse.

Contributions to SPE_X: When an out-of-control situation is detected on the SPE_X plot, the contribution of each variable of the original data set is simply given by $(x_{new,j} - \hat{x}_{new,j})^2$. Variables with high contributions are investigated.

Contributions to Hotelling's T^2: Contributions to an out-of-limit value in the Hotelling'sT^2 chart are obtained as follows: a bar plot of the normalized scores

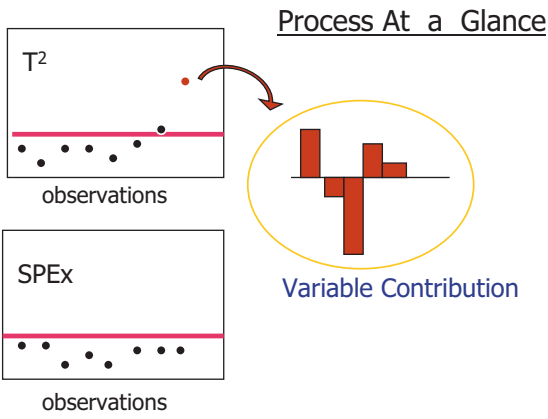

Fig. 22.3 The process may be monitored with two charts; when deviation from typical operation is observed, contribution plots may be used to identify the variables responsible for the deviation

$(t_i / s_{t_i})^2$ is plotted and scores with high-normalized values are further investigated by calculating variable contributions as shown in Eq. (22.3).

Variables on this plot that appear to have the largest contributions to it, but also the same sign as the score should be investigated (contributions of the opposite sign, will only make the score smaller). When there are K scores with high values, an "overall average contribution" per variable is calculated, over all the K scores (Kourti 2005a).

Utilizing contribution plots, when an abnormal situation is detected the source of the problem can be diagnosed such that corrective action is taken. Some actions can be taken immediately, in real time. Others may require interventions to the process equipment or procedures.

22.4 Latent Variable Modeling of Batch Process Trajectory Data

Variable trajectories are obtained by taking measurements over time (Fig. 22.4). Such information may be necessary for process monitoring and for developing models for real time control purposes (mid course correction). Including trajectory information introduces a complex data structure and therefore it requires appropriate methodology for modeling. Historical data collected from batches of the same duration (same length of time or same number of aligned observations), where all J process variables are measured at K time intervals, or K aligned observation numbers (A.O.N.), can be represented by a three-dimensional data matrix $\underline{\mathbf{X}}$ $(I \times J \times K)$, as shown in Fig. 22.5.

The variable trajectories measured over the duration of the batch are nonlinear with respect to time and form a multivariate time series with dynamic nature. The product quality y_{it} at the end of the batch at time t is a function of the process conditions at time t, but also a function of the process conditions several lags before,

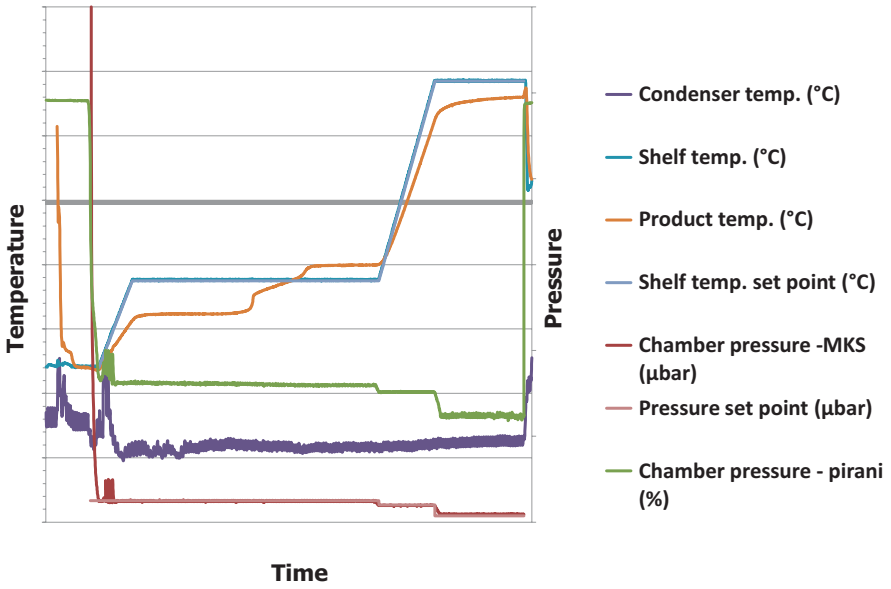

Fig. 22.4 Many variable trajectories may be recorded during a batch process

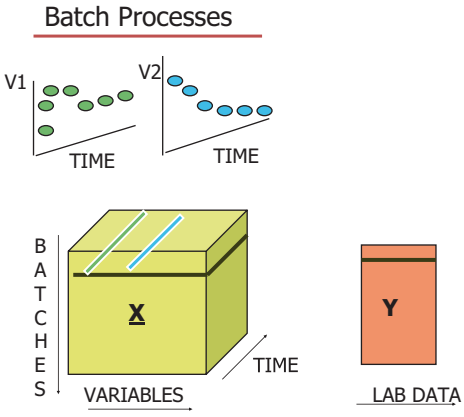

Fig. 22.5 Three-dimensional arrays are formed from process variable trajectories collected on several batches

and in most cases a function of the conditions that existed during the entire batch. In modeling, when we deal with dynamic multivariate time series data, in order to relate input **X** to output **Y** and to capture the dependence of the final product on events that took place during different time intervals, the **X** matrix is expanded to include values of the x variables at several lags (MacGregor et al. 1991). For a batch process where J process variables are measured at K time intervals, or K aligned observation numbers (A.O.N.), for each one of I batches, then if the number of lags are the same for all the x variables the expanded matrix would be $\mathbf{X}$ ($I \times JK$), as shown in Fig. 22.6. The data could also be folded and represented by a three-dimensional data array $\boldsymbol{X}(I \times J \times K)$.

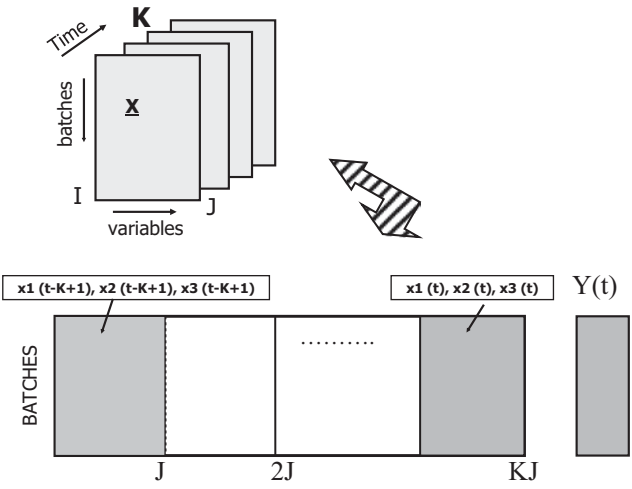

Fig. 22.6 The quality at the end of a batch process depends on the process conditions that were experienced during the batch; it can be expressed as a function of the process variables values at different time lags

Very often, batches do not have the same time duration. Different runs of the same batch process may take different time. Although a significant number of batch processes work with very reproducible cycles, even in these processes we may occasionally have problems. An alignment of the batch trajectories is required before the analysis. This is discussed in a later section. For the discussion here, we assume that the batches have been aligned, therefore we use time intervals or aligned observation numbers, interchangeably.

Finally, there are variables that may not be recorded for all the steps of the batch and the user should consider what the appropriate modeling way to address such situations is. In Fig. 22.1 for example, the pressure chamber variable is recorded after the freezing step. In that case we may have the situation which for illustration purposes is simplified in Fig. 22.7; here there are three variables, recorded at four time instances; variable x_3 however, is not recorded in the first time interval. When such a case exists, instead of a full cube (three-dimensional array of all variable measurements at all time intervals) we have a three-dimensional structure with some of the columns of the cube missing, as shown at the bottom right of the figure (incomplete cube). Ways of modeling such cases are discussed below.

22.4.1 Modeling Data from Batch Processes. The Implications of Unfolding

There are several approaches for modeling three-way data derived from batch processes. The choice of the method depends on the purpose of the model (i.e., prediction of final quality, statistical process control, etc.) and the types of the data sets available.

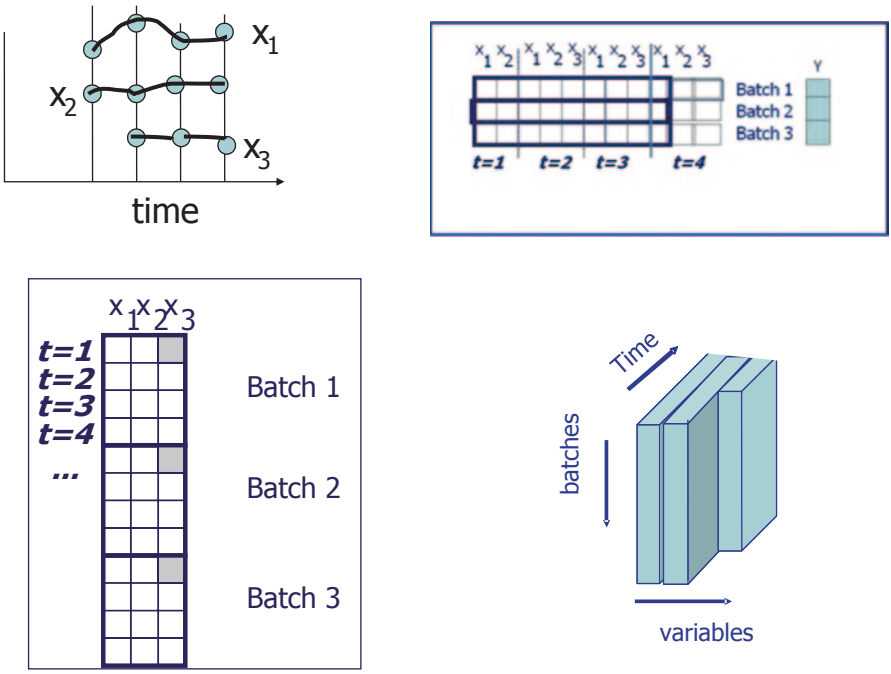

Fig. 22.7 Illustration of a case where some variables are not recorded at all time intervals. This results in an incomplete cube. Depiction of batchwise and variable-wise unfolding

Critical discussions on batch process modeling procedures can be found in selected publications (Nomikos 1995; Westerhuis et al. 1999a; Kourti 2003a, 2003b).

In various approaches, the three-way data matrix $\underline{\mathbf{X}}(I \times J \times K)$ is unfolded to a two-way array first, and then PCA, or PLS, is applied to this unfolded array. Each of the different rearrangements of the three-way data matrix $\underline{\mathbf{X}}$ into a large two-dimensional matrix followed by a PCA on this matrix, corresponds to looking at a different type of variability. The following ways of creating unfolded two-dimensional matrices are most in use for batch processes:

Batchwise unfolding: A method presented by Nomikos and MacGregor (1994, 1995a, b) is termed in the literature "batchwise unfolding." The method unfolds the three-dimensional structure into a two-dimensional array, $\mathbf{A}(I \times KJ)$. In this new array, different time slices are arranged next to each other; variables observed at a given time interval are grouped in one time slice; the number of variables in each time slice may vary. This arrangement is best for real-time monitoring and process control (midcourse correction). Another way of unfolding results to matrix $\mathbf{B}(I \times JK)$; the two matrices are equal with just the columns rearranged. Rearranging the data by matrix **B** allows for a good interpretation of the loadings for process understanding. In batchwise unfolding (**A** or **B**), the number of variables considered for each time interval may vary and therefore this approach can account for variables present and/or measured for a fraction of the duration of the batch. This approach is shown in the upper-right side of Fig. 22.7. Notice that in this arrange-

ment one batch is represented by one row of data in the unfolded $\underline{\mathbf{X}}$ matrix and the corresponding row in the **Y** matrix. When L quality data are available per batch, they can be arranged in a $\mathbf{Y}(I \times L)$ matrix and a PLS between **A** (or **B**) and **Y** is straightforward. When other information is also available (i.e., formulation parameters, information on equipment) another matrix **Z** may be introduced, and the data may be represented by multiblock structure, discussed in a later section. Again, one line of data in the matrix **Z** corresponds to one batch. Modeling with this type of unfolding takes into account simultaneously both the auto- and cross-correlation of all the variables; this is because it allows for analyzing the variability among the batches by summarizing the information in the data with respect to both variables and their time variation. With this particular representation, by subtracting the mean of each column prior to performing the multiway PCA/PLS, the average trajectory of each variable is subtracted, and we look at the deviations from the average trajectory. This way a nonlinear problem is converted to one that can be tackled with linear methods such as PCA/PLS.

Variable wise unfolding: This unfolding results to matrix $\mathbf{C}(IK \times J)$ or $\mathbf{D}(KI \times J)$—the two matrices are equal with just the rows rearranged. (Wold et al. 1998). This way of unfolding was discussed in Nomikos (1995): "the only other meaningful unfolding of $\underline{\mathbf{X}}$ is to arrange its horizontal slices, corresponding to each batch, one below the other into a two-dimensional $\mathbf{X}(IK \times J)$ where the first K rows are the measurements from the first batch in the database. A PCA performed on this unfolded matrix is a study of the dynamic behavior of the process about the overall mean value for each variable. Although this variation might be of interest in some situations, it is not the type of variation of interest in SPC of batch processes." In Fig. 22.7, the variablewise unfolding is shown for a set of three batches with three variables at the bottom-left corner. Recall that variable x_3 is not measured at $t=1$. Notice that when a variable is measured or present for a fraction of the duration of the run, this two-way matrix has empty spaces. They cannot be treated as missing data as they consistently appear at the same location. In another alternative, one could break the batches in phases (each phase with different number of variables) and to model each phase separately.

The implications of unfolding variablewise are discussed by Westerhuis et al. (1999a), Kourti (2003a), and Kourti (2003b), Albert and Kinley (2001). The fact that the batchwise approach gives rich information about the process is discussed also by Albert and Kinley (2001); they had implemented both unfolding approaches and commented that "loading plots produced by the batch-to-batch model (batchwise unfolding) enable the visualization of the complex dynamic correlation structure between variables throughout the batch duration."

22.4.2 Batch Trajectory Synchronization/Alignment

Sometimes batches for the same product may have variable time duration. This occurs because the progress of the process is a complicated function of several phenomena and not simply a function of time. For example, sometimes longer time

may be required to achieve the same moisture content. Therefore, it is very often the case that the batch trajectories have to be expressed against some other variable (or combination of variables) in order to be aligned.

Several approaches have been suggested to align batches. Nomikos (1995) suggested to replace time by another measured variable which progresses monotonically in time and has the same starting and ending value for each batch, and to perform the analysis and on-line monitoring relative to its progress. This variable is being termed indicator variable. Any variable that changes monotonically during the batch can be used as indicator variable. Indicator variables may be measured variables, as for example the cumulative monomer fed (Kourti et al. 1996) or, calculated from other measured variables based on process knowledge, like the extent of reaction (Neogi and Schlags 1998) or another calculated quantity in fermentations (Jorgensen et al. 2004). However, in some situations the indicator variable approach is not adequate. Methodologies developed in the speech recognition literature where they encounter similar problems were explored for the alignment of the variable trajectories. Several approaches that utilize dynamic time warping (DTW) methods from that literature and combine it with latent variable methods have been very successful in aligning batches of different duration, and allowing for the analysis and diagnosis of operating problems (Kassidas et al. 1998). After the alignment, the new $\underline{\mathbf{X}}$ matrix contains the aligned variable trajectories. Taylor (1998) suggested using the cumulative warped information as an extra variable in the *new aligned* $\underline{\mathbf{X}}$ space and this makes DTW powerful for fault detection and also easier to use it for on-line monitoring. The same is true when using the cumulative time deviation from average time as an extra variable in the new aligned $\underline{\mathbf{X}}$ for the indicator variable method. This was applied by Westerhuis et al. (1999b) and Garcia-Munoz et al. (2008). Ündey et al. (2003) address the problem of discontinuity in process variable measurements due to operation switching (or moving to a different phase) that causes problems in alignment and modeling. When using either DTW or the indicator variable, the raw data are being manipulated before being used for the latent variable analysis. The issues related to this manipulation are discussed in Kourti (2003a). The batch alignment issue has been revisited by González-Martínez et al. (2011), introducing an adaptation to the Kassidas et al. (1998) approach.

22.4.3 Centering and Scaling the Data from Batch Processes

Based on the definition of multivariate SPC, the current variable trajectories should be compared to their corresponding average trajectories to detect deviations beyond common cause variation. This corresponds to mean centering the data of the two-way matrix created by batchwise unfolding. This way of mean centering, effectively subtracts the trajectory, thus converting a nonlinear problem to one that can be tackled with linear methods such as PCA and PLS. We should stress here that the above discussion is for modeling that will be used for batch process monitoring.

The requirements are different if PCA or PLS is used to model data collected from designed experiments during development; in this case, several recipes and

process conditions are tried in a set of designed experiments and the product quality is obtained for these different conditions. For these cases where we intentionally vary the recipe, chances are that the trajectories of the same variable obtained with different recipes are significantly different from each other. In this case, the shape of the trajectory does matter as discussed in Duchesne and MacGregor (2000), as we are trying to find the optimal combination of recipe and trajectory to obtain the desired quality product.

After the designed experiments, we select the optimal recipe and the desired trajectories and we proceed to routine manufacturing. In routine production, we wish to keep repeating the same recipe and trajectory profiles to produce the product with the desired specifications. At this point we implement monitoring schemes, to detect deviations from target trajectories. During production, what prevents us from repeating the exact desired trajectories are disturbances (e.g; impurities, problems in raw materials, inability to heat/cool at desired rates) or unit problems (faulty sensors, plugged pipes). These are the problems we try to detect and isolate by implementing monitoring schemes, like multivariate statistical process control (MSPC).

Scaling will also define the problem we try to solve. For a set of dynamic data unfolded batchwise, the two-way array is scaled to unit variance, by dividing each column by its standard deviation. Nomikos (1995) discussed this scaling and mentioned that: "The variables in each column of the unfolded $\underline{\mathbf{X}}$, after they are mean centered, are also scaled to unit variance by dividing by their standard deviation, in order to handle differences in the measurement units between variables and to give equal weight to each variable at each time interval." However, if one wishes to give greater or less weight to any particular variable, or to any particular period of time in the batch, these weights are easily changed. Another way of scaling is to scale each variable at each time interval by its overall (throughout the batch duration) standard deviation. It is the author's experience that scaling per variable means that periods of high noise will be weighted more and periods under tight control will get a small weight. The reader should consider that variables that are under tight control are the ones that matter the most; when a variable is kept under tight control for a given period in a process, this variable is very important in defining the quality (or meeting other constraints in the process like environmental and safety). Hence, small fluctuations are not acceptable for such variable. It is therefore of paramount importance to detect even small deviations for that variable at that crucial period. However if the variable is given a small weight in the model for that period, this may not be feasible. The effect of centering and scaling is discussed in detail in Kourti (2003a).

22.4.4 Analysis Historical Data Bases Containing Batch Process Variable Trajectories

With batchwise unfolding, the data are converted into a two-way matrix. PCA or PLS can be performed on this matrix and score plots can be obtained. Therefore one can plot scores against each other, as described earlier for two-way data arrays, and

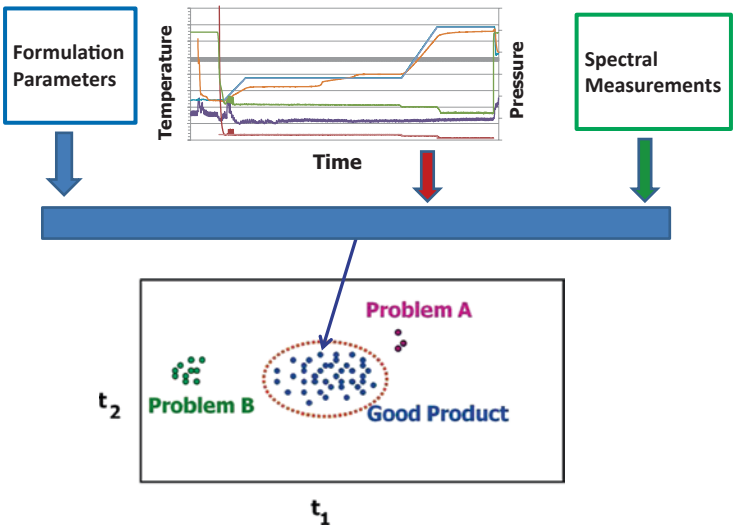

Fig. 22.8 Several dozens of measurements on variables vs. time and other information collected for each batch, project to *a single point* on a principal component space; this allows for very simple visualization of the process performance

identify good batches and unusual batches, as depicted in Fig. 22.8. For each one of the batch runs, there are several dozens of measurements collected: process variables vs. time, formulation parameters, data from in-line analyzers. By projecting this information utilizing latent variables, the dozens of measurements are converted to *a single point* on the projection space and allow for visualization of process performance. The loadings of the unfolded matrix also present special interest for process understanding. The loadings obtained with this setup give a detailed picture of the auto- and cross-correlations of the variables for the duration of the batch.

22.4.5 Multivariate Batch Statistical Process Control

Once a typical operation has been established, control charts can be developed to monitor future batches. The limits for these charts are derived from data collected from past batches operating at typical conditions and producing acceptable quality product. The procedure is as follows: (i) Historical data from past batches are collected; these typically include batches that produced both good and bad quality product. (ii) After trajectory alignment, centering and scaling, a multiway PCA is performed to examine the score plots for "observability"; that is, to see if bad product batches and batches with faulty operation project away from the good product batches. By doing this, we make sure that "bad product batches" and "faulty operation" as designated by the operators and/or other company personnel, can be detected from the collected process measurements. This is an important step to decide if representative data is being collected and how the model will be used for

monitoring. (iii) Following the observability test, we should investigate the reasons for bad product batches and faulty operation in the past, using contribution plots. This investigation may result in making changes in the process. (iv) If changes are made, then new data must be collected to derive control limits for MSPC. If no changes are made, the existing historical data can be used. (v) The model will be built by utilizing the good batches and limits will be calculated for control charts. It is important to make sure that these charts are capable of detecting problems for past batches that produced bad quality product or had unusual operation. (vi) Future batches are checked against good operation limits. This may happen either in real time (on-line monitoring) or after the batch is completed. Contribution plots are used to diagnose the reasons for deviation for out-of-limit batches.

When setting the control charts, the user should keep in mind that by definition, in statistical process control we check that the deviation from the target is within certain limits. Therefore, we need to subtract from a known target and check the deviations of the process variables against limits. The known target in the case of batch processes are the average variable trajectories (calculated from a set of training data, from batches corresponding to good operation), or the desired trajectories derived by optimization procedures (Duchesne and MacGregor 2000).

Industrial applications reported in the literature have mainly used the variable-wise unfolding for process monitoring due to the availability of commercial software packages based on this approach. Readers interested in applications utilizing batchwise unfolding can find theoretical principles in Nomikos and MacGregor (1994, 1995b) and examples of industrial applications in Albert and Kinley (2001) and Zhang and Lennox (2004). Commercial packages on this approach are becoming available. When utilizing commercial software, it is important to understand which one of the approaches is used in order to be able to interpret the results appropriately.

22.5 Multistage Operations—Mutiblock Analysis

Lyophilization involves three steps. In batch analysis, one may also include formulation parameters and information from spectral data, as shown in Fig. 22.8. When the data come from multistep processes or from different sources, one may consider utilizing multiblock approach. Rather than building a model for each step, one can build a model for the full process that will take into account the interactions between steps and their relative importance to the final product quality by weighting them differently. This is the approach of multiblock PLS (MB-PLS).

In the MB-PLS approach, large sets of process variables (**X**) are broken into meaningful blocks, with each block usually corresponding to a process unit or a section of a unit. MB-PLS is not simply a PLS between each **X** block and **Y**. The blocks are weighted in such a way that their combination is most predictive of **Y.** Several algorithms have been reported for multiblock modeling and for a good review it is suggested that the reader consult the article by Westerhuis et al. (1998).

Multivariate monitoring charts for important subsections of the process, as well as for the entire process, can then be constructed, and contribution plots are used for fault diagnosis as before. In a multiblock analysis of a batch process for example, one could have the combination of three blocks (**Z**, **X**, and **Y**); block **Z** could include information available on formulation parameters, as well as information of the shifts (which operator was in charge) or the vessels used, and other information relevant to the batch, **X** would include process variable trajectories, and **Y** would be quality. Analysis of this type of data could even point to different ways the operators operate the units and relate product quality to operator, or different process behavior of vessels and identify faulty vessels, etc. The reader is referred to the work of García-Muñoz et al. (2003, 2008) for detailed examples where the multiblock analysis is utilized in batch processes for troubleshooting and for determining the batch operating policies in order to achieve specific product quality while minimizing the duration of the batch run.

Several alternative ways to perform multiblock appear in commercial software. One approach that is being frequently used to deal with a data structure of several blocks, involves two stages: PCA is performed for each one of the **Z** and **X** blocks and then the scores and/or residuals derived from these initial models are related to **Y** with a PLS. In an alternative version, PLS is performed between **Z** and **Y**, **X** and **Y** and the resulting scores are related to **Y**. The users should exercise caution, because these approaches may fail to take into account combinations of variables from different blocks that are most predictive of **Y**. For example, in situations where process parameters in **X** are modified to account for variability of raw material properties in **Z** (i.e., when **X** settings are calculated as a feed forward control to deviations of **Z**), a PLS between **Z** and **Y** will show that **Z** is not predictive of **Y** variability; similarly a PLS between **X** and **Y** will show that **X** is not predictive of **Y**; a MB-PLS of [**Z, X**] and **Y** will identify the correct model. Finally MB-PLS handles missing data in a very effective way.

22.6 Process Control to Achieve Desired Product Quality

The term "control" currently appears in the biopharmaceutical literature to describe a variety of concepts such as, endpoint determination, feedback control, statistical process control, or simply monitoring. Process control refers to a system of measurements and actions within a process intended to ensure that the output of the process conforms with pertinent specifications.

Here the terms related to process control are used as follows:

- Feedback control, to indicate that the corrective action is taken on the process based on information from the process output
- Feed forward control, to indicate that the process conditions are adjusted based on measured deviations of the input to the process (as for example, information on raw material)

22.6.1 Feed Forward Estimation of Process Conditions

The concept of adjusting the process conditions of a unit based on measured disturbances (feed forward control) is a concept well known to the process systems engineering community for several decades. The methodology is also used in multistep (multiunit) processes where the process conditions of a unit are adjusted based on information of the intermediate quality achieved by the previous unit (or based on raw material information). There are several unpublished examples in the chemical and other industries where information on the raw data **Z** is used to determine the process conditions **X** or $\underline{\boldsymbol{X}}$ in order to achieve the desired quality **Y**, utilizing projection methods. Sometimes such information from **Z** may simply be used to determine the length of the run, while in other cases it may be a multivariate sophisticated scheme that determines a multivariate combination of trajectories for the manipulated variables. To achieve this, historical databases can be used to develop multiblock models **Z**, $\underline{\boldsymbol{X}}$ (or **X**) and **Y**.

22.6.2 Endpoint Determination

Endpoint detection or endpoint control has been addressed by several industries. There have been reports in the literature where real-time analyzers are usually applied for such purpose. In most of these situations, a desired target concentration is sought, as for example the percentage of moisture in drying operations.

Examples from Lyophilization Process

i. The determination of endpoint of primary drying is discussed by Patel et al. (2010). The authors state that one of the objectives during freeze-drying process development is to minimize the primary drying time, which is the longest of the three steps in freeze-drying, because freeze-drying is a relatively expensive process requiring long processing time. However, increasing the shelf temperature into secondary drying before all of the ice is removed from the product will likely cause collapse or eutectic melt. Thus, from product quality as well as process economics standpoint, it is very critical to detect the end of primary drying. Several endpoint detection techniques were investigated and it was concluded that the Pirani is the best choice of the methods tested for evaluation of the endpoint of primary drying.
ii. De Beer et al. (2007, 2009) report the use of in-line Raman in combination with PCA and NIR. Raman spectroscopy was able to supply information about the endpoint of freezing (endpoint of mannitol crystallization). NIR spectroscopy proved to be a more sensitive tool to monitor endpoint of ice sublimation during drying.

Latent variable methodology allows for taking into consideration the process signatures in a multivariate way for endpoint detection problems. Combinations of process measurements and other sensors are used to develop a “process signature” that

has to signal that the desired target is achieved. Marjanovic et al. (2006) described a preliminary investigation in to the development of a real-time monitoring system for a batch process. The aim of the work conducted in that study was to develop a data-based system able to accurately identify the endpoint of the batch. This information can then be used to reduce the overall cycle time of the process. Approaches based upon multivariate statistical techniques were shown to provide a soft sensor able to estimate the product quality throughout the batch and a prediction model able to provide a long-term estimate of the likely cycle time. This system has been implemented on-line and initial results indicate that it offers the potential to reduce operating costs.

22.6.3 Manipulation of Process Variables

Examples from Lyophilization Processes

i. Fissore et al. (2008) discuss the control of a freeze-drying process in vials. First, they predict the optimum constant chamber pressure and shelf temperature of the process using mathematical simulations. Further improvements can then be obtained if the shelf temperature is varied during the process in such a way that the product temperature is always maintained at the maximum allowable value. This strategy for the in-line control of the process allows for minimization of the time required for the primary drying, while satisfying the process constraints. The possibility of manipulating the chamber pressure for control purposes was also discussed by the authors. An alternative strategy based on a simple feedback controller, with proportional-integral action, is also investigated: it is able to control the product temperature at a predetermined value, giving stable and fast responses. The controller uses a soft sensor to get a reliable in-line estimate of the controlled variable; i.e., of the maximum temperature of the product.
ii. Barresi et al. (2009) presented a soft sensor monitoring of the lyophilization process and determined the optimal shelf temperature for primary drying, ensuring the fastest drying time without overcoming the maximum allowable product temperature both in scouting and production cycles.

Lately, latent variable methods have found their way to control batch product quality and have been applied in industrial problems. Zhang and Lennox (2004) utilized latent variable methodology for soft sensor development that could be used to provide fault detection and isolation capabilities and that it can be integrated within a standard model predictive control framework to regulate the growth of biomass within a fermenter. This model predictive controller is shown to provide its own monitoring capabilities that can be used to identify faults within the process and also within the controller itself. Finally, it is demonstrated that the performance of the controller can be maintained in the presence of fault conditions within the process.

Work has also been reported for complicated control problems where adjustments are required for the full manipulated variable trajectories (Flores-Cerrilo and MacGregor 2004). Control through complete trajectory manipulation using

empirical models is possible by controlling the process in the reduce space (scores) of a latent variable model rather than in the real space of the manipulated variables. Model inversion and trajectory reconstruction is achieved by exploiting the correlation structure in the manipulated variable trajectories. Novel multivariate empirical model predictive control strategy (LV-MPC) for trajectory tracking and disturbance rejection for batch processes, based on dynamic PCA models of the batch processes has been presented. The method presented by Nomikos and MacGregor (1994, 1995a, b) is capable of modeling three-way structures generated when formulating the control problem of batch processes using latent variables.

22.6.4 Setting Raw Material Multivariate Specifications as a Means to Control Quality

Duchesne and MacGregor (2004) presented a methodology for establishing multivariate specification regions on raw/incoming materials or components. The thought process here is that if the process remains fixed, we should control the incoming material variability and other components that may affect the process. PLS is used to extract information from databases and to relate the properties of the raw materials supplied to the plant and the process variables at the plant to the quality measures of the product exiting the plant. The specification regions are multivariate in nature and are defined in the latent variable space of the PLS model. The authors emphasize that although it is usually assumed that the raw material quality can be assessed univariately by setting specification limits on each variable separately, this is valid only when the raw material properties of interest are independent from one another. However, most of the times, the properties of products are highly correlated. To develop models to address the problem, multiblock PLS is used for **Z**, **X** and **Y**; **Z** contains measurements on N lots of raw material data from the past; **X** contains the steady state processing conditions used to process each one of the N lots; **Y** contains final product quality for these N lots. The methodology could be easily extended to batch process $\underline{\boldsymbol{X}}$.

22.7 Using Latent Variable Methods for Optimization

22.7.1 Exploiting Data Bases for Causal Information

For process optimization causal information must be extracted from data, so that a change in the operating variables can be made that will lead to a better quality product, or higher productivity and profit. There has been a lot of interest in exploiting historical databases to derive empirical models (using tools such as neural networks regression or PLS) and use them for process optimization. However, databases obtained from routine operation contain mostly noncausal information. Inconsistent

data, range of variables limited by control, noncausal relations, spurious relations due to feedback control and dynamic relations are some of the problems the user will face using such happenstance data. In spite of this, several authors have proposed approaches to optimization and control based on interpolating historical bases. However, in all these cases their success was based on making strong assumptions which allowed the database to be reorganized and causal information to be extracted. One approach was referred to as "similarity optimization" which combined multivariate statistical methods for reconstructing unmeasured disturbances and nearest neighbor methods for finding similar conditions with better performance. However, it too was shown to fail for many of the same reasons. In general, it was concluded that one can only optimize the process if there exist manipulated variables that change independently of the disturbances and if disturbances are piecewise constant, a situation that would be rare in historical process operations.

The reader should therefore exercise caution of how historical data bases are used when it comes to retrieving causal information. However, databases obtained from routine operation are great source of data for building monitoring schemes.

22.7.2 Product Design

Given the reservations about the use of historical databases, one area where some success has been achieved is in identifying a range of process-operating conditions for a new grade of product with a desired set of quality properties and in matching two different production plants to produce the same grade of product. If fundamental models of the process exist, then these problems are easily handled as constrained optimization problems. If not, optimization procedures based on response surface methodology can be used. However, even before one performs experiments, there exists information within the historical database on past operating conditions for a range of existing product grades (García-Muñoz et al. 2006).

In this case, the historical data used are selected from different grades and therefore contain information on variables for several levels of past operation (i.e., there is intentional variation in them, and are not happenstance data). The key elements in this empirical model approach is the use of latent variable models that both reduce the space of **X** and **Y** to a lower dimensional orthogonal set of latent variables and provide a model for **X** as well as **Y**. This is essential in providing solutions which are consistent with past operating policies. In this sense, PC regression and PLS are acceptable approaches, while MLR, neural networks, and reduced rank regression are not.

The major limitation of this approach is that one is restricted to finding solutions within the space and bounds of the process space **X** defined by previously produced grades. There may indeed be equivalent or better conditions in other regions where the process has never been operated before, and hence where no data exists. Fundamental models or more experimentation would be needed if one hopes to find such novel conditions.

A very good discussion on these issues can be found in García-Muñoz et al. (2008). The authors illustrate a methodology with an industrial application where the batch trajectories are designed to satisfy certain customer requirements in the final product quality properties while using the minimal amount of time for the batch run. The cumulative time or, used time, is added as an extra variable trajectory after the alignment of the batches.

22.8 Site Transfer and Scale-Up

Product transfer to different sites and scale-up, fall in to the same class of problems: one needs to estimate the process operating conditions of plant B in order to produce the same product that is currently produced in plant A.

Lyophilization Examples There are different approaches to scale-up.

i. Rambhatla et al. (2006) state: "an important objective of freeze-drying process design is the development of a process that is robust, is economical, and can be easily transferred to all freeze dryers irrespective of size and design. To be fully transferable, the process should be equivalent—that is, the product temperature vs. time profile should be identical—when the same freeze-drying process is performed on different freeze dryers. Achieving this objective poses some challenges." Rambhatla et al. (2006) presented a study aimed to provide guidelines for convenient scale-up of the freeze-drying process. They estimated differences in heat and mass transfer between freeze dryers due to inherent design characteristics using data obtained from sublimation tests. Steady-state heat and mass-transfer equations were used to study a combination of different scale-up issues pertinent during lyophilization cycles commonly used for the freeze-drying of pharmaceuticals.
ii. Mockus et al. (2011) discuss a Bayesian model for the prediction of primary drying phase duration and they suggested that the model be used during scale-up activities in order to minimize trial and error and reduce costs associated with expensive large scale experiments

Attempts have been made to solve such problems with latent variable methods, utilizing historical data from both locations from transferring other products. The main points to keep in mind when addressing such a problem are:

- The quality properties of the product should always be checked within a multivariate context, because univariate charts may be deceiving. The multivariate quality space for both the sites should be the same. Correct product transfer cannot be achieved by comparing endpoint quality on univariate charts from the two sites (or from pilot scale and manufacturing). The product quality has to be mapped from site to site in a multivariate way (the products in both sites have to project on the same multivariate space).
- The endpoint quality may not be sufficient to characterize a product. The path to end product is important. Whenever full mechanistic models exist, these models

describe the phenomena that are important for the process and therefore determine this path. When changing sites, the full mechanistic model will describe the desired path in the new site taking into account size, mass, and energy balances and/or other phenomena related to the process. When mechanistic models do not exist, this mapping of the "desired process paths" or "process signatures" has to happen with empirical data.

A methodology has been developed for product transfer and scale-up, based on latent variables (García-Muñoz et al. 2005). The methodology utilizes data bases with information on previous products and their corresponding process conditions from both sites. The two sites may differ in equipment, number of process variables, locations of sensors, and history of products produced.

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Chapter 23
Using Mathematical Modeling and Prior Knowledge for QbD in Freeze-Drying Processes

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Abbreviations

CFD	Computational fluid dynamics
DSMC	Direct Simulation Monte Carlo
PRT	Pressure rise test
A_v	Cross section area of the vial, m^2
a	Specific surface of the dried product, $m^2\ kg^{-1}_{\text{dried product}}$
C_s	Residual moisture, $kg_{\text{water}}\ kg^{-1}_{\text{dried product}}$
$C_{s,0}$	Residual moisture at the beginning of secondary drying, $kg_{\text{water}}\ kg^{-1}_{\text{dried product}}$
$C_{s,eq}$	Weight fraction of sorbed water in the solid that would be in local equilibrium with the partial pressure of water in the drying chamber, $kg_{\text{water}}\ kg^{-1}_{\text{dried product}}$
$C_{s,t}$	Target value of the residual moisture in the product, $kg_{\text{water}}\ kg^{-1}_{\text{dried product}}$
C_1	Parameter used in Eq. (23.8), $W\ K^{-1}\ m^{-2}$
C_2	Parameter used in Eq. (23.8), $W\ K^{-1} m^{-2}\ Pa^{-1}$
C_3	Parameter used in Eq. (23.8), Pa^{-1}
$c_{p,\text{liquid}}$	Specific heat of the liquid, $J\ kg^{-1}\ K^{-1}$
$c_{p,p}$	Specific heat of the product, $J\ kg^{-1}\ K^{-1}$
D	Duct diameter, m
$E_{a,d}$	Activation energy of the desorption reaction, $J\ mol^{-1}$
ΔH_d	Heat of desorption, $J\ kg^{-1}_{\text{water}}$
ΔH_s	Heat of sublimation, $J\ kg^{-1}_{\text{water}}$
J_q	Heat flux to the product, $W\ m^{-2}$
J_w	Mass flux, $kg\ s^{-1}\ m^{-2}$
K	Parameter used in Eq. (23.19)

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K_v Overall heat transfer coefficient between the heating fluid and the product at the vial bottom, W m^{-2} K^{-1}
k_d Kinetic constant of the desorption rate, $kg_{dried\ product}^{-1}$ s^{-1} m^{-2}
$k_{d,0}$ Pre-exponential factor of the kinetic constant of the desorption rate, $kg_{dried\ product}^{-1}$ s^{-1} m^{-2}
L Duct length, m
L_0 Product thickness after freezing, m
L_{dried} Thickness of the dried product, m
L_{frozen} Thickness of the frozen product, m
M_w Water molar mass, kg mol^{-1}
m Mass, kg
m_{dried} Mass of dried product, kg
P_c Chamber pressure, Pa
P_1 Parameter used in Eq. (23.10), s^{-1}
P_2 Parameter used in Eq. (23.10), m^{-1}
$p_{w,c}$ Water vapor partial pressure in the drying chamber, Pa
$p_{w,i}$ Water vapor partial pressure at the interface of sublimation, Pa
R_p Resistance of the dried product to vapor flow, m s^{-1}
$R_{p,0}$ Parameter used in Eq. (23.10), m s^{-1}
R Ideal gas constant, J K^{-1} mol^{-1}
r_d Water desorption rate, kg_{water} $kg_{dried\ product}^{-1}$ s^{-1}
$r_{d,PRT}$ Water desorption rate measured through the test of pressure rise, kg_{water} $kg_{dried\ product}^{-1}$ s^{-1}
T Temperature, K
T_B Product temperature at the vial bottom, K
T_c Temperature of the vapor in the drying chamber, K
T_{fluid} Heating fluid temperature, K
T_g Glass transition temperature, K
$T_{g,s}$ Sucrose glass transition temperature, K
$T_{g,w}$ Ice glass transition temperature, K
T_i Product temperature at the interface of sublimation, K
T_p Product temperature, K
t Time, s
$t_{0,PRT}$ Initial time of the PRT, s
t_d Duration of secondary drying, h
V_c Free volume of the chamber, m^3
V_p Volume of the product, m^3

Greeks

λ_{frozen} Heat conductivity of frozen product, W m^{-1} K^{-1}
λ_{liquid} Heat conductivity of liquid product, W m^{-1} K^{-1}
ρ_{dried} Apparent density of the dried product, kg m^{-3}
ρ_{frozen} Density of the frozen product, kg m^{-3}
ρ_{liquid} Density of the liquid product, kg m^{-3}

23.1 Introduction

Freeze-drying is widely used in pharmaceuticals manufacturing to provide long-term stability to formulations containing an active pharmaceutical ingredient. At first, the aqueous solution containing the drug and the excipients is put in vials, loaded onto the shelves of the drying chamber of the freeze-dryer. Then, product temperature is decreased by means of a cold fluid flowing through the shelves: part of the water ("free water") crystallizes, and part ("bound water") remains unfrozen. Ice sublimation (primary drying) is then obtained by decreasing the chamber pressure: during this step the temperature of the fluid flowing through the shelves is increased, and the fluid is used to supply heat to the product as the sublimation is an endothermic process. As a result of ice sublimation, a porous cake is obtained: water vapor flows through this cake, moving from the interface of sublimation (the boundary between the frozen product and the cake) to the drying chamber, and then to a condenser, where it sublimates over cold surfaces. Finally, the target value of residual moisture in the product is obtained by further increasing product temperature in order to desorb the bound water (secondary drying).

The values of the operating conditions of the freeze-drying process, i.e., the temperature of the heating fluid (T_{fluid}) and the pressure in the drying chamber (P_c) during primary and secondary drying, as well as the duration of both drying steps, can significantly affect final product quality. In particular, the following issues have to be taken into account:

i. Product temperature has to remain below a limit value that is a characteristic of the formulation being processed, during both primary and secondary drying stages. This is required to avoid product denaturation, melting (in case of crystalline products), or collapse of the dried cake (in case of amorphous products). Cake collapse can be responsible for blockage of cake pores, thus increasing cake resistance to vapor flow, and retarding the end of primary drying due to the lower sublimation rate. Moreover, a collapsed cake can retain a higher amount of water in the final product, the reconstitution time can increase, and the physical appearance is unattractive (Bellows and King 1972; Tsourouflis et al. 1976; Adams and Irons 1993; Pikal 1994; Franks 1998; Wang et al. 2004).
ii. The sublimation rate has to be compatible with the condenser capacity, and choking flow has to be avoided in the duct connecting the chamber to the condenser (Searles 2004; Nail and Searles 2008; Patel et al. 2010).
iii. A target value of residual moisture has to be obtained in the final product in order to maximize product stability.
iv. The duration of the whole process has to be minimized in order to maximize plant productivity.

Finally, it must be evidenced that the final quality of the product may be also related to the design of the equipment: the chamber design (and in particular shelf size and interdistance, shelf-wall clearance, duct size, and location) may affect the intrabatch variability. Moreover, duct and valve type and size and condenser design may affect

pressure drop and, thus, they determine the minimum controllable pressure in the chamber and the quality of pressure control and, in the worst case, they are responsible for choked flow and lose of pressure control.

According to the "Guidance for Industry PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance" issued by FDA in September 2004, a true quality by design manufacturing principle, rather than the classical quality-by-testing approach, should be implemented to have safe, effective, and affordable medicines. By this way product quality is built-in, or is by design, and it is no longer tested in final products.

This chapter is focused on obtaining quality-by-design in a pharmaceuticals freeze-drying process. To this purpose, it is necessary to determine the design space of the process. According to "ICH Q8 Pharmaceutical Development Guideline" (2009), a design space is the multidimensional combination of input variables and process parameters that have been demonstrated to provide assurance of quality. Generally, the empirical approach is used to determine the design space: various tests are carried out using different values of T_{fluid} and P_c, and final product properties are measured experimentally (Chang and Fisher 1995; Nail and Searles 2008; Hardwick et al. 2008). Obviously, this approach is expensive and time consuming, even if the number of tests can be reduced by using the experimental design technique (Box et al. 1981) and the multi-criteria decision making method (De Boer et al. 1988, 1991; Baldi et al. 1994). Moreover, the experimental approach does not guarantee to obtain the optimal cycle, and in case the cycle is determined in the lab-scale freeze-dryer, the scale-up to the industrial-scale freeze-dryer is required, and this is a challenging and complex task.

Mathematical modeling can be effectively used to determine the design space for a pharmaceuticals freeze-drying process as it allows studying in silico the evolution of the product (i.e., how the temperature, the residual amount of ice, and the sublimation flux change during time) as a function of the operating conditions. Clearly, a suitable model has to be used to perform the calculations. Primarily, the mathematical model has to be accurate, i.e., it has to account for all the heat and mass transfer phenomena occurring in the product, and a deep mechanistic understanding is required for this purpose. Secondly, the mathematical model has to involve few parameters, whose values can be easily and accurately determined by means of theoretical calculations or (few) experimental investigations: the accuracy of complex and very detailed models can be impaired by the uncertainty on the value of the parameters. Finally, the time required by the calculations can be an important concern.

The structure of the chapter is the following: at first, mathematical modeling of product evolution in the vial (during freezing, primary and secondary drying) and of freeze-drying equipment is addressed; second, the use of mathematical models to calculate the design space for primary and secondary drying is discussed, pointing out how the design space can be used to optimize the cycle, as well as to analyze the effect of any deviation of process variables from their set-point values.

23.2 Mathematical Modeling

23.2.1 Freezing

The ice crystals morphology (mean size, shape, and size distribution) obtained in the freezing step can significantly influence both primary and secondary drying stages. In fact, in case small-size ice crystals are obtained, then the resistance of the cake to vapor flow will be increased (as small cake pores are obtained from ice sublimation), thus decreasing the sublimation rate, and increasing the duration of primary drying. The larger cake-specific surface obtained in this case can be beneficial in the secondary drying stage, when water desorption takes place. As a general trend, large ice crystals are obtained from slow cooling rates, while faster cooling results into smaller and more numerous ice crystals (Kochs et al. 1991). Kochs et al. (1993) mentioned that nucleation temperature has no great impact on a macroscopic sample freezing, which was controlled mainly by cooling conditions. In case of small scale frozen systems, like pharmaceutical freeze-drying in vials, many authors consider the nucleation temperature as a key factor: the undercooling degree of the solution determines the number of nuclei and, thus significantly influences the ice crystals size distribution (Searles et al. 2001a, b). Nakagawa et al. (2007) proposed a simple model for the freezing process to calculate the temperature profile in the vial during the freezing stage. They used a commercial finite element code in two-dimensional axial-symmetric space to take into account the actual vial geometry. In the cooling step, the well-known conductive heat equation is solved:

$$\rho_{\text{liquid}} c_{p,\text{liquid}} \frac{\partial T}{\partial t} = \nabla \left(\lambda_{\text{liquid}} \nabla T \right). \tag{23.1}$$

Nakagawa et al. (2007) assume that nucleation starts at the vial bottom at a given temperature (which is a parameter of the model); the freezing model is based on Eq. (23.1), where $c_{p,\text{liquid}}$ is replaced by an apparent heat capacity (Lunardini 1981) that takes into account the coexistence of liquid and ice, and the heat generation due to ice nucleation and ice crystallization is added on the right-hand side. From calculated experimental temperatures profiles, a semi-empirical model was set up to estimate the mean ice crystal size and, consequently, the water vapor dried layer permeability (using standard diffusion theory). It is assumed that the ice crystals mean size is proportional to the freezing rate and to the temperature gradient in the frozen layer (Bomben and King 1982; Reid 1984; Kochs et al. 1991; Kurz and Fisher 1992; Woinet et al. 1998). The model was validated against experiments carried out using mannitol and BSA-based formulations. The results obtained by means of a numerical simulation confirmed that nucleation temperature is the key parameter that determines the ice morphology, and that an increase of the cooling rate leads to smaller ice crystal sizes (Nakagawa et al. 2007).

23.2.2 Primary Drying

During the primary drying stage ice sublimation occurs: water vapor flows from the interface of sublimation to the drying chamber, going through the dried layer. As primary drying goes on, the interface of sublimation moves from the top surface of the product to the bottom of the vial.

Detailed multidimensional model were proposed in the past to study in silico the process. A bidimensional axial-symmetric model was first proposed by Tang et al. (1986) to investigate the freeze-drying of pharmaceutical aqueous solutions in vials, but no results were shown. This model was also proposed by Liapis and Bruttini (1995) to demonstrate that radial gradients of temperature exist when the radiative flux at the vial side is taken into account, and that the sublimation interface is always curved downward at the edges of the vial. A finite-element formulation was used by Mascarenhas et al. (1997) and by Lombraña et al. (1997) to solve the bidimensional model: an arbitrary Lagrangian–Eulerian description is proposed, treating the finite-element mesh as a reference frame that may be moving with an arbitrary velocity. According to Sheehan and Liapis (1998), this formulation has major problems related to the way the problem is treated from a numerical point of view. In fact, it fails to describe the dynamic behavior of the primary drying stage in a vial when the moving interface does not extend along the whole length of the diameter of the vial; moreover, it cannot describe properly the dynamic behavior of the geometric shape and position of the sublimation interface because the water vapor mass flux is considered to be time invariant when the position of the moving interface is located between mesh points of the grid. Thus, a different numerical method, based on the orthogonal collocations, was proposed by Sheehan and Liapis (1998): they evidenced that when there is no heat input from the vial sides, as in the majority of the vials of the batch, the geometry of the moving interface is flat. Only in case vials are heated by radiation from chamber walls (i.e., for vials at the edges of the shelf), a curvilinear shape is obtained for the sublimation interface, but, in any case, the difference between the position of the interface at the center and at the side of the vial is less than 1 % of the total thickness of the product. These results were confirmed also by Velardi and Barresi (2011), who evidenced that even in case of radiation from chamber walls, radial gradients of temperature are very small. This is in agreement with the results given by Pikal (1985), where it was found with a series of experiments that the temperature at the bottom center of the vial was equal to the temperature of the bottom edge, within the uncertainty of the temperature measurement (0.5 °C). Thus, taking also into account that the numerical solution of a multidimensional model can be highly time consuming, and that they involve many parameters whose values are very often unknown, and/or could be estimated only with high uncertainty, various monodimensional models were proposed in the literature (see, among the others, Pikal 1985; Millman et al. 1985; Sadikoglu and Liapis 1997): they are based on the heat and mass balance equations for the frozen and the dried product, neglecting radial gradients of temperature and composition, as well as the effect of heat transfer in the sidewall of the vial, although it has been argued that this could play an important role as energy can be transferred to

the product from the vial wall as a consequence of conduction through the glass (Ybema et al. 1995; Brülls and Rasmuson 2002). Recently, a monodimensional model including the energy balance for the vial glass has been proposed by Velardi and Barresi (2008). Also in case of monodimensional models, it is possible to vary the complexity of the model itself by neglecting some heat and mass transfer phenomena (that not significantly affect the dynamics of the process), thus obtaining simplified models that can be really useful as they involve few parameters that can be measured experimentally.

The product in a vial is heated from the fluid flowing through the shelf, and the heat flux is proportional to a driving force given by the difference between the temperature of the fluid and that of the product at the vial bottom:

$$J_q = K_v \left(T_{\text{fluid}} - T_B \right). \tag{23.2}$$

The sublimation flux from the sublimation interface is assumed to be proportional to a driving force given by the difference between the water vapor partial pressure at the interface and in the drying chamber:

$$J_w = \frac{1}{R_p} \left(p_{w,i} - p_{w,c} \right), \tag{23.3}$$

where the water vapor partial pressure at the sublimation interface is a well-known function of product temperature at that position (Goff and Gratch 1946), while water vapor partial pressure in the drying chamber can be assumed to be equal to total chamber pressure. Thus, a simple model of the process (Velardi and Barresi 2008) consists of the heat balance at the interface of sublimation and of the mass balance for the frozen layer:

$$J_q = \Delta H_s J_w \tag{23.4}$$

$$\frac{dL_{\text{frozen}}}{dt} = -\frac{1}{\rho_{\text{frozen}} - \rho_{\text{dried}}} J_w. \tag{23.5}$$

Heat accumulation in the frozen layer is assumed to be negligible and, thus, heat flux is constant in the frozen layer. This allows to determine the relationship between T_i (and, thus, $p_{w,i}$) and T_B:

$$T_B = T_{\text{fluid}} - \frac{1}{K_v} \left(\frac{1}{K_v} + \frac{L_{\text{frozen}}}{\lambda_{\text{frozen}}} \right)^{-1} \left(T_{\text{fluid}} - T_i \right). \tag{23.6}$$

In order to calculate the evolution of product temperature and frozen layer thickness vs. time, it is required to know the values of the operating conditions (T_{fluid} and P_c), of some physical parameters (ρ_{frozen}, ρ_{dried}, λ_{frozen}, ΔH_s), and of the two parameters

of the model, namely, the overall heat transfer coefficient between the heating fluid and the product at the vial bottom (K_v), and the total resistance (including the contribution of dried layer, stopper, and chamber) to the vapor flow (R_p).

A simple experiment can be carried out to determine the value of K_v for the various vials of the batch (Pikal et al. 1984; Pikal 2000; Pisano et al. 2011a). It is required to fill the vials with water, and to measure the weight loss Δm after ice sublimation for a time interval Δt:

$$K_v = \frac{\Delta m \Delta H_s}{A_v \int_0^{\Delta t} \left(T_{\text{fluid}} - T_B \right) dt}. \tag{23.7}$$

In order to use Eq. (23.7), ice temperature at the vial bottom has to be measured: wired thermocouples can be used in lab-scale and pilot-scale freeze-dryers, while wireless sensors are much more suitable in industrial-scale units (Vallan et al. 2005; Corbellini et al. 2010). An example of the results that can be obtained is shown in Fig. 23.1: it appears that the value of the heat transfer coefficient K_v is not the same for all the vials of the batch. This is due to the fact that Eq. (23.2) assumes that the product in the vial is heated only by the fluid flowing through the shelf, but, actually, it can be heated also by radiation from the chamber walls and the upper shelf, and by conduction from metal frames, in case they are used to load/unload the batch. As a consequence, the value of K_v for the vials of the first raw is higher than that obtained for vials in the central part of the shelf due to the contribution of radiation from chamber walls. Thus, it is possible to classify the vials of a batch in various groups, depending on their position over the shelf (Table 23.1). The gravimetric test has to be repeated at least at three different values of chamber pressure, as for a given vial-freeze-dryer system P_c significantly affects the value of K_v:

$$K_v = C_1 + \frac{C_2 P_c}{1 + C_3 P_c}. \tag{23.8}$$

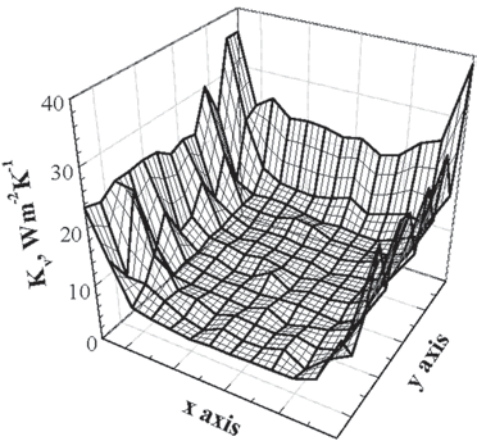

Fig. 23.1 Values of the heat transfer coefficient K_v for the vials of the batch (tubing vials, internal diameter = 14.25 mm, $T_{\text{fluid}} = -15$ °C, $P_c = 10$ Pa). The batch is composed of 26 vials (along the y axis) x 13 vials (along the x axis) Values obtained for half of the batch are shown

Table 23.1 Characteristics of the various groups of vials considered in the case study

Group	Position over the shelf	Additional mechanisms to heat transfer		
		Radiation from chamber walls	Contact with the metal frame	Contact with "hot" vials
a	Core	No	No	No
b	Core	No	No	Yes
c	Peripheral	Yes	No	Yes
d	Peripheral	Yes	Yes	Yes

Figure 23.2 shows an example of results obtained by measuring experimentally K_v for the various group of vials identified in Table 23.1 at different values of P_c.

The heat transfer coefficient K_v can also be determined using the measurement of the sublimation flux J_w obtained with the Tunable Diode Laser Absorption Spectroscopy (TDLAS) (Kessler et al. 2006; Gieseler et al. 2007; Kuu et al. 2009):

$$K_v = \frac{J_w \Delta t \Delta H_s}{A_v \int_0^{\Delta t} \left(T_{\text{fluid}} - T_B \right) dt}. \tag{23.9}$$

In this case a "mean" value of K_v is obtained, as it is assumed that the value of this parameter is the same for all the vials of the batch (this value is an approximation of the heat transfer coefficient of central vials, as they represent the majority of the batch). Similarly, a "mean" value of K_v is obtained also when using one of the algorithms proposed to monitor the process using the pressure rise test (PRT): the valve in the duct connecting the drying chamber to the condenser is closed for a

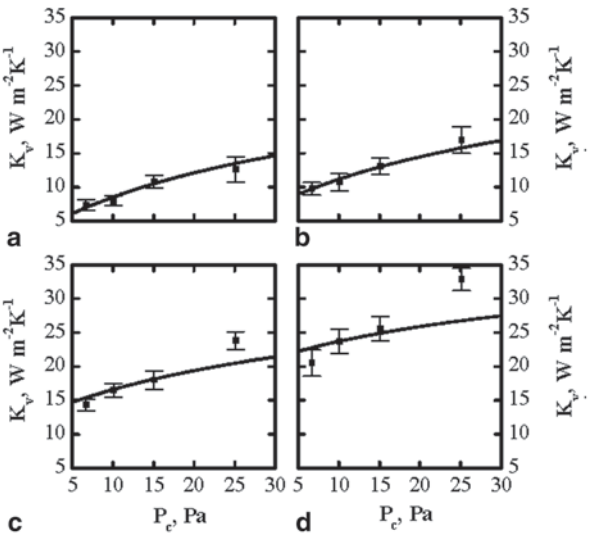

Fig. 23.2 Effect of chamber pressure on the values of K_v for the various groups of vials described in Table 23.1 (tubing vials, internal diameter = 14.25 mm). *Symbols* refer to the experimentally measured values; *lines* correspond to the values calculated using Eq. (23.8)

short time interval (e.g., 30 s), and various variables (temperature and residual ice content of the product, and model parameters K_v and R_p) are determined looking for the best fit between the measured and the calculated values of pressure rise (Milton et al. 1997; Liapis and Sadikoglu 1998; Chouvenc et al. 2004; Velardi et al. 2008; Fissore et al. 2011a).

The parameter R_p depends on the freezing protocol, on the type of product and of freeze-dryer, and on the thickness of the cake according to the following equation:

$$R_p = R_{p,0} + \frac{P_1 L_{\text{dried}}}{1 + P_2 L_{\text{dried}}}. \tag{23.10}$$

The parameters $R_{p,0}$, P_1, and P_2 have to be determined by means of experiments, looking for the best-fit between the reference curve and the measured values of R_p vs. L_{dried}. The PRT can be used for this purpose, as well as the TDLAS sensor using the following equation:

$$R_p = \frac{p_{w,i} - p_{w,c}}{J_w}. \tag{23.11}$$

A weighing device placed in the drying chamber can be used to measure the sublimation flux and, in case also product temperature is measured, to estimate R_p through Eq. (23.11). The Lyobalance (Vallan 2007; Barresi and Fissore 2011; Fissore et al. 2012) can be effectively used to this purpose as the weighed vials are frozen with all the other vials of the batch, they remain almost always in contact with the shelf (they are lifted just during the measurement), and the geometrical characteristics of the weighed vials are the same as the rest of the batch. Figure 23.3 shows the values of R_p vs. L_{dried} in case of the freeze-drying of a 5 % by weight mannitol aqueous solution: a good agreement is obtained when comparing the values obtained using Lyobalance and the PRT.

23.2.3 Secondary Drying

The secondary drying stage involves the removal of the bound (unfrozen) water. For an amorphous solid, the water removal rate per unit of mass can be dependent on:

- Water molecular diffusion in the glassy solid from the interior of the solid to the surface;
- Evaporation at the solid–vapor interface;
- Water vapor transport through the porous dried cake;
- Water vapor transport from the headspace in the vial to the condenser.

Generally, water desorption from the solid is assumed to be the rate-determining step, as it has been evidenced by extensive investigations carried out with crystalline (mannitol) and amorphous (moxalactam di-sodium and povidone) products

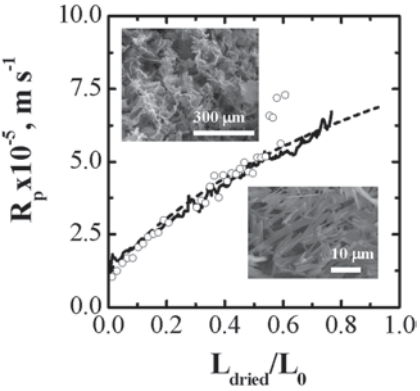

Fig. 23.3 Comparison between the value of R_p vs. L_{dried} measured by Lyobalance (*solid line*), estimated by the pressure rise test technique (*symbol*), and the value calculated using Eq. (23.10) (*dashed line*) in case of the freeze-drying of a 5% by weight mannitol solution ($T_{\text{fluid}} = -22\,°\text{C}$, $P_c = 10$ Pa) processed into ISO 8362-1 2R tubing vials (internal diameter = 14.25 mm), filled with 1.5 mL of solution. The internal structure of the solid is also shown (*Scanning Electron Microscope images*)

(Pikal et al. 1980), and various equations were proposed to model the dependence of r_d on C_s, assuming that the desorption rate is proportional either to residual moisture:

$$r_d = ak_d C_s \tag{23.12}$$

or to the difference between residual moisture and the equilibrium value:

$$r_d = ak_d \left(C_s - C_{s,eq} \right). \tag{23.13}$$

Equation (23.12) has been demonstrated to be able to describe the process adequately (Sadikoglu and Liapis 1997): this has a very important practical advantage when compared with Eq. (23.13), because its expression does not require detailed information about the structure of the porous matrix of the dried layer of the material being freeze-dried. In fact, in order to use Eq. (23.13) one would have to construct an expression for the equilibrium concentration $C_{s,eq}$, and this requires tedious and time consuming adsorption–desorption equilibrium experiments (Millman et al. 1985; Liapis and Bruttini 1994; Liapis et al. 1996)

A detailed multidimensional model of the secondary drying was proposed by Liapis and Bruttini (1995): results evidenced that radial gradients of temperature and concentration are very small, even in those vials placed at the edges of the shelf where lateral heating, due to radiation from the chamber walls, is significant (Gan et al. 2004, 2005). A detailed monodimensional model was proposed by Sadikoglu and Liapis (1997), even if also axial gradients of temperature and concentration were shown to be small (Gan et al. 2004, 2005). Thus, a lumped model can be effective to describe the evolution of product temperature and of the amount of residual

moisture during secondary drying. The energy and mass balances for the product in the vial are given by the following equations:

$$\rho_{\text{dried}} c_{p,p} V_p \frac{dT_p}{dt} = K_v A_v \left(T_{\text{fluid}} - T_p\right) + V_p \rho_{\text{dried}} r_d \Delta H_d \tag{23.14}$$

$$\frac{dC_s}{dt} = -r_d \tag{23.15}$$

where r_d is given by Eq. (23.12). The kinetic constant k_d is dependent on product temperature, e.g., according to an Arrhenius-type equation (Pisano et al. 2012):

$$k_d = k_{d,0} \exp\left(-\frac{E_{a,d}}{\mathrm{R}T_p}\right). \tag{23.16}$$

The effect of chamber pressure on desorption rate is assumed to be negligible, at least in case P_c is lower than 20 Pa as reported by Pikal et al. (1980) and Pikal (2006).

In order to use Eqs. (23.14) and (23.15) to calculate the evolution of the product during secondary drying, it is required to determine the value of the kinetic constant k_d, or better the Arrhenius parameters $k_{d,0}$ and $E_{a,d}$, and to check how r_d depends on C_s. A soft sensor, recently proposed to monitor secondary drying (Fissore et al. 2011b, c), can be effectively used to this purpose. It is based on the measurement of the desorption rate obtained from the pressure rise curve measured during the PRT:

$$r_{d,PRT} = 100 \cdot \frac{\left.\frac{M_w V_c}{\mathrm{R}T_c} \frac{dP_c}{dt}\right|_{t=t_{0,PRT}}}{m_{\text{dried}}} \tag{23.17}$$

and on a mathematical model describing the water desorption from the product (Eqs. (23.12)–(23.15)). The value of the residual moisture in the product at the beginning of secondary drying ($C_{s,0}$) and of the kinetic constant k_d are obtained looking for the best fit between the measured and the calculated values of desorption rate. In case the test is repeated during secondary drying, it is possible to determine the evolution of C_s vs. time. By this way it is possible to determine also how r_d depends on C_s. Finally, in case the test is repeated using different values of T_{fluid}, it is possible to calculate the Arrhenius parameters (looking for the best fit between the measured values of k_d and values calculated using Eq. (23.16)).

Figure 23.4 (*graph a*) shows the dependence of r_d on C_s for different set-points of the heating fluid temperature in case of drying of 5 % w/w aqueous solutions of mannitol, thus proving that a linear equation like Eq. (23.12) is suitable to model this dependence. The desorption rate is measured using the PRT, and the soft-sensor designed by Fissore et al. (2010, 2011b) to monitor secondary drying is used to

determine k_d. The Arrhenius plot (not shown) points out that Eq. (23.16) is able to model the dependence of k_d on T_p. In this case $k_{d,0}$ is equal to 54720 s^{-1}, while the activation energy ($E_{a,d}$) is equal to 5920 J mol^{-1}. Figure 23.4 (*graphs b and c*) compares the values of residual moisture in the product with the values obtained extracting vials from the chamber and using Karl Fisher titration, as well as the calculated product temperature with the values obtained through a T-type thermocouple inserted in some vials: the agreement between measured and calculated values is particularly good and satisfactory.

23.2.4 Freeze-Dryer Equipment Modeling

As anticipated in the introduction, the final product quality may be related to the design of the equipment and, in any case, the selection of the operating conditions required to obtain the desired product characteristics may be significantly influenced by the equipment design (chamber, duct and valve, condenser), and thus the drying time may be affected. In this section, the state-of-the-art in modeling the

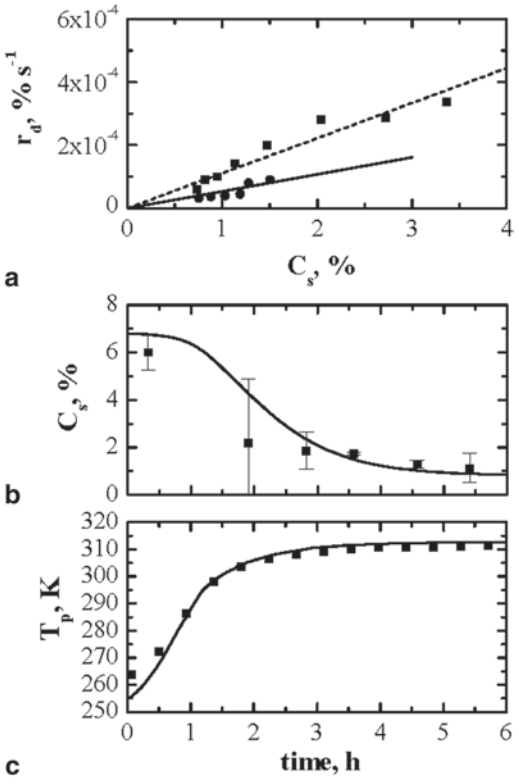

Fig. 23.4 *Graph a*: Desorption rate vs. residual moisture for different temperatures of the heating fluid (*solid line*, *black circles*: T_{fluid} = 20°C; *dashed line*, *black squares*: T_{fluid} = 40°C. P_c = 5 Pa) *Graphs b* and *c*: Comparison between calculated (*lines*) and measured (*symbols*) values of residual moisture (*graph b*), and product temperature (*graph c*) when T_{fluid} = 40°C *and* P_c = 5 Pa. Data refer to a 5 % by weight mannitol aqueous solution processed into ISO 8362-1 2R tubing vials (internal diameter = 14.25 mm), filled with 1.5 mL of solution; primary drying was carried out at T_{fluid} = -10°C *and* P_c = 5 Pa.

different parts of the freeze-dryer, or the dynamic behavior of the whole equipment, will be summarized.

Recently, Computational Fluid Dynamics (CFD) started to be applied to model either single parts or the whole equipment in steady-state conditions (Barresi et al. 2010b): with this approach, continuity and Navier–Stokes equations, along with other relevant governing equations (e.g., enthalpy balance), are solved through a finite-volume numerical scheme. The transport properties appearing in these equations (e.g., gas viscosity and thermal conductivity) are calculated with the standard kinetic theory (often resorting to simple molecular potentials), as explained in the book of Chapman and Cowling (1939).

The main limitation of this technique stands in its description of the sublimating gas as a continuum (Batchelor 1965); to establish whether or not a fluid is in the continuous regime, it is sufficient to compute the Knudsen number (Kn), usually calculated as the ratio of the molecular free path length to a certain representative macroscopic length-scale of the flow (Knudsen 1909): if $Kn < 10^{-3}$ the medium can be considered as a continuum (when $Kn < 10^{-4}$ the Euler equations can be used instead of the Navier–Stokes equations).

If the mean free path of the gas molecules is neither very large nor very small as compared to the macroscopic length-scale of the flow, a more complicated law applies, in particular with respect to the gas flow near solid surfaces. It is generally accepted that the range of applicability of the continuum approach can be extended into the rarefied regime ($10^{-3} < Kn < 10^{-1}$) if special boundary conditions, taken into account the possibility of having a velocity slip or a temperature jump at the walls, are adopted. This is called the slip regime (Maxwell 1879).

When Kn assumes very high values (larger than 1) the flow is in the molecular regime. Under this regime collisions between molecules are not very frequent and the molecule velocity distribution is not Maxwellian; the Boltzmann equation has to be solved. In these cases, it is necessary to resort to alternative simulation frameworks, such as the Lattice–Boltzmann scheme. For the transition regime ($10^{-1} < Kn < 1$), no reliable model exists.

In the drying chamber, as the characteristics size is relatively large and the gas pressure not so low, the CFD approach is generally feasible, even if in case of small clearance between the shelves, the slip boundary conditions must be adopted. In the past, the role played by water vapor fluid dynamics was assumed to be negligible, also because of the difficulty to identify and isolate its effects from the experimental results. Rasetto et al. (2008, 2010) and Rasetto (2009) studied the effect of some geometrical parameters of a drying chamber (clearances between the shelves and position of the duct leading the vapor to the condenser) on the fluid dynamics of the water vapor as a function of the sublimation rate, both in a small-scale and in an industrial-scale apparatus. Their results evidenced the presence of significant pressure gradients along the shelves, in particular in the large-scale units (Barresi et al. 2010a).

Also, the addition of inert to control the pressure may have a significant influence not only on the fluid dynamics in the chamber but also on the local composition of the atmosphere, and thus on the local partial pressure of water; this can be an

additional source of variability in the batch. This aspect is very important especially for the laboratory-scale apparatus, where the inert is typically introduced in the drying chamber only by one inlet, as shown by Barresi et al. (2010a). Furthermore, these concentration gradients can worsen the performance of all those sensors that use a local measurement of water vapor concentration to monitor the process. These sensors are usually confined to peripheral positions to allow the shelves movements for vials stoppering, or are connected to the chamber by short pipes. Rasetto et al. (2009) showed that these phenomena can be more or less marked depending on freeze-dryer geometry and size.

The evolution of product temperature and of residual ice content in the various vials of a batch during a freeze-drying process in some cases may be significantly affected by local conditions around each vial. In fact, vapor fluid dynamics in the drying chamber determines the local pressure that, taking into account the heat flow from the shelf and, eventually, radiation from chamber surfaces, is responsible for the sublimation rate and product temperature. It is very important to be able to predict the expected variability in certain conditions, and to evaluate the effect of a change in the design of the apparatus (for example, in the distance between shelves, and thus in the maximum loading) in product temperature and in the drying time of the vials of the batch.

To this purpose, a dual-scale model which couples a three-dimensional model, describing the fluid dynamics in the chamber, and a second mathematical model, either mono- or bidimensional, describing the drying of the product in the vials, can significantly improve the understanding of a pharmaceutical freeze-drying process. A two-scales model can be useful to simulate the dynamics in single vials placed in particular positions (e.g., where the radiation effects are more important or where the pressure is higher), as well as that of the whole batch, thus calculating the mean value of the product temperature and of the residual water content, as well as the standard deviation around this mean value. An example of the results obtainable with this approach, suitable for process transfer and scale up has been already presented (Rasetto et al. 2010; Barresi and Fissore 2011): in this case, the dependence of local pressure on geometry and operating condition has been given by empirical correlations obtained by CFD preliminary simulations. This approach is valid in case of "one-way coupling," that is in case the general hydrodynamics is not significantly affected by the distributions of the local sources.

When different sublimation fluxes in different vials affect the fluid dynamics in the chamber, resulting in what is usually known as "two-way coupling," a simplified model describing the evolution of each single vial could be directly implemented in the CFD code, e.g., by means of user-defined functions. Only simple models can be implemented in a CFD code and, therefore, this approach is preferable only when a certain degree of uncertainty on the results about the time evolution of the product is acceptable, and the focus is on the equipment design (Barresi et al. 2010b).

As already discussed, great care must be paid to the possibility of choked flow in the duct connecting chamber and condenser. In fact, due to the very low pressure values (and therefore very high water vapor velocities) critical sonic flow conditions may be encountered (Searles 2004; Nail and Searles 2008). The diameter and

length of the duct, as well as the geometry of the isolating valve, must be properly designed in order to guarantee under a wide range of operating conditions that the desired sublimation rate is evacuated.

Recently Alexeenko et al. (2009) investigated fluid flow in the duct connecting the drying chamber of a freeze-drying apparatus to the condenser both in an industrial scale and in a lab-scale unit. The flows under continuum gas conditions were analyzed using the Navier–Stokes equations, whereas the rarefied flow solutions were obtained by the Direct Simulation Monte Carlo (DSMC) method the Boltzmann equation. The comparison of the results showed that under extreme operating conditions the continuum approach (used by CFD) can be no longer valid.

Recent unpublished work carried out by the authors and by other researchers (Patel et al. 2010; Barresi et al. 2010b) has confirmed that in many cases the CFD approach is still suitable, even if discrepancies (not completely explained) have been evidenced between experimental results and predictions.

Figure 23.5 reports an example of the mass flow rate as a function of condenser and chamber pressures. As it is possible to see, when the pressure difference increases, critical flow conditions are reached, resulting in a maximum flow rate, known as "critical flow rate," which increases with the pressure in the chamber, because this affects the static density of the fluid. This critical flow rate depends on the chemical composition of the vapor (it is modified by the presence of inert) and is strongly influenced by the length-to-diameter ratio of the duct and the geometry of the isolating valve. To this purpose, it must be evidenced that even if it has been suggested that the conductance of a duct is independent on the duct diameter, if the results are plotted in term of mass flow (Oetjen 1999; Oetjen and Haseley 2004) the duct size actually affects the conductance. Another aspect that must be carefully considered is that the conductance of an empty duct is strongly affected by the inlet conditions, as the largest part of the pressure drop takes place just in the inlet, then accurate simulations must include also the inlet of the chamber and the exit. Finally, the real conductance may be much larger than that estimated according to the procedure proposed by Oetjen (1997, 1999).

In case of the valves, the type (mushroom or butterfly) and even more the shape of the disk strongly affect the conductance. Thus, in case of scale up or process transfer from a freeze-dryer to another, significantly different limitation to maximum sublimation rate may occur. Figure 23.6 shows an example of the critical flows that can be estimated by CFD for two different valves: the case of an empty duct is also shown for comparison, to show that the concept of duct equivalent length, often adopted to handle the case of valves, must be used with great care. As the slope of the curves is different, the correct equivalent length would change with chamber pressure.

The performance of the condenser may have a significant effect on the drying cycle and on the final product quality. If its efficiency is low, it may be difficult to reach the minimum pressure required in the chamber, and in case, it is not able to condense all the vapor sublimated; in fact, the pressure in the chamber will increase up to when the pressure control is lost (and a pressure increase is always related to a fast increase in the product temperature). The factors that influence the condenser

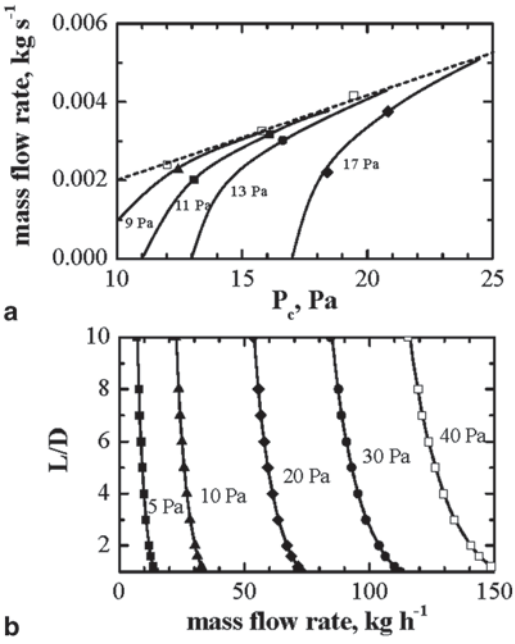

Fig. 23.5 *Graph a*: Mass flow rate as a function of the chamber pressure for different condenser pressures. Straight duct, DN 350, $L/D=2$. The *dashed line* with empty symbols corresponds to sonic flow and represents the asymptote. *Graph b*: Dependence of critical mass flow rate on chamber pressure and duct geometry. (Data published by permission of Telstar Technologies S. L.)

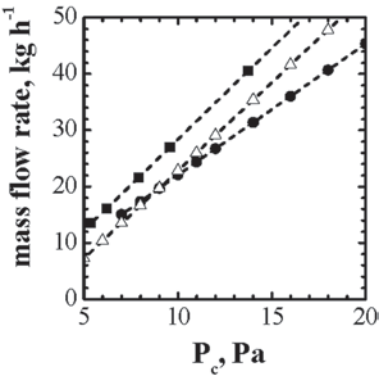

Fig. 23.6 Examples of critical flow conditions, estimated using CFD, with empty duct and different valve geometries. The filled symbols refer to two different valve shapes, the open symbols to a straight duct with $L/D=10$. (Data published by permission of Telstar Technologies S. L.)

efficiency are condenser geometry, fluid dynamics of sublimated vapor, chamber design, duct size, location and type of closing valve used, and the dynamics of ice deposition, but little work has been done to investigate in detail the influence of the condenser geometry on its efficiency (Kobayashi 1984).

The design of the condenser can largely benefit from the knowledge of the real fluid dynamics inside the condenser itself and from the evaluation of the ice deposition rate on coils and surfaces, but very little work has been done on modeling of the condenser up to now: due to the very low pressure, depending on the geometry considered, the continuum approach may be valid or not. Ganguly et al. (2010) focused

on the simulation of ice deposition in a laboratory-scale condenser by means of DSMC, comparing the efficiencies of two different geometries, but without investigating the role played by the inert gas. Computational fluid dynamics was instead used by Petitti et al. (2013) to model both a whole lab-scale apparatus (including drying chamber, duct, valve, and condenser) and an industrial condenser, with the purpose to achieve a better comprehension of the flow dynamics and of the process of ice condensation and deposition in the condenser, in order to evaluate condenser efficiency. Computations can become extremely heavy in this case, especially in the complex geometry of an industrial apparatus, due to the necessity of modeling the vapor disappearance (and the ice formation) with a realistic mechanism that takes into account the proper kinetics.

Finally, a multiscale model of the process, which couples a lumped model of the dryer and of the condenser, with a detailed model of the vial, can be used for better understanding the dependence of process/product dynamics upon processing conditions, or to predict the product quality in presence of unexpected variations in the fluid temperature and/or in the chamber pressure due, for example, to a plant malfunctioning (Sane and Hsu 2007, 2008).

23.3 Design Space Calculation for the Primary Drying Stage

The design space for the primary drying stage can be defined as the set of operating conditions (temperature of the heating fluid and pressure in the drying chamber) that allows to maintain product temperature below the limit value, beside avoiding choking flow in the duct connecting the drying chamber to the condenser. Process simulation allows calculating quickly the design space: the accuracy of the results is affected by model accuracy and by parameters uncertainty. As a consequence, the mathematical model used for the calculations has to be accurate, and it should involve few parameters that could be easily measured (or estimated) with few experimental runs. To this purpose, the simplified model previously described (Eqs. (23.2)–(23.6)) can be effectively used.

When calculating the design space for the primary drying stage two different approaches can be used. In fact, it is possible to look for the values of T_{fluid} and P_c that maintain product temperature below the limit value throughout the primary drying stage, or it is possible to take into account that the design space changes as drying goes on due to the increase of the cake thickness. In fact, Eqs. (23.4) and (23.6) can be written as:

$$\left(\frac{1}{K_v}+\frac{L_{\text{frozen}}}{\lambda_{\text{frozen}}}\right)^{-1}\left(T_{\text{fluid}}-T_i\right)=\Delta H_s\,\frac{1}{R_p}\left(p_{w,i}-p_{w,c}\right) \tag{23.18}$$

evidencing that the same couple of operating conditions (T_{fluid} and P_c) can determine different values of product temperature (and of sublimation flux) depending

on the value of L_{dried} and, thus, of R_p. This means that a couple of values of T_{fluid} and P_c can be inside the design space at a certain time instant during primary drying, and they can be outside in a different time instant.

In case T_{fluid} and P_c are kept constant throughout the primary drying stage, the following calculations can be done to investigate if their values are inside the design space (Giordano et al. 2011):

1. Selection of the range of values of T_{fluid} and P_c of interest.
2. Selection of a couple of values of operating conditions $T_{fluid,k}$ and $P_{c,j}$.
3. Calculation of the evolution of product temperature and sublimation flux until the end of primary drying.
4. The operating conditions $T_{fluid,k}$ and $P_{c,j}$ belong to the design space in case maximum product temperature is lower than the limit value, and the maximum sublimation flux is lower than the limit value.
5. Repetitions of steps 3–4 for all the values of $T_{fluid,k}$ and $P_{c,j}$ of interest.

The approach proposed by Giordano et al. (2011) can be effectively used to account for model parameters uncertainty in the calculation of the design space. It has to be remarked that as the batch is nonuniform, mainly due to the different heat transfer mechanisms to the product, the previously described procedure has to be repeated for each group of vials, characterized by a specific value of the heat transfer coefficient.

Figure 23.7 shows the design spaces calculated for the vials of group a (center of the shelf) and of group d (first external raw) in case of freeze-drying of a 5% mannitol aqueous solution. Various iso-flux curves are shown, and they can help in identifying the operating conditions that minimize the duration of primary drying. It is evident that the design space of vials of group a is larger than that of vials of group d: this is due to the fact that for a given couple of values of T_{fluid} and P_c product temperature is lower, due to the lower value of K_v. As a consequence, for a value of chamber pressure of 5 Pa it could be possible to set $T_{fluid} = -15\,°C$ without breaking the constraint on maximum product temperature in vials of group a, but vials of group d would be overheated. Thus, if the goal is to maintain product temperature below the limit value in the whole batch, it is required to set a lower value of T_{fluid}, e.g., $-20\,°C$, even if this will result in a higher drying time. In fact, primary drying is completed first in the vials of group d, and then in vials of group a, where the sublimation flux is lower than that obtained in case $T_{fluid} = -15\,°C$, as product temperature is lower.

Figure 23.8 shows the results obtained when the operating conditions (shown in graph A) are selected in such a way that product temperature remains below the limit value throughout the primary drying stage in the whole batch as shown by the thermocouple measurement in vials of groups a and d (shown in graph c), as well as by the temperature estimated using the PRT (that can be assumed to be equal to that of vials in the central position of the shelf, as they are the most numerous). In this case, the duration of the primary drying is equal to about 31 h, as determined by the ratio between the pressure signal of a capacitive and of a thermal conductivity gauge (shown in graph b).

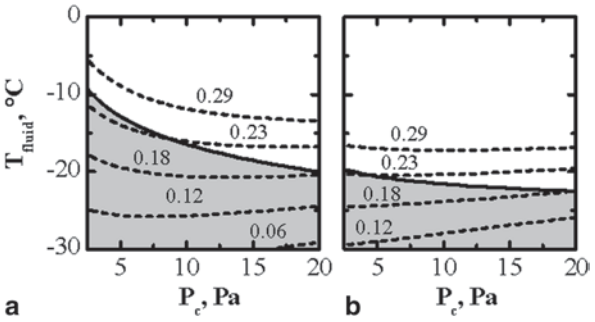

Fig. 23.7 Design space for the freeze-drying of a 5 % by weight mannitol solution for vials of group A (on the *left*) and of group D (on the *right*). Isoflux curves (in kg $h^{-1}m^{-2}$) are also shown (*dashed lines*)

The choice of the container type is an important aspect to be considered during the design process. This decision is dictated by the filling volume used in manufacturing, and by the volume of liquid required for the product reconstitution. With this regard, various solutions are feasible, as the same solid content per vial can be obtained varying both the solution concentration and the filling volume. However, these various combinations are not equivalent in terms of drying length, as they entail a different value of the product resistance to vapor flow and of the total amount of water to be removed. To better clarify this aspect, let us consider an example, that is, the freeze-drying of a mannitol-based formulation. Let us imagine that the objective is to get 50 mg of dried product per vial, and that the same type of vials

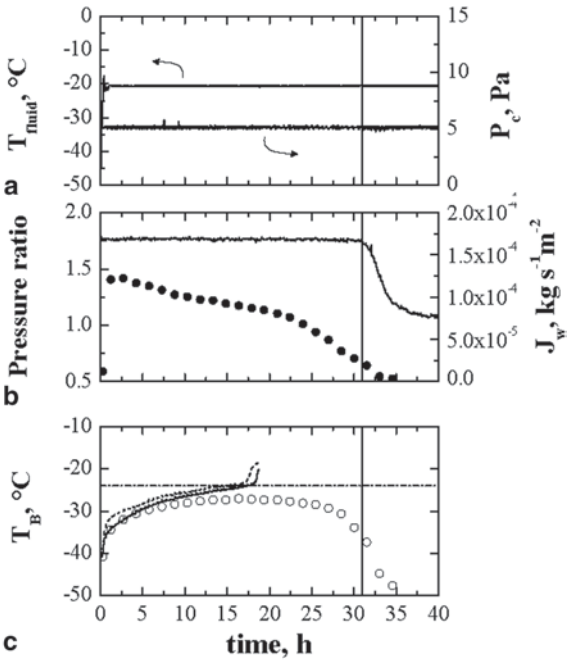

Fig. 23.8 Example of freeze-drying cycle carried out using a 5 % by weight mannitol solution. Evolution of: (*graph a*) T_{fluid} and P_c; (*graph b*) Pirani-Baratron pressure ratio (*solid line*) and J_w as estimated by the PRT technique (*symbols*); (*graph c*) T_B as measured by thermocouples (*solid line*: vial of group a, *dashed line*: vial of group d) or estimated by PRT technique (*symbols*). The *vertical line* evidences the completion of ice sublimation as detected by the pressure ratio

used above for carrying out the experimental study is considered here. To achieve this objective, two different configurations are investigated: (1) 1.5 mL of a 5 % by weight mannitol solution per vial, thus L_0 = 10.6 mm; and (2) 0.38 mL of a 20 % by weight mannitol solution, thus L_0 = 2.65 mm. The product resistance to vapor flow measured for the two formulations is displayed in Fig. 23.9 (graph a): as expected, the value of R_p of the second formulation increases faster with L_{dried}. These values have been then used to build the design space, and hence to design an appropriate cycle for the drying of the two formulations, see Fig. 23.9 (graphs b). Figure 23.9 (graph c) shows how the drying time varies with the solid content, and for two different values of chamber pressure. As expected from the design spaces displayed in Fig. 23.9 (graph b) for 5 and 20 % by weight mannitol solutions, the value of chamber pressure does not significantly modifies the drying time. On the contrary, the duration of the sublimation step significantly reduces as the solid content increases and the filling volume decreases. In order to optimize the drying duration, these results suggest to use, when possible, a high solid content and a low filling volume. Of course, in a similar way it is possible to evaluate the influence of a change in the vial geometry and size.

In case the variation of the design space vs. time is taken into account, the approach proposed by Fissore et al. (2011d) can be effectively used. It is based on the use of L_{dried} (or L_{frozen}) as third coordinate of the diagram instead of time. In fact, at the same time instant, the value of L_{dried} can be different due to the past history of the product and, thus, it would be impossible to get a unique diagram using time as coordinate of the diagram. On the contrary, the use of L_{dried}, beside T_{shelf} and P_c, allows obtaining a unique diagram. The following calculations are required:

1. Selection of the range of values of T_{fluid} and P_c of interest.
2. Selection of the range of values of L_{dried} of interest.
3. Selection of a couple of values of operating conditions $T_{\text{fluid},k}$ and $P_{c,j}$.
4. Calculation of product temperature and sublimation flux for the ith value of L_{dried}.
5. The operating conditions $T_{\text{fluid},k}$ and $P_{c,j}$ belong to the design space for the selected value of L_{dried} in case product temperature is lower than the limit value, and the sublimation flux is lower than the limit value.
6. Repetitions of steps 4–5 for all the values of $T_{\text{fluid},k}$ and $P_{c,j}$ of interest, thus obtaining the design space for the selected value of L_{dried}.
7. Repetitions of steps 4–6 for all the values of L_{dried} of interest.

Figure 23.10 shows the results of the calculations for the vials of group A of the previous case study: each curve identifies the highest value of T_{fluid} that keep product temperature below the limit value for the values of L_{dried} and P_c considered. As far as the primary drying goes on, i.e., L_{dried} increases, the design space shrinks because of the variation of R_p with time. Figure 23.11 shows an example of results obtained when using a cycle selected using the design space shown in Fig. 23.10. It appears evident that it is possible to carry out the first part of primary drying using higher values of T_{fluid} and P_c with respect to the values required for the second part, and this could be useful to further optimize the process (in this case primary drying is

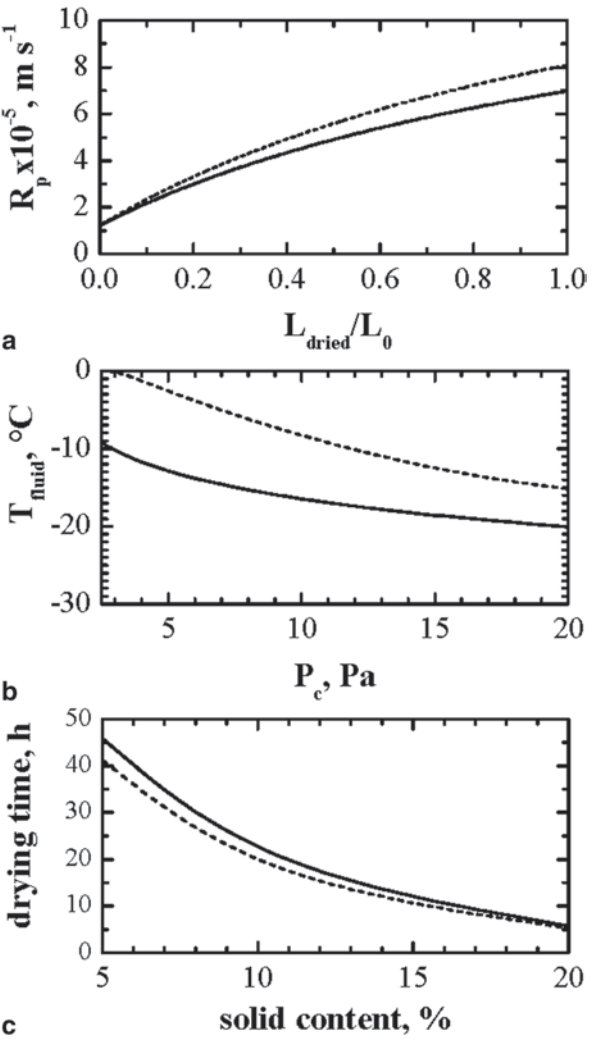

Fig. 23.9 *Graph a*: Comparison between the value of R_p vs. L_{dried} in case of the freeze-drying of a 5% (*solid line*) and of a 20% (*dashed line*) by weight mannitol solution. *Graph b*: Design space for the freeze-drying of a 5% (*solid line*) and of a 20% (*dashed line*) by weight mannitol solution for vials of group a. *Graph c*: Duration of the primary drying stage for mannitol solutions having a different solid content and processed at (*dashed line*) P_c = 5 Pa and (*solid line*) P_c = 20 Pa. The fluid temperature is chosen according to the design space of each formulation. The filling volume is fixed to get 50 mg of dried product per vial

completed in 25 h, 6 h less than in case the operating conditions are kept constant throughout primary drying).

Beside cycle design and optimization, the design space can be effectively used for process failure analysis, i.e., to evaluate if the product remains inside the design space after an unexpected variation of T_{fluid} and/or P_c due to some kind of failure or disturbances. In this case, misleading results can be obtained if the design space calculated without taking into account the variation of L_{dried} during primary drying is used. As an example it is possible to consider the following case study. Let us consider the case of $T_{fluid} = -20\,°C$ and $P_c = 5$ Pa and that at about one-half of primary drying, the temperature of the heating fluid increases to $-10\,°C$. According

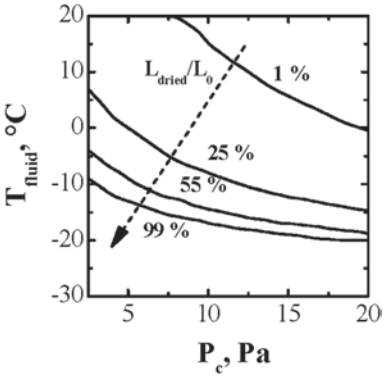

Fig. 23.10 Design space for 5% by weight mannitol solution calculated at various values of L_{dried}/L_0

to the design spaces shown in Fig. 23.7, the new values of the operating conditions are outside the design space and, thus, the cycle can be stopped and the product discarded. According to the design space shown in Fig. 23.10, the product is still in the design space, at least until L_{dried}/L_0 is lower than 55%.

A final remark concerns the scale-up of the design space. Actually, as the design space is calculated using the model of the process, and the results depends on the

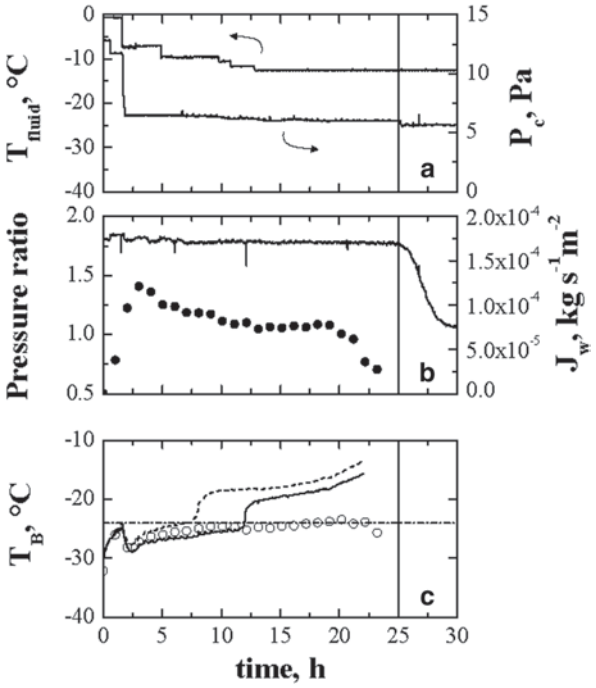

Fig. 23.11 Example of freeze-drying process carried out using a 5% by weight mannitol solution. Evolution of: (*graph a*) T_{fluid} and P_c; (*graph b*) Pirani–Baratron pressure ratio (*solid line*) and J_w as estimated by the PRT technique (*symbols*); (*graph c*) T_B as measured by thermocouples (*solid line*: vial of group a, *dashed line*: vial of group d) or estimated by PRT technique (*symbols*). The *vertical line* evidences the completion of ice sublimation as detected by the pressure ratio

values of the parameters K_v and R_p and also on the limit curve to avoid the occurrence of choked flow in the duct. As the heat transfer coefficient K_v takes into account all the heat transfer mechanisms to the product, and some of these can vary in different freeze-dryers (e.g., radiation from chamber walls, radiation from upper shelf,…), then this parameter has to be experimentally measured also in the industrial-scale freeze-dryer. Actually, in case the coefficients C_1, C_2, and C_3 of Eq. (23.8) have been determined in the lab-scale freeze-dryer, only one gravimetric test might be necessary for a different equipment (to determine the value of the coefficients C_1), as the parameters C_2 and C_3 gives the dependence of K_v on P_c, and their dependence on the type of equipment can be neglected. Once also the parameter R_p has been determined in the industrial-scale freeze-dryer, then previous algorithms can be used to determine the new design space.

As concerns the choked flow conditions, it must be evidenced that these are generally reached more easily in an industrial apparatus than at laboratory and pilot scale; and as shown in Fig. 23.6, changes in the geometry of the duct, and in the characteristics of the valves installed, may modify significantly the conductance.

23.4 Design Space Calculation for the Secondary Drying Stage

The design space for the secondary drying stage can be defined as the set of operating conditions (temperature of the heating fluid and duration of the secondary drying) that allows to get the target value of residual moisture in the product, beside maintaining product temperature below the limit value. This requires to know how the glass transition temperature changes as a function of the residual moisture content in the product. In case of sucrose solutions, the equation proposed by Hancock and Zografi (1994) can be used:

$$T_g = \frac{C_s T_{g,w} + K\left(1 - C_s\right) T_{g,s}}{C_s + K\left(1 - C_s\right)} \tag{23.19}$$

with $K = 0.2721$, $T_{g,w} = 135$ K, and $T_{g,s} = 347$ K.

The design space for the secondary drying stage can be calculated using the lumped model previously described (Eqs. (23.14)–(23.15)) according to the following procedure (Pisano et al. 2012):

1. Selection of the range of values of T_{fluid} of interest.
2. Determination of the maximum allowed value of product temperature as a function of the residual moisture content.
3. Selection of the value of $C_{s,0}$.
4. Calculation of the evolution of T_p and C_s, using the model of the process, for the ith value of fluid temperature $T_{\text{fluid},i}$.

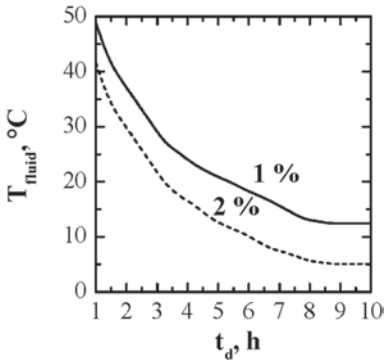

Fig. 23.12 Design space calculated for the secondary drying of a 5% by weight mannitol solution in case $C_{s,0}$ = 5% and the target value of residual moisture is 1% (*solid line*) or 2% (*dashed line*).

5. Determination of the time ($t_{d,i}$) required to get the target value of residual moisture ($C_{s,i}$) for the selected value of heating fluid temperature.
6. The point corresponding to the couple of values ($t_{d,i}$, $T_{\text{fluid},i}$) belongs to the design space in case product temperature remains below the limit value throughout the drying phase.
7. Repetition of steps 4–6 for all the values of $T_{\text{fluid},i}$ of interest.
8. Repetition of steps 4–7 for different values of $C_{s,0}$, as this variable can be hardly known and it can be not the same for the various vials of the batch.

When the design space has been calculated, it is possible to optimize the secondary drying stage by selecting the value of T_{fluid} that minimizes the drying time.

Figure 23.12 shows the design space obtained for the secondary drying of a 5% w/w aqueous solutions of mannitol (the maximum temperature of the heating fluid is assumed to be 40 °C). For a target value of residual moisture (e.g., 1 or 2%), the design space is coincident with the area of the diagram below the solid line. In case the target value of residual moisture must be comprised between two values, e.g., 1 and 2%, then the design space corresponds to the area comprised between the two curves.

23.5 Conclusions

Mathematical modeling can be really effective in obtaining quality-by-design in a freeze-drying process. In fact, mathematical simulation of the freezing stage, as well as of the primary and secondary drying stages can allow determining the effect of the operating conditions of the process and, thus, to preserve product quality, beside optimizing the process. Evidently, this approach requires a preliminary investigation to determine the values of the parameters of the model: model accuracy and level of parameters uncertainty influence the quality of the results. As an alternative, it could be possible to design the freeze-drying cycle inline, using a suitable monitoring system (e.g., the PRT) and a control algorithm (Pisano et al. 2010,

2011b). Obviously, in this case, only the best cycle (according to the target specified in the control algorithm) is obtained, and the additional information supplied by the design space, concerning the robustness and the effect of eventual deviations, are not available. In addition, the feasibility of the approach based on the use of control algorithms is limited by the availability of a suitable monitoring system, in particular in industrial-scale freeze-dryers.

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Chapter 24
Application of Multivariate Statistical Process Monitoring to Lyophilization Process

Fuat Doymaz

24.1 Introduction

Multivariate statistical process monitoring (MSPM) has found applications in a variety of industrial applications (Duchesne and MacGregor 2000; Doymaz et al. 2001; Machin et al. 2011; Ündey et al. 2010, 2012; Zhang et al. 2004). One of the critical steps in manufacturing biological drug products is lyohilization process step that involves several phases such as temperature equilibration, freezing, primary drying, and secondary drying (Jameel and Searles 2010). Lyophilization data generated during manufacturing have three-dimensional structure (Kourti 2015). Variables measured at each of the freezing, primary drying, and secondary drying phase of a batch have records of data per sampling instance that creates the batch evolution variable, time. Trajectories of the lyophilization process variables within each phase are dictated by the recipe developed for a lyophilized drug product. When sufficient data from successfully completed batches become available, an MSPM model can be developed to effectively monitor the process. Thereafter, every new batch can be monitored either in real-time or offline. Where infrastructure allows for real-time data collection, synchronization, and data analysis, it is possible to detect undesirable trends, identify root cause(s), and subsequently make mid-course changes to the process. Significant savings is possible if corrective action is taken timely to manufacture a batch with good product quality characteristics. This chapter aims to provide the practitioner with the elements of how MSPM is applied to a lyophilization process.

Batch data collected from a lyophilization process can come from various sources and have a range of formats. For example, process variable trajectories such as, measurements obtained at frequent intervals for the duration of freezing, primary drying, and secondary drying are formed from variables like shelf

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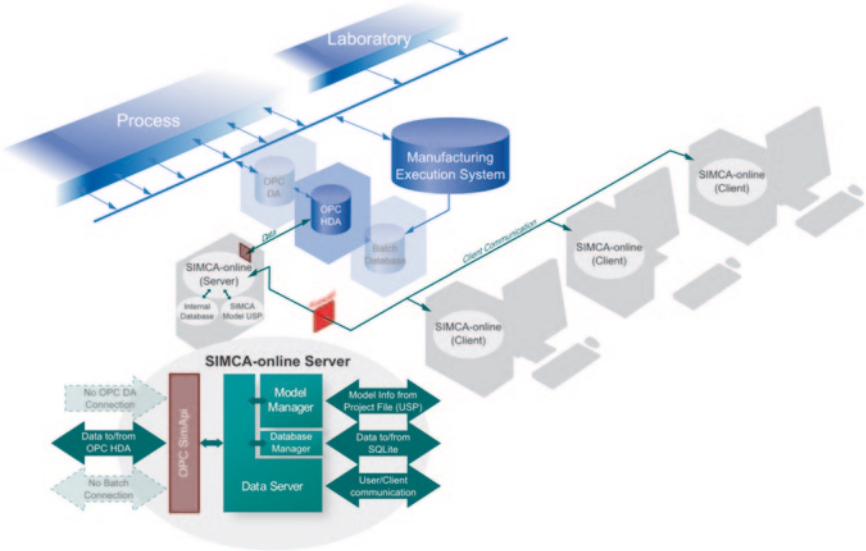

Fig. 24.1 An interface developed by Umetrics, Inc. to access online and offline process and laboratory data makes quicker and healthier analysis of the data possible for process performance assessment during manufacturing

temperature, chamber pressure, condenser temperature, heater input, or Near Infrared (NIR)-based spectral moisture data. As a result, large datasets are accumulated via use of data acquisition systems connected to historians such as the OSIsoft, Inc.'s PI system (OSIsoft Inc 2015) that stores data in a process database called manufacturing execution system (MES) (see Fig. 24.1). These datasets or, subsets of them, may be used in different ways to build models to analyze process and product performance behavior. Access to these datasets is made possible using data servers. Umetrics Inc. (Umetrics Inc. 2013) has developed a server called SIMCA-online server to facilitate access to such data through its two versions of (online-and offline) SIMCA softwares. Users (operators or process engineers) analyze such high-dimensional and complex datasets to summarize process performance either on a client PC, website, or hand-held devices. These softwares allow multivariate data analysis in a fast, reliable, and in a manner that is compliant with the United States Food and Drug Administration's Code of Federal Regulation (CFR) Part 11 (Food and Drug Administration of the United States 2003).

24.2 Data Pretreatment and Analysis

To build an MSPM model, two datasets (modeling and test) are needed. Modeling dataset should contain batches of normal operation and test dataset would ideally comprise both good and failed batches data. This would allow building an MSPM model whose performance is checked prior to installation for online

process performance monitoring purpose. Prior to model building step, data matrix would need to be unfolded batch-wise or variable-wise (Kourti 2015), and subsequently the variables are scaled to have zero mean and unit variance. If time factor is selected as a batch evolution parameter, a technique called dynamic time warping approach is used to track batch performance at specified time intervals (Kassidas et al. 1998). Scaled data are then projected onto orthogonal (principal components) subspace for creating few latent variables that capture most of the variability in the original data. The process of selecting the number of latent variables may sometimes involve leave-one-out type cross-validation within the training dataset, or by projecting new batches data (test dataset) onto the model space to assess model performance for a given number of principal components.

24.3 Process Performance Monitoring for Fault Detection and Isolation

Once model is built, monitoring of the process is possible by plotting the first two latent variables (t_1 and t_2) on a scatter plot with limits in the form of an ellipse, as shown in Fig. 24.2. When the process is in state of statistical control, the points (latent variables' scores) will fall within the ellipse. In case of an anomaly in the process, the scores will plot outside the ellipse. The region within the ellipse is also called normal operating region (NOR). For real-time process monitoring, it is preferable to create a Hotelling's T^2 statistic chart and a square prediction error (SPE) chart to define the NOR based on a number of latent variables. In some software packages the term distance to model (DModX) is also used in place of SPE. The T^2 and SPE charts shown in Fig. 24.2 are two complementary indices; together they indicate how the process is performing at a time point during batch evolution. If points are within their respective limits (i.e., inside NOR), the process is considered under control. (Doymaz et al. 2001) proposed the use of T^2 and SPE measures in a single plot with their respective limits for classifying in- or out-of-control events. In the SPE vs. T^2 plot, values that simultaneously violate both T^2 and SPE limits usually point to process disturbances while sensor/test method issues mostly trigger the SPE limit excursions. Once a point is detected out of limit, then contribution plot can be utilized to provide us with a list of the variables that mainly contributed to the out-of-control point, and hence, assists in diagnosing the problem immediately. For both charts, contribution plots can be created for out-of-control points.

24.4 MSPM Application to a Lyophilization Process

In the following example, data from five historical batches that represent normal operations during freezing, primary drying, and secondary drying of a biopharmaceutical drug product lyophilization process were considered in the model-building

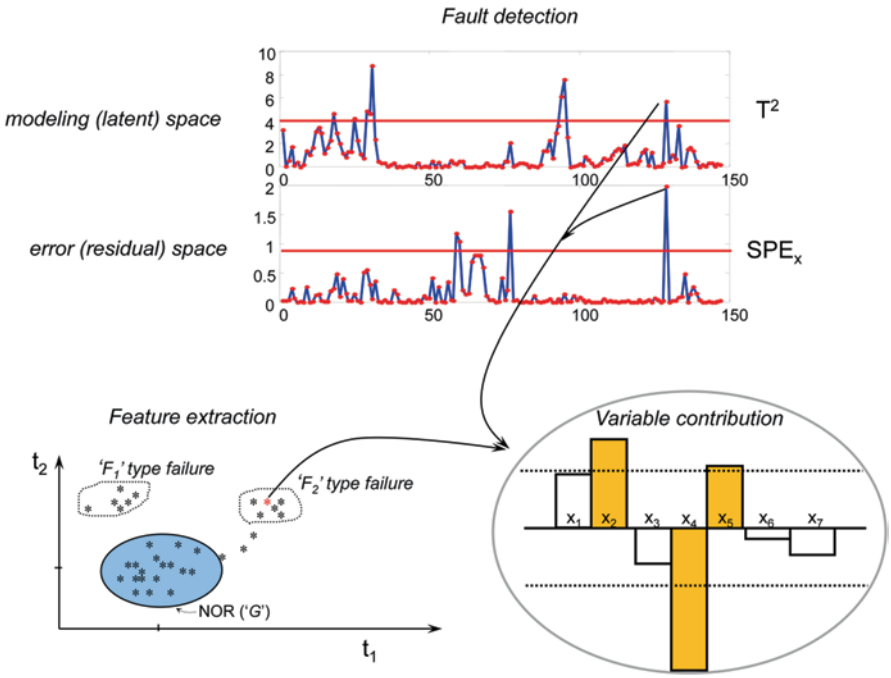

Fig. 24.2 The process may be monitored with two charts (Hotelling's T^2 and SPEx). When deviation from NOR is observed, contribution plots can be used to identify the variables responsible for the deviation

step. Performance of the model built was tested by mapping the data from a batch, which underwent a process deviation, onto the NOR (T^2 and DModX). In general, model building requires more data than only from five batches. However, for several reasons, it is impractical and unnecessary to wait to manufacture say 15 batches or more lots in order to build first multivariate model. In cases where data are limited to around five batches, one can build the model and then update the model as more batch data become available. Having limited data can only increase false alarm rate which understandably better than not reacting to an issue that could compromise the quality of a batch.

Lyophilization dataset consists of time-course data from temperature (product [x5], condenser [x3], shelf heating liquid –prior to entering [x6] and after exiting [7] shelf), chamber pressure (from viatran) [x1], chamber vacuum [x2], and input from silicon controlled rectifier (SCR) power control unit [x4] sensors across freezing, primary drying, and secondary drying phases of the lyophilization process. Elapsed time was chosen to be the batch maturity variable in this example, hence batch PLS is the appropriate choice of modeling to create NOR for process monitoring. Data were analyzed using Umetrics SIMCA version 13 software. To construct the MSPM batch model, three PCs were retained for three lyophilization

phases which accounted for 83 %, 69 %, and 89 % of the variability in **X** matrices of the freezing, primary drying, and secondary drying phases, respectively. Fig. 24.3 depicts the Hotelling T^2 and DModX plots of the modeling segments of three phases. For process performance monitoring, these two measures provide complementary information about the overall status of the process, hence both charts should be watched for any signal that appears out-of-control. The SIMCA software calculates two limits for the Hotelling T^2 chart: 95 % upper control limit and 99 % upper control limit to define the model space boundary of the NOR. Process owner may want to react to the tighter control limit given by the T2Crit(95 %) and then decide to switch to T2Crit(99 %) in cases it gives too frequent false alarms. The DModX limits are established based on variability around the mean squared prediction error of each time point. Hence, the + 3Std Dev limits shown in solid red color defines the upper limit of the NOR at a particular point in time of batch evolution for each lyophilization phase.

In testing step of the MSPM model, data from another batch (Lot T1) known to have encountered issues during its production were used to assess the capability of the model built using good batches data. In SIMCA program software, the dataset is imported from its source and labeled as prediction set. Batch control charts are created fast by the SIMCA software. Figure 24.4 shows how the process fared against NOR regions defined in both Hotelling T^2 and DModX measures. While time evolution of Lot T1 during freezing and secondary drying phases seemed relatively in control, the process appears to have encountered a disturbance that triggered both Hotelling's T^2 and the DModX measures to exceed their control limits. The peak point highlighted in the Hotelling's T^2 plot of the primary drying phase (marked as Event A) was selected for drill down of its cause. To do this, SIMCA simplified the calculations of the contribution to Hotelling's T^2 and displays them in a bar graph. The trend chart for the variables (sensors) with bar heights that exceed ± 3 standardized unit can further be analyzed for their conformance to historical levels during batch progression. The contribution plot for Event A in Fig. 24.4 is depicted in the upper left of Fig. 24.5. At this time point, variable x4 (SCR heater controller input) contributed more than 3Std Dev unit to the T^2 and the trend chart on lower left of Fig. 24.5 clearly shows the span of this variable's nonconforming behavior to historical range. Post investigation to this behavior found a failed transformer in the SCR heater that led the control fuse to blow which subsequently reduced heating capacity of the heater. Another significant out-of-control signal was also seen in the DModX plot which almost covered the entire time of the primary drying period (Fig. 24.4). The point marked as Event B was similarly investigated by looking at the contribution plot of the variables to DModX (see Fig. 24.5 top right bar chart). The contribution of x3 (condenser temperature) has contribution that exceeded the 3 Std Dev unit from the center. Trend chart of the x3 shown in the bottom right of Fig. 24.5 indicates different temperature levels that are below the historical range at early periods and after mid range of the primary drying phase.

The example presented above shows the utility of MSPM batch model for monitoring process performance offline and also for identifying issues after they have

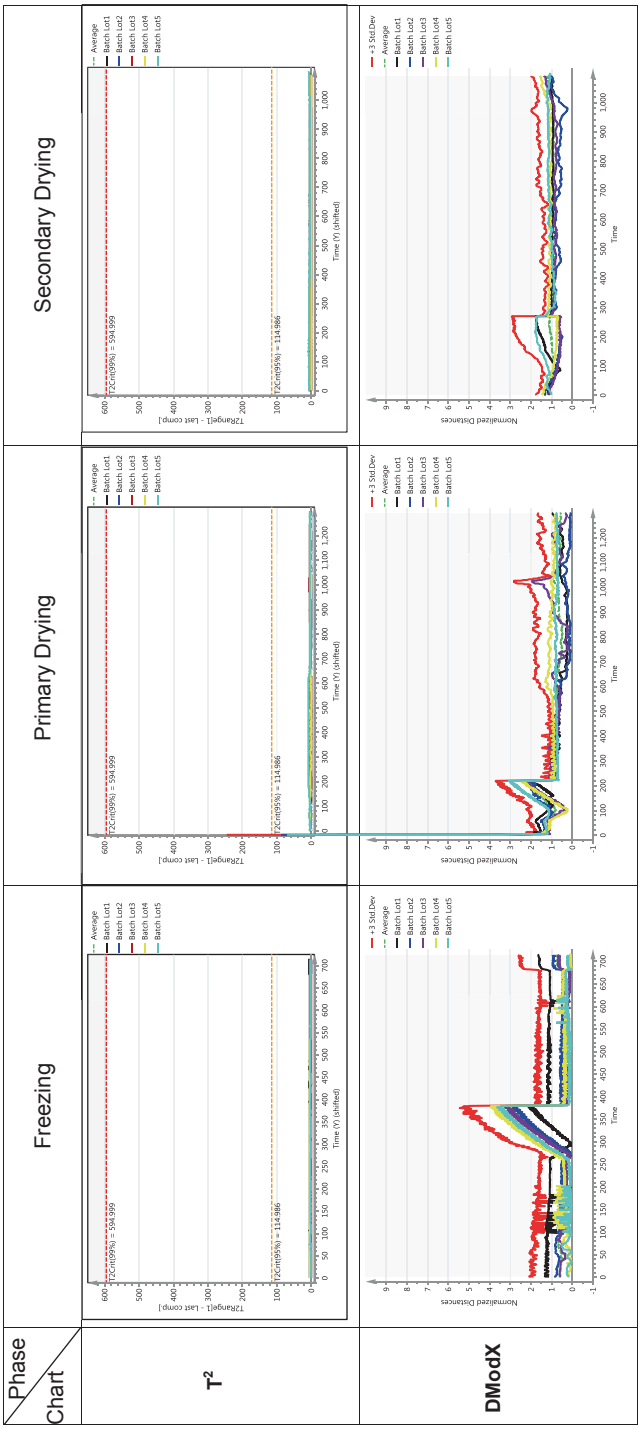

Fig. 24.3 Hotelling's T^2 and DModX for each lyophilization phase and control limits that define NOR. These plots summarize several dozens of measurements on variables (and other information collected for each batch) at a given time point by projecting the information down to a single point in principal component space and renders simple but succinct way of visualizing the process performance during batch manufacturing

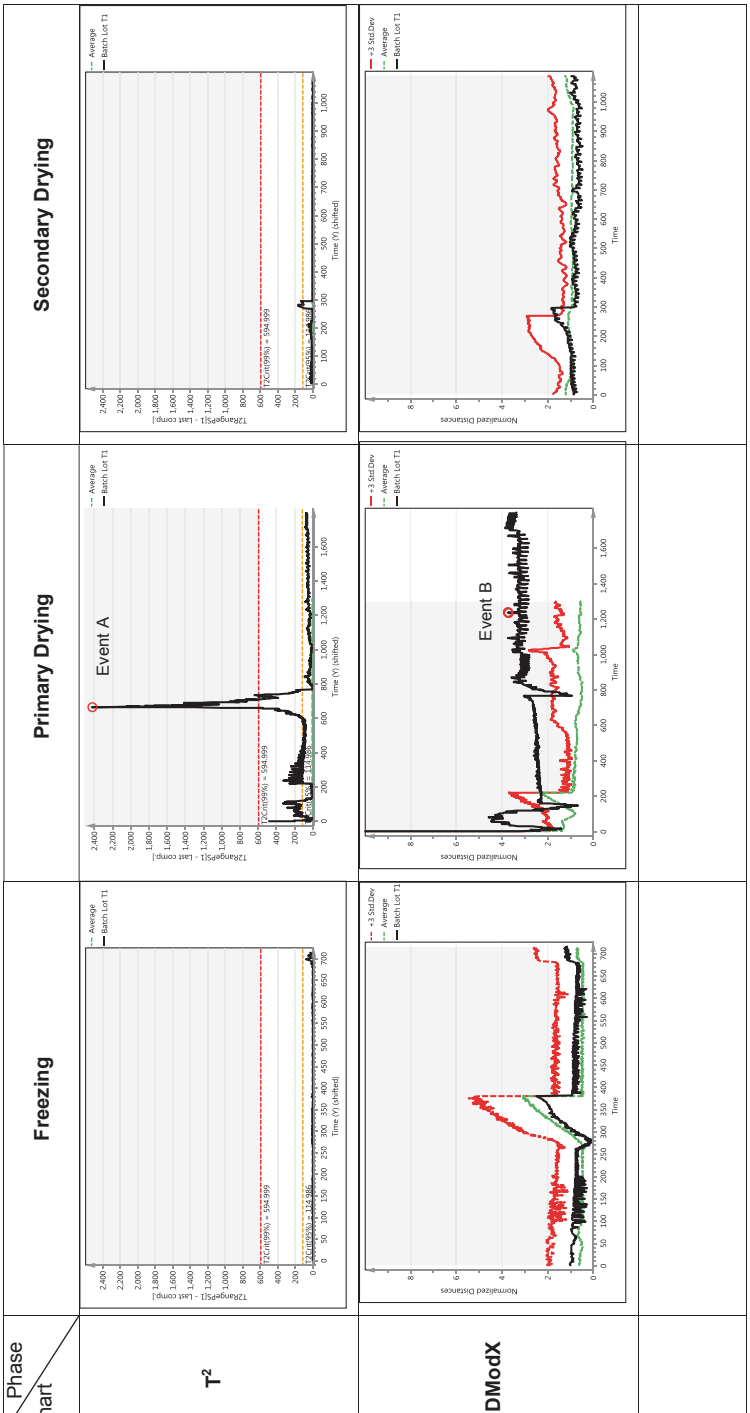

Fig. 24.4 Process performance assessment on a new batch (Lot T1) data using the MSPM batch model that was built from the data of historical good batches

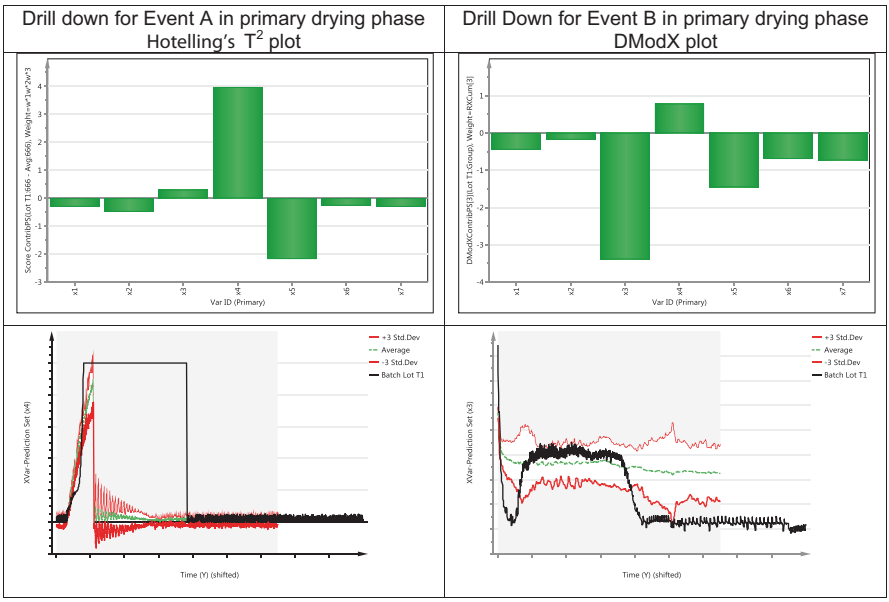

Fig. 24.5 Contribution plots help to identify variables that have drifted from away from their center lines at specific time point. Trend chart of the impacted variables appear to have caused the Hotelling's T^2 and DModX measures to exceed established multivariate control limits

occurred. However, the MSPM batch model built this way can also be used in real-time via SIMCA-online version. The infrastructure needed to make this possible was described in the Introduction section.

24.5 Conclusions

This chapter provided an MSPM application for monitoring a biopharmaceutical drug product lyophilization process. For each of the lyophilization process phase (freezing, primary drying, and secondary drying) a batch evolution model was built. The offline models have successfully identified issues with a testing batch that was later manufactured. The use of a commercially available software streamlined analysis of batch data and the drill down steps to identify issues driving the process out of control.

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Chapter 25
Application of PAT in Real-time Monitoring and Controlling of Lyophilization Process

Feroz Jameel, William J. Kessler and Stefan Schneid

25.1 Introduction

In the recent years, the field of biopharmaceuticals has experienced a significant increase in interest and growth and products resulting from this growth have been shown to provide significant improvement in patient health. As the addition of biomolecules for therapeutic uses is growing, the expectations of the regulatory agencies are also growing. With the recent rollout of the US FDA initiated QbD guidelines and process analytical techniques (PAT), and the International Committee on Harmonization (ICH) activities, notably Q8, Q9, and Q10 guidelines, it is expected that the product and process performance characteristics should be scientifically designed to meet specific objectives, not empirically derived from the performance of test batches (FDA 2002; ICH 2005a, b; ICH 2007; International Conference on Harmonization 2007; http://www.fda.gov/cder/guidance/6419fnl.pdf). This requires definitions of the target process based upon a target product profile and critical process parameters based on critical quality attributes (CQA) and tools to monitor and control them. The FDA defines PAT as a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality (http://www.fda.gov/cder/guidance/6419fnl.pdf). In the absence of PAT, processes are generally designed empirically without a thorough understanding of the relationship between critical product qualities and process parameters. In commercial manufacturing, the

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value of the product processed in the freeze-dryer may exceed several million dollars per batch. Thus, an approach that does not use PAT places high value-added pharmaceutical product at risk for loss, due to unanticipated process deviations and a lack of knowledge of how these deviations may affect product quality. The use of PAT tools will not only help to monitor and control the manufacturing process but also enhance scientific understanding.

Therapeutic proteins that exhibit marginal pharmaceutical stability in liquid dosage form are often dried to enhance their stability. Lyophilization, which is also referred to as freeze-drying, remains one of the most preferred stabilization methods relative to other drying technologies due to the fact that it is a low temperature process and allows processing of biological solutions that are otherwise susceptible to damage (Pikal 2002; Franks 1990). However, lyophilization is a complex process and itself can cause in-process and storage instabilities if it is not properly understood and designed. The process of lyophilization is comprised of three phases: (1) freezing, (2) primary drying, and (3) secondary drying. In the freezing phase the water in the solution is converted into solid ice by exposing the solution to temperatures $\leq -40\,^{\circ}C$ and holding until nearly all water is frozen. The primary drying phase constitutes the sublimation phase during which the ice is sublimed through the application of heat and vacuum. At the end of primary drying, depending upon the composition of the formulation, there will still be a significant amount of water left that did not form ice and is removed through desorption using elevated temperatures during the secondary drying phase. The design of a target lyophilization process requires in-depth understanding of material science, the multiple processes occurring during lyophilization, the effect of independent/dependent variables on the process and product as well as the challenges associated with scale-up and manufacturing operations. These challenges stem from the differences in environment (e.g., effect of particle free environment), differences in load size (scale related issues), differences in equipment (dryer) design, and time and procedural differences between laboratory-based lyophilization and production. From the commercial manufacturing point of view, the manufacturing process should be short (i.e., economically viable and efficient), operate within the capabilities of the equipment with appropriate safety margins and efficiently and reproducibly utilize plant resources within the established "design space."

During process development the thermal response characteristics of proposed formulations are characterized, relationships between critical process parameters and product quality attributes are established and a robust "design space" is identified that enables process operation within the operating constraints of the processing equipment. This is accomplished through a combination of prior knowledge, experimentation, and a risk-based assessment which identifies all the parameters that have the potential to influence process performance and product quality attributes. They typically fall into four categories: (a) Freeze-drying process operating parameters (shelf temperature, chamber pressure, ramp rates, and hold-times), (b) product-related parameters (protein concentration, excipients and their concentrations, vial configuration, stopper design, fill volume), (c) equipment (capabilities and limitations, batch load/size, scale effects), and (4) component preparation and devices. Multivariate experiments are designed and supported with stability studies to determine the degree of impact each parameter has on the CQAs. This

evaluation may be based on the statistical significance in the experiments and process parameters that significantly impact CQAs that are categorized as critical process parameters (CPP). This is the key to process understanding and an expectation by regulators. The CPPs driving the variability of the CQAs must be identified and understood during process characterization, so that they can be measured and controlled in real time during the manufacturing process. Thus, the measurement and control of the critical parameters should be enabled using a broad spectrum of analytical technologies interfaced to the production plant control networks and assimilated into standard procedures (Jameel and Mansoor 2009).

The FDA Office of Pharmaceutical Science guidance on the application of PAT tools includes:

1. Multivariate data acquisition and analysis tools:
   These are the tools that enable the determination of multiple critical factors and their influence on the quality attributes of the product in combination with multivariate mathematical approaches such as statistical design of experiments and process simulation in conjunction with knowledge management systems.
2. Modern process analyzers or process analytical chemistry tools:

These tools can be either conventional systems measuring one variable (e.g., temperature, pressure) or advanced tools that determine biological or chemical attributes. The location where these measurements are performed can be categorized into at-line (removal of samples and analysis close to the process), on-line (diversion of samples from the process, measurement and return to the process), and in-line (invasive or non-invasive measurement in the process stream without removal of samples). The most important benefit of these systems is their capability to determine relative differences and changes of process attributes and to enable adjustments to the process parameters to compensate for variability. Real-time process adjustments are made based upon product quality attributes and real time process information via feedback and/or feed forward mechanisms.

3. Process and end point monitoring and control tools:

These tools are designed to monitor the state of a process and actively manipulate it to maintain a desired state. The strategy is based on the identification of critical material and process attributes and process measurement systems that can provide real-time determination of all critical parameters. Information from these sensors can be used to adjust the process, account for material variability, and control the product quality through mathematical relationships between critical material and process attributes. The end point of a process is not a fixed time, but the achievement of the desired material attribute within a reasonable process time. Validation can be demonstrated by continuous quality assurance for a continually monitored and adjusted process using validated in-process measurements and process end points.

4. Continuous improvement and knowledge management tools:

These tools are used for continuous improvement over the life cycle of a product and are required for post-approval changes and additional understanding of the process and potential problems or variations.

25.2 PAT for Freeze-Drying Process Monitoring and Control

25.2.1 Dependent Variables/Critical Process Parameters of Freeze-Drying

The process parameters that directly impact the critical quality attributes are termed critical process parameters (CPP), and those quality attributes that impact the target product profile are called critical quality attributes (CQA). The independent critical process parameters are shelf temperature and chamber pressure, and the dependent critical process parameters relating to lyophilization process are product temperature, nucleation temperature (degree of under-cooling), product resistance, and sublimation rate which are briefly described below. In addition to critical parameters the end points of primary and secondary drying phase need to be controlled and monitored as they influence the process performance and product quality attributes.

25.2.1.1 Product Temperature

Product temperature is a critical process parameter that determines the process performance and product quality attributes. It is expected that the lyophilized product should look pharmaceutically elegant with low residual moisture content, short reconstitution time, in-process retention of activity, and adequate shelf-life. To achieve these goals, the product must be dried below the maximum allowable product temperature, which is the collapse temperature for a predominantly amorphous system or eutectic melt for a crystalline system (Pikal and Shah 1990). Hence, it is critical that the product temperature needs to be accurately measured, controlled, and monitored during the process to control the product quality.

25.2.1.2 Product Resistance

Product resistance is defined as the resistance that the already dried product layer develops against the flow of water vapor, and is commonly displayed as a function of the dry-layer thickness. Product resistance depends on formulation composition, solid content, and process characteristics such as freezing rate. Occurrence of collapse or microcollapse within the dried structure as well as cake cracking can affect the product resistance profile. Modification of ice crystal size and interconnection, induced for example, by application of an annealing step during freezing, can also affect the product resistance. Development of product resistance over the primary drying process depends on the formulation, and may show significant increase towards the end of primary drying with a corresponding increase in product temperature due to reduced sublimative cooling.

25.2.1.3 Sublimation Rate

The determination of the drying rate or sublimation rate of ice during the primary drying phase is critical for process performance and valuable to enable comparison of processes during scale-up and technology transfer. One of the criteria used to establish the performance equivalency of the two lyophilizers or processes is the demonstration of identical sublimation rates (per vial or unit surface area). From the process performance perspective the sublimation rate needs to be monitored to ensure the primary drying phase is complete within the allocated time before advancing to secondary drying. Additionally, it needs to be maintained below a certain level which is dependent upon the equipment capabilities including the diameter of the duct between the chamber and condenser and the water vapor-capturing capacity of the freeze-dryer condenser. Control of the sublimation rate through shelf temperature and pressure control will avoid condenser overload and a "choked flow" condition. The choked flow point is defined as process condition when the velocity of water vapor traveling through the duct that connects the chamber to the condenser approaches the Mach one speed of sound limit. Under these conditions, the flow velocity no longer increases and the water vapor pressure within the chamber increases, leading to a loss of pressure control. Since a primary source of the transfer of heat to the vials is through gas collisions, a rise in chamber pressure leads to increased heat transfer to the product vials, a further increase in the sublimation rate, and a positive feedback runaway condition. Condenser overload occurs when the rate of incoming water vapor is faster than the rate at which the refrigeration system is able to remove heat from rapidly condensing water vapor and maintain the condenser coil temperature at a temperature which provides a sufficient negative gradient in vapor pressure on the condenser surface to the vapor pressure in the product to sustain sublimation, and to the chamber pressure to enable gas transport. Both the conditions are characterized by a loss of chamber pressure control (Searles 2004).

25.2.1.4 Nucleation Temperature

The nucleation temperature is the temperature at which ice crystals first form in the solution during cooling, and combined with the rate of ice growth it determines the size and morphology of the ice crystals. The difference between the equilibrium freezing point and the nucleation temperature is defined as the degree of undercooling. Nucleation temperature affects the product resistance to mass transfer which in turn impacts the subsequent process performance and product quality attributes. The nucleation temperature depends on the cooling/freezing rate: the faster the cooling/freezing rate the higher the degree of undercooling resulting in smaller ice crystal size. Smaller ice crystals result in smaller pores and channels for the escape of water vapor during sublimation and higher product resistance to drying. Higher resistance leads to lower sublimation rates and increased processing time. Ice nucleation is a random process and variability in the design of the vials, contact with the shelf, heat transfer coefficients, and the level of particulate matter in the product solution may

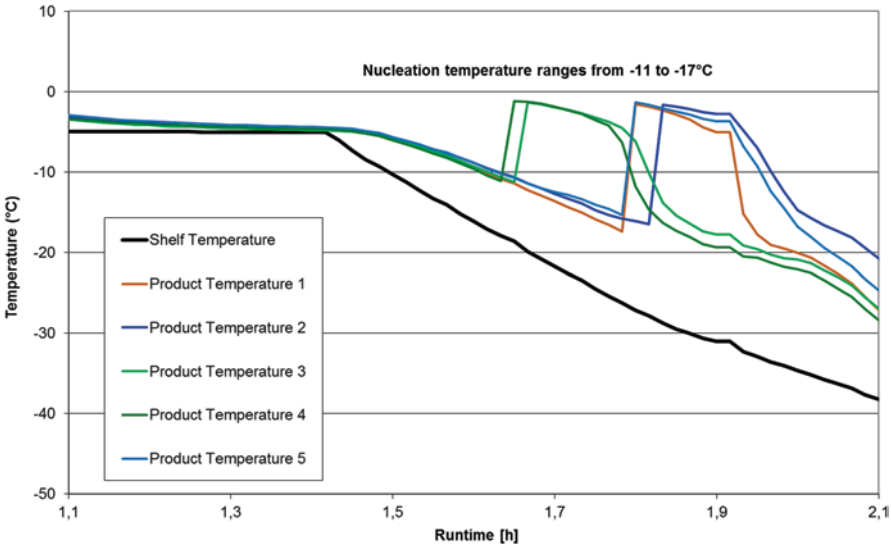

Fig. 25.1 Example data highlighting the heterogeneity in the nucleation temperature in a lyophilizer

contribute to variability in the degree of undercooling (see Fig. 25.1) which in turn may contribute to the heterogeneity in the product from vial to vial and batch to batch (Rambhatla et al. 2004). Hence it is valuable to determine the distribution of nucleation temperatures in a production lyophilizer and if possible to monitor and control it in order to eliminate heterogeneity and improve drying efficiency.

25.2.1.5 End Point Determination of Primary Drying Phase

The end point determination of the primary drying phase is important information both during the design/optimization of the process as well as during manufacturing. It is directly related to the ice sublimation rate and is a dependent variable affected by the independent variables such as chamber pressure, shelf temperature, heat transfer coefficient of vials, fill volume, and product resistance. A method that precisely determines when all of the ice within the product vials is sublimed is important not only for maximizing the throughput of a process, but also from the product quality perspective. Advancement of the drying process to the secondary drying phase through an increase in the shelf temperature without the completion of ice sublimation carries the risk of product collapse, degradation in product quality, and the risk of a batch failure and monetary loss.

25.2.1.6 End Point Determination of Secondary Drying Phase

Most biopharmaceuticals are sensitive to residual moisture and elevated temperatures and require optimal processing time and temperature conditions during secondary

drying. Unnecessary exposure of the product to elevated temperatures for prolonged periods of time can compromise the stability. Additionally, drying to below or above the desired/optimal residual moisture content can compromise the stability of the product. Hence, precise knowledge of the end point of the secondary drying phase is beneficial not only for process efficiency but also for product quality.

25.3 PAT for Freeze-Drying Process Monitoring and Control

There are several commercially available analytical tools that are designed to determine critical process and product parameters of the lyophilization process; some of them have theirapplications limited to process development and some are designed to be useful for monitoring and controlling the process in a commercial manufacturing setting. These tools can be further categorized into single vial and batch process monitoring technologies. The batch monitoring techniques are often preferable as they have the advantage of providing information related to all of the vials within the dryer, representative of the entire batch. Single vial measurements not only lack true representation of the batch but also in some instances the measurement technique itself may influence the drying profile of the chosen vial. However, information from single vial technologies may also provide information on position effects and batch heterogeneity which is not accessible for batch methods. PAT measurement tools for lyophilization include those based on either heat transfer (thermocouples, RTDs, Tempris, etc.), mass transfer (microbalance, TDLAS), pressure (capacitance manometer, Pirani, dew point sensor, manometric temperature measurement), or composition of gas (mass spectrometer, plasma emission spectroscopy, near infrared (IR)/Raman spectroscopy, TDLAS). It is quite obvious that a single PAT tool currently will not provide all of the process information required for adequate process monitoring and understanding. Sometimes a combination of tools needs to be utilized to acquire all the information needed, and the choice of technique used is dependent upon not only on the target process parameters, but also on the available resources.

There are certain requirements and preferable instrument attributes for successful utilization and acceptance as an on-line monitoring tool including: (1) Provides a measurement representative of the entire batch, not only for a single vial, (2) provides an absolute, quantitative measurement, (3) measurement capability for monitoring the slowest drying vials within a batch (see Table 25.1), (4) compatible with the process procedures and flow, e.g., loading and unloading of trays/vials, (5) compatible with cleaning and steam sterilization, (6) does not compromise lyophilizer vacuum or sterility, and (7) scalable for use and integration with laboratory through production scale freeze-drying equipment.

In the following section of this chapter a brief review of a subset of the available single vial and batch PAT monitoring techniques is presented, focusing on those techniques most commonly used and the techniques that have the highest potential to significantly enhance monitoring of laboratory and production scale drying. A

Table 25.1 Advantages and disadvantages of process analytical tools for monitoring and control of freeze-drying process

Techniques	Batch method	Single vial method	Commercially viable	Product temperature	Sublimation rate	Primary drying end point indicator	Secondary drying end point indicator
Thermocouples/RTD	No	Yes	Yes	Yes	No	Yes	No
TEMPRIS	No	Yes	Yes	Yes	No	Yes	No
Near IR	Yes	No	Yes	No	No	Yes	Yes
Microbalance	No	Yes	No	No	Yes	Yes	Yes
Pirani/capacitance differential pressure	Yes	No	Yes	No	No	Yes	Yes
MTM	Yes	No	Yes	Yes	Yes	Yes	No
Thermodynamic lyophilization control (TLC)	No	Yes	Yes	Yes	No	Yes	Yes
Dew point	Yes	No	Yes, Possible	No	No	Yes	Yes
Lyotrack	Yes	No	Yes	No	No	Yes	Yes
Residual gas analyzer, mass spec (Lyoplus)	Yes	No	Yes, but cannot be steam sterilized	No	No	Yes	Yes
TDLAS	Yes	No	Yes	Yes	Yes	Yes	Yes

peer-reviewed publication by Patel and Pikal provides a comprehensive review of available process-monitoring devices that have been used to measure critical process parameters (Patel and Pikal 2009). Many PAT techniques are well established and have been used to monitor freeze-drying for decades. One of the newest batch monitoring techniques that has been applied to freeze-drying is tunable diode laser absorption spectroscopy (TDLAS). A detailed description of this batch monitoring technique is provided. TDLAS may be applied to all scale dryers and it holds great promise for providing information that can be linked to numerous parameters that affect product quality.

25.4 Single Vial Methods

As indicated in the discussion above single vial methods may often not be compatible with production equipment and often do not comply with manufacturing requirements. They are commonly used during process development with the understanding that they can be biased and not representative of the entire batch. The following section reviews some of the single methods that are currently available for obtaining process and product information.

25.4.1 Thermocouples and RTDs

Temperature sensors in the form of thermocouples of various gauges may be manually placed in vials at selected locations and used to monitor the product temperature. Thermocouple-based temperature measurements provide a measurement technique to ensure that the product temperature remains below the collapse temperature of the product during primary drying. If the product temperature exceeds the collapse temperature there is a potential to significantly alter the product quality attributes. Based on thermocouple readings, one of the input critical process parameters, specifically either the shelf temperature or the chamber pressure, may be adjusted to ensure that the product temperature is maintained below the collapse temperature. Product temperature readings may also serve as one of the indicators of the end point of primary drying, and can be used to assess the extent and variation of undercooling during the freezing step in development. The precision and accuracy of the probes is important and the use of 30-gauge thermocouples is recommended (e.g., Omega: 5SRTC-TT-T-30-36 or a similar model with 30 gauges). Product temperature-based end point monitoring provides an approach for maximizing the throughput of a process and for ensuring product quality, because advancing to the secondary drying phase without the completion of ice sublimation carries the risk of product collapse. Additionally, thermocouples are commonly used during lyophilizer equipment qualification shelf mapping studies and during process development.

Although one can obtain very useful data through the use of thermocouples there are certain drawbacks to the use of in-situ temperature probes. The first drawback

is that the vials with probes behave differently than those without probes. The presence of the sensor changes the ice nucleation behavior, resulting in less supercooling and more rapid freezing. This changes the ice structure, resulting in larger pore sizes within the product matrix, reducing the product resistance to drying, increasing the ice sublimation rate and resulting in product temperature measurements that are not representative of the entire batch. This behavior is most important in the sterile production environment because the super-cooling bias between vials with and without sensors becomes more significant due to the particle-free environment within sterile manufacturing facilities. Thus, in a production environment, it is misleading to use thermocouple-based product temperature profiles as an indicator of end of primary drying without the use of a 10–15% primary drying time "soak period" to ensure that all vials completed primary drying.

The second drawback is that the thermocouples are commonly placed in the front row in a production scale freeze-dryer to avoid the risk of contamination. However, the atypical front-row vials facing the door of a freeze-dryer are exposed to elevated heat transfer, which also increases the product temperature and water vapor mass flux. Lastly, a thermocouple measures the product temperature at the bottom of the vial rather than at the sublimation interface. It is the temperature at the constantly moving sublimation interface that must be maintained below the collapse temperature to ensure product quality. Thus vials containing thermocouples are not representative of the overall batch product temperature and the use of thermocouples to determine product temperature in manufacturing-scale freeze-dryers is not appropriate. The use of in-situ temperature probes is appropriate for laboratory-scale experiments and process development.

Resistance thermal detectors (RTDs) are used in production freeze-dryers due to their good stability and compatibility with sterilization. However, the errors associated with their measurements are even higher than for thermocouples, as the sensors are significantly larger which increases their influence on nucleation and supercooling. In addition, they only measure average temperatures over total sensor area, instead of the point measurements obtained by correctly placed thermocouples, and generate heat during their operation which leads to nonrepresentative drying end points. The measured values are also not directly comparable with data from thermocouples from development runs. Therefore, RTDs cannot be regarded as a useful PAT tool for lyophilization.

25.4.2 TEMPRIS

TEMPRIS which stands for temperature remote interrogation system is a wireless and battery-free tool designed for the measurement of product temperatures in the vial during the process of freeze-drying (Schneid and Gieseler 2008a, b). The novel sensor which is connected to a quartz crystal encased in a sterilizable cover is powered via passive transponder excitation using an amplitude-modulated electromagnetic signal in the internationally available at the 2.4 GHz ISM band.

The sensor provides a large number of temperature measurements per minute that are instantaneously available during the drying process. Its performance has been evaluated as a function of fill volume and solid content and the values were found to be in agreement with the values obtained through the use of standard thermocouples and the manometric temperature measurement (MTM) technique described below (Milton et al. 1997). Although it has the advantage of being a wireless sensor and the same sensor can be used in laboratory and manufacturing environments, it still does not ease all constraints associated with aseptic handling. It requires manual placement in the vial under sterile conditions, is a single vial measurement, its placement within a vial may affect freezing behavior and thus ice structure and product resistance to drying and thus its measurements are not representative of the entire batch. However, it can be introduced into automatic loading system in any position within the vial array, and is a useful tool to shelf mapping studies and to determine the comparability and quantify edge effects of both small- and large-scale freeze-dryers.

25.4.3 Near-Infrared Spectroscopy (NIR)

Near-infrared absorption spectroscopy (NIR) has been successfully employed as one of the PAT tools for lyophilization process monitoring for several decades (Ciurczak 2002, 2006). It is based upon water vibrational spectroscopy and implemented by transmitting a light beam possessing a wavelength in the range of 1100–2500 nm and monitoring changes in the reflected radiation due to the presence of water or ice from the product throughout the drying process. NIR technology has been successfully used to determine the moisture content in the vial and thereby predict either the sublimation rate and/or end of the primary or secondary drying phase. The measured values have been shown to be in agreement with other techniques such as Karl Fisher (Lin Tanya and Hsu Chung 2002). A recent study by De Beer et al. employed NIR measurements from a probe placed adjacent to a vial in the array throughout the drying phase. The data were evaluated in conjunction with Raman measurements on a different vial in the same batch (De Beer et al. 2009). They found that the NIR data was more valuable for the determination of the primary drying end point as well as for monitoring of release of hydrate water during storage (De Beer et al. 2007). NIR measurements also confirmed observations made by Raman spectroscopy such as crystallization of ice and excipients as well as by solid state characterization of the dried cake.

The measurement technique and calibration factor need to be robust enough to accommodate variations arising from the measurement configuration and from the formulation and manufacturing processes. The use of peak area analysis rather than peak height analysis gives more accurate predictions and agreement with the measured values. Although the technique is nondestructive and does not require sample preparation, it is formulation dependent and requires the development of calibration curves, specific to the product, in conjunction with the commonly acceptable

method such as Karl Fisher. Another drawback of this technique is the large size of the sensor and its placement adjacent to the vial. This requires the usual array to be changed, and the monitored vial may be subjected to higher atypical radiation effects and heat introduction from the NIR tip. The modified array also leads to an atypical drying behavior in the monitored vial and reduced representativeness for the rest of the batch. This technique is most suitable as a development tool in laboratory-scale freeze-dryers and for troubleshooting during transfer to pilot scale for optimization of the secondary drying step, but may not be suitable under GMP manufacturing settings (Derksen et al. 1998). However, it has the potential to be employed as an on-line technique for 100% verification of the moisture content at the end of the process during automated unloading which provides immediate process verification and release testing.

25.4.4 Microbalance Technique

The microbalance technique was first employed by Pikal et al. (Pikal et al. 1983) to determine the sublimation rates and resistance behaviors of different materials during freeze-drying. Later they also used the technique for investigation of the drying rate during secondary drying and the delineation of drying kinetics and influencing factors (Pikal et al. 1990). The microbalance utilizes gravimetric measurement technique, i.e., measures the difference in mass as a function of time to monitor the drying rate during the freeze-drying process by periodically weighing a single vial within the freeze-dryer (Roth et al. 2001). The microbalance instrument is placed on the surface of the dryer with a single vial located within the reach of the balance weighing arm. The microbalance is programmed to lift and weigh the vial at user-defined time intervals. Although this technique has been successfully commercialized as Christ microbalance (CWS-4099) (Christ 2000) and utilized for the determination of heat transfer/drying rate homogeneity across the shelf, its use is limited by its inability to provide representative data that captures the effects of surrounding vials (within the hexagonal array) and the effect of vial location on the dryer shelf (edge vs. center vials). In addition, the application of this technology is limited by barriers associated with integrating it into commercial freeze-drying equipment, including the requirement for compatibility with clean-in-place and sterilize-in-place (CIP/SIP) systems.

25.5 Batch PAT Methods

As previously indicated, PAT tools that can be used for real-time, at-line or on-line process monitoring and control of the freeze-drying process, and can provide information that is representative of the entire batch may be more meaningful and preferable. Not all of the critical information pertaining to the progress and performance of the process can be obtained through monitoring a single PAT tool. The output

data from the controls of the freeze-dryer and process parameters such as nitrogen flow rate at constant pressure, Pirani vs. capacitance gauge data comparison, pressure rise and partial water vapor measurements can be utilized in conjunction with the newly developed PAT devices to determine critical process information. Some of the traditional and newly developed batch methods that are available as PAT are discussed below:

25.5.1 Pressure Measurements: Capacitance Manometer and Pirani Gauge

The pressure in the chamber and the condenser during drying may be measured and controlled using a capacitance manometer (e.g., MKS Baratron gauge). The capacitance manometer is a transducer device consisting of a metal diaphragm, typically Inconel, placed between the two fixed electrodes. One side of the diaphragm is evacuated and the other side is exposed to the chamber or condenser pressure. The deflection of the diaphragm determines the force per unit area providing the absolute pressure in the range of 0–760 Torr with the variability of $<\pm 1$ mTorr.

Pirani gauge pressure measurements are based on the thermal conductivity of the gas. Their data is often used to determine the end of the primary drying, and in most cases also secondary drying, by using comparative pressure measurement (Pikal 2002). Pirani pressure gauges are typically calibrated against air or nitrogen and since the thermal conductivity of water vapor is roughly 1.6 times that of air or nitrogen, the gauge outputs a higher pressure reading during primary drying when most of the gas within the lyophilizer chamber is water vapor. When the last piece of ice is sublimed and the gas composition within the lyophilizer chamber is mostly nitrogen, the Pirani gauge reading approaches the true pressure determined using a capacitance manometer indicating the end of the primary drying phase. Similar behavior can be observed during water desorption in secondary drying. Traditional Pirani gauges provide useful pressure readings between 10^{-3} and 10 Torr. Sterilizable Pirani sensors are available which can be operated in a GMP environment, and are relatively inexpensive, providing a cost-effective approach to end point monitoring.

25.5.2 Dew Point Monitor

The temperature at which water starts to condense from the gas phase on a colder surface is defined by a physical parameter called dew point. During freeze-drying, the dew point may be used to indicate the gas phase water concentration. Similar to a Pirani gauge, a dew point sensor can be used as a tool to determine the end point of the primary or secondary drying phase based on the principle of changes in the capacitance of a thin film of aluminum oxide due to adsorption of water at a given partial pressure (Roy and Pikal 1989). The change of vapor composition from water vapor to nitrogen leads to a temperature decrease of the dew point and it has been

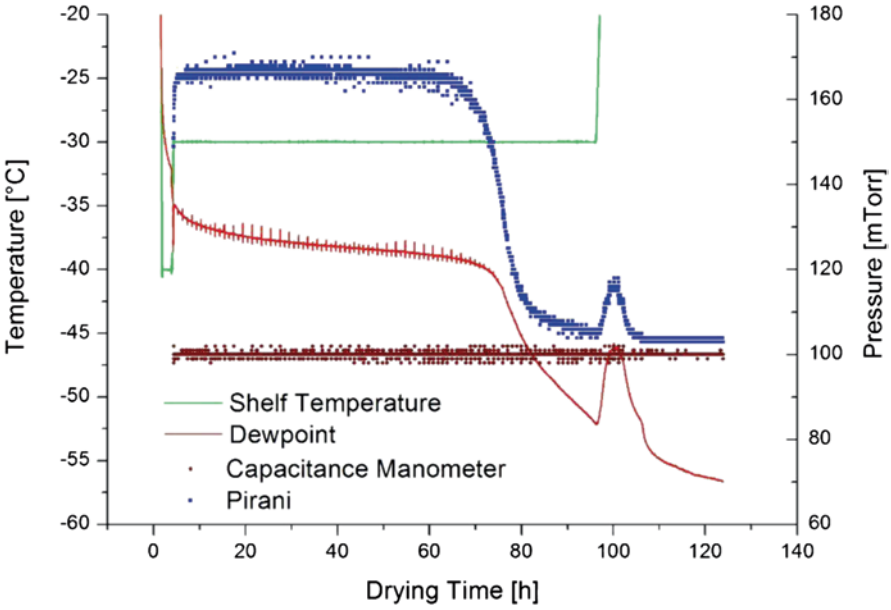

Fig. 25.2 Comparison of primary drying phase end point detection using both Pirani gauge and dew point sensors

reported to be more sensitive than comparative pressure measurements (Bardat et al. 1993). During the primary drying phase moisture continues to evolve from product vials and is indicated by the sensor as a steady dew point temperature, usually between −35 °C and −65 °C. The end of the primary drying is indicated when the dew point drops and all of the product ice has sublimed. Water desorption during secondary drying can also be measured by a lower increase in dew point temperature. These sensors not only work for aqueous systems but also work for mixed systems where the removal of a mixture of organic solvents and water is required. The challenge associated with the use of these moisture probes is their inability to survive steam sterilization during SIP operation, however new sensor models incorporate a method of isolating the probe with a special fixture and include a biological barrier that can be sterilized within the fixture. These improvements may enable increased application of these relatively inexpensive sensors. Figure 25.2 shows good agreement in the determination of the primary drying phase end point as indicated by Pirani gauge pressure and by dew point sensor measurements.

25.5.3 Manometric Temperature Measurement (MTM)

Manometric temperature measurement (MTM) is a technique during which the valve between the dryer chamber and the condenser is momentarily closed for 25–30 s and the pressure rise data is collected. The MTM equation, Eq. (25.1), is

fitted to the pressure rise data through nonlinear regression to determine the vapor pressure of ice at the sublimation temperature and the sum of product and stopper resistance (Milton et al. 1997; Tang et al. 2005, 2006).

$$\overbrace{P(t) = P_{\text{ice}} - (P_{\text{ice}} - P_0)\cdot\exp\left[-\frac{3.461\cdot N\cdot A_{\text{p}}\cdot T}{V\cdot(R_{\text{p}}+R_{\text{s}})_{\text{s}}}\cdot t\right]}^{\text{Team1}} + \underbrace{+0.465\cdot P_{\text{ice}}\cdot\Delta T\cdot\left[1-0.811\cdot\exp\left(-\frac{0.114}{L_{\text{ice}}}\cdot t\right)\right]}_{\text{Team 2}}\underbrace{+\text{X}\cdot t}_{\text{Team 3}} \tag{25.1}$$

P_{ice} is the vapor pressure of ice at the sublimation interface (an output to be determined), P_0 is the chamber pressure setpoint, N is the total number of sample vials, A is the internal cross sectional area of the vials, T_{s} is the set shelf temperature, V is the product chamber volume, $R_{\text{p}}+R_{\text{s}}$ is the area normalized product and stopper resistance (an output to be determined), ΔT is the temperature difference across the frozen layer, L_{ice} is the thickness of the ice, X is a constant parameter characterizing the linear component of the pressure rise (an output to be determined). The MTM pressure rise is due to contributions coming from three sources, firstly, the pressure rise controlled by dry-layer resistance and the ice temperature at the sublimation interface indicated by term 1 in the MTM Eq. 25.1, secondly, the pressure rise caused by the temperature rise at the sublimation surface arising from the dissipation of the temperature gradient across the frozen layer indicated by the term 2 in the MTM equation, thirdly, the pressure rise due to the increase in ice temperature by heat transfer from the shelf during MTM operation. Once the vapor pressure of ice is determined using the MTM equation, the product temperature at the sublimation interface is determined using the following pressure–temperature relationship shown in Eq. 25.2.

$$T = \frac{-6144.96}{\ln(P_{\text{ice}}) - 24.01849} \tag{25.2}$$

Since the MTM technique determines the vapor pressure of ice at the sublimation interface and since at the end of primary drying no ice remains in the product, a sharp drop in the ice vapor pressure will be indicative of end of primary drying. Additionally, based upon the determinations of vapor pressure of ice at the product temperature and the total resistance to mass transfer one can deduce additional valuable information such as the heat transfer into the product, i.e., dQ/dt, remaining ice thickness (L_{ice}), vial heat transfer coefficient (K_{v}) and sublimation rate (dm/dt).

One of the advantages of the MTM technique is that it gives the average product temperature of the entire batch as opposed to thermocouples which are biased and not representative of the variation across all of the vials within the dryer. As displayed in Fig. 25.3, during laboratory-scale freeze-drying, when the insertion of

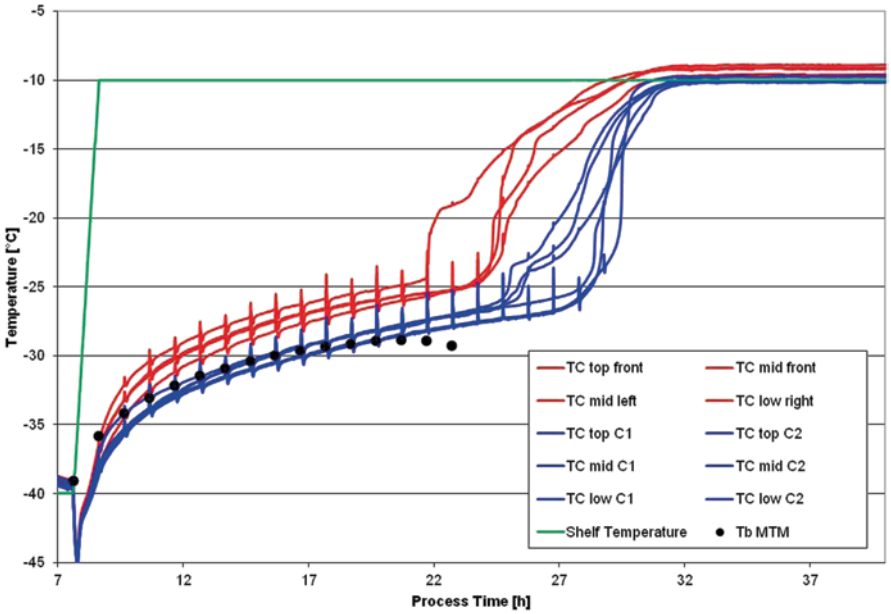

Fig. 25.3 Example freeze-drying profile of a 10% glycine formulation, comparison of product temperature, MTM vs. thermocouple

the thermocouple into the product vial has little effect on the ice morphology (due to the particle loading within the laboratory environment), the agreement between MTM and thermocouple-based measurements is within ± 2 °C. The other advantage of MTM-based product temperature is that it gives the value at the interface as opposed to thermocouples which measure the temperature at the bottom of the vial which may be somewhat warmer than at the interface. One of the limitations of the MTM technique is that the determinations are only accurate up to ~ 2/3 of the primary drying phase. This is due to the reduced pressure rise near the end of primary drying, and a fraction of vials becoming free of ice. There is also a potential for erroneous measurements for products that have low collapse temperatures, as the lowest product temperature that can be reliably measured by MTM is −35 °C. In addition, products that are predominantly amorphous in nature tend to reabsorb sublimed water vapor during the pressure rise when the valve between the condenser and chamber is closed, resulting in erroneous vapor pressure data (Milton et al. 1997).

Pressure rise data has been historically used to approximate the end point of the primary drying phase in commercial scale freeze-dryers, but its use for MTM technique requires swift closure of the valve to monitor the pressure rise and meeting this requirement is a challenge within large-scale production dryers. Additionally, since the pressure rise rate is dependent upon the product dry-layer resistance, the ice sublimation area, and the chamber volume, large volume freeze-dryers and small sublimation areas or small number of vials can significantly limit its applicability.

Thus, using MTM one can obtain valuable information such as product temperature, product dry-layer resistance to drying, the sublimation rate, heat transfer

coefficient of vials, and the end points of the primary and secondary phases of the drying process. This information is useful for improved process understanding, development, technology transfer, and for obtaining critical process data that can be later applied to assess the impact of deviations and control strategies of freeze-drying process. (Fig 25.3)

25.5.4 Thermodynamic Lyophilization Control (TLC)

The thermodynamic lyophilization control (TLC) pressure rise method is based on the pressure rise analytical method developed by Oetjen and Haseley (Neumann and Oetjen 1958; Haseley et al. 1997). Using this technique, the valve between the chamber and condenser is closed for only 3 s. The analysis of the pressure rise uses barometric temperature measurements (BTM) to calculate the product temperature at the sublimation front during primary drying. The calculation includes adaptation to the chamber volume, load conditions, and correction for leak rate. Furthermore, the calculated temperature may be used as a feedback for automatic adaptation of the cycle conditions to optimize the process time. One of the advantages of the TLC method is the short measuring time of 3 s that prevents any potential warming and reabsorption of water vapor effects on the product. The end point of primary drying phase is determined by monitoring the ice temperature correlated with a lower amount of pressure increase.

TLC is a commercial product offered by GEA Lyophilizers. When compared to the MTM-based SMART™ freeze-dryer technology offered by SP Scientific, it does not fit pressure rise data to determine the product temperature and product resistance, but uses several other parameters for optimization of the lyophilization cycle based on a derivation of the pressure rise profile. The freezing step time is controlled based on the energy input required for freezing of the product, calculated from the temperature difference between shelf inlet and outlet temperatures. During primary drying, the chamber pressure is adjusted to obtain the desired temperature at the sublimation interface while keeping the shelf temperature constant. TLC is not designed to determine product resistance or the mass flow rate which is possible using MTM.

The secondary drying step may also be monitored using TLC and the end point can be estimated through the determination of the desorption rate by measuring the pressure rise over a predetermined period. The measuring time is longer than that used during primary drying, but has no impact on the product as only absorptively bound water is present. The desorption rate is calculated using the chamber volume of the lyophilizer and the mass of the dry product.

25.5.5 Gas Plasma Spectroscopy (Lyotrack)

Similar to Pirani gauge and dew point sensors, Lyotrack measures the concentration of water vapor in the drying chamber at pressures ranging from 4 to 400 mTorr using a cold plasma source, and determines the end point of both primary and secondary

drying (Mayeresse et al. 2007). The device consists of a plasma generator and an optical spectrometer. The plasma generator ionizes the gas present in the chamber while the spectrometer analyzes the gas species based on the wavelength-dependent fluorescence emitted by the ionized gas. A few advantages of the Lyotrack sensor are that it can be easily calibrated against a reference system and readily implemented into existing freeze-dryers, it is robust (sterilizable and compatible with SIP/CIP) and has a good measurement sensitivity that allows detection of ice in less than 1 % of the vials (Hottot et al. 2009). However, its broader applicability is restricted due to its creation of free radicals that can negatively impact the stability of the product through free radical-induced oxidation. This effect is especially important when drying protein products. This problem can be moderated or eliminated by installing the device in the spool that connects the chamber to the condenser instead of having it in the chamber. Since the gas composition profile of the Lyotrack is the same as the pressure profile measured by a Pirani gauge, its added value is not obvious.

25.5.6 Residual Gas Analyzer, Mass Spectrometer (LYOPLUS™)

Mass spectrometry has also been used as an on-line tool to monitor the composition of the gas in the freeze-dryer chamber to determine drying progress and the primary and secondary drying end points (Nail and Johnson 1992). In addition to the end point determination, it has also been considered for the detection of leaks and ingress of other gases and solvents arising from vacuum pump oils, heat transfer fluid, and solvents used for cleaning. It consists of a quadrupole mass spectrometer which analyses the residual gases based on mass to charge ratio and quantifies its measurements into partial pressures which can be further correlated with residual water. This enables online determination of moisture content. Since the profiles of partial pressure of water obtained by Pirani gauge and the residual gas analyzer are comparable and the inflection points are the same, the use of the more expensive mass spectrometer for end point detection is questionable as the same information can be obtained through the use of a less expensive Pirani gauge.

25.5.7 Tunable Diode Laser Absorption Spectroscopy

Tunable diode laser absorption spectroscopy (TDLAS), which is based on absorption of electromagnetic energy by gas molecules at a specific wavelength in the electromagnetic spectrum (absorption line) allows detection and quantification of trace concentrations of a gas or gas component. Historically, TDLAS technology has been used in the measurement of gas concentrations in the atmosphere and in the chemical industry, in leak detections for natural gas pipelines, and process control in petrochemical manufacturing to measure concentrations of methane, ethane, and other gas components (Harward et al. 2004). Recently this technology became commercially available as the LyoFlux™ product for the monitoring of freeze-

drying processes at all scales. Two optical pathways are installed within the duct connecting the lyophilizer chamber and condenser. Two laser beams originating from a single-diode laser beam are transmitted through antireflection (AR) coated windows in the spool piece wall at an angle with respect to the direction of vapor flow, usually 45° and 135°, and detected on the opposite side of the duct using two photodiode detectors. TDLAS has been demonstrated for monitoring the water vapor mass flow rate in the duct connecting the lyophilizer product chamber and the dryer condenser (Gieseler et al. 2007). Using near infrared absorption spectroscopy, TDLAS provides direct measurements of the water vapor temperature (K), concentration (molecules/cm^3) and gas flow velocity (meters/second) within the duct connecting the lyophilizer chamber and condenser. These measurements are combined with knowledge of the cross-sectional area of the duct to calculate the instantaneous water vapor mass flow rate, d*m*/d*t* (g/s). The mass flow rate is integrated as a function of time to provide a continuous determination of total water removed. The mass flow rate may also be combined with freeze-drying heat and mass transfer models and additional process measurements (e.g., product chamber shelf temperature) and process specific parameters (e.g., vial cross-sectional area and heat transfer coefficients) to determine the batch average product temperature. In the remainder of this chapter, a detailed description of the TDLAS technique and its application to real-time, continuous lyophilizer monitoring is provided.

25.5.8 TDLAS Measurements of Vapor Mass Flow

TDLAS sensors rely on well-known spectroscopic principles and sensitive detection techniques to continuously measure concentrations of selected gases. The quantitative absorption measurement is described by the Beer–Lambert law shown in Eq. 25.3:

$$I_\nu = I_{o,\nu} \exp[-S(T)g(\nu - \nu_o)N\ell] \tag{25.3}$$

where $I_{o,}\nu$ is the initial laser intensity at frequency ν, I_ν is the intensity recorded after traversing a path length, ℓ, across the measurement volume, $S(T)$ is the temperature-dependent absorption line strength, $g(\nu - \nu_0)$ is the spectral line shape function, and N is the number density of absorbers (the water concentration). The lineshape function describes the temperature- and pressure-dependent broadening mechanisms of the fundamental linestrength. For the low pressure conditions present during lyophilization, $g(\nu - \nu_0)$ is primarily described by a Gaussian function. In addition, by scanning the laser frequency across the entire absorption lineshape, any pressure dependency of the lineshape function is removed from the number density measurement, $\int g(\nu - \nu_0)\, d\nu = 1$. Scanning the fully resolved absorption lineshape also reduces the effect of broadband absorbers in the background gas and nonresonant scattering from any aerosols or particulates that may be present in the flow.

The water concentration, [H_2O], in molecules per cm^{-3}, is calculated using Eq. 25.4,

$$N = \frac{-\int \ln\left[\frac{I(\nu)}{I_o(\nu)}\right] d\nu}{S(T)\ell} \tag{25.4}$$

where dν is the laser frequency scan rate per data point (cm^{-1}/point).

The near IR 1.3925 μm water vapor absorption feature, which arises from the $3_{03} \leftarrow 2_{02}$ rotational line within the $\nu_3+\nu_2$ vibrational band, was chosen to monitor water vapor due to the availability of robust, fiber-coupled telecommunications grade diode lasers to probe the transition, its strong absorption linestrength, and the relative temperature insensitivity of the transition. The linestrength for this transition changes by ~ 2.7 % per 10 K gas temperature change under conditions of interest during lyophilization (Rothman et al. 1994). The water absorption lineshape is analyzed to determine the gas temperature and calculate the linestrength during drying to correct for temperature fluctuations. A Gaussian lineshape profile is given by Eq. (25.5).

$$\varphi_D = \frac{2}{\Delta\nu_D}\sqrt{\frac{\ln 2}{\pi}} \exp\left[-4\ln 2\left(\frac{\nu - \nu_o}{\Delta\nu_D}\right)^2\right] \tag{25.5}$$

where $\Delta\nu_D$ (cm^{-1}) is the Doppler full width at half maximum given by Eq. (25.6)

$$\Delta\nu_D = 7.162\times10^{-7}\nu_0\sqrt{\frac{T}{M}} \tag{25.6}$$

where ν_0 is the line center frequency (7181 cm^{-1}), T is the gas temperature (K), and M is the molecular weight (g/mole) of the absorbing species (water vapor).

Figure 25.4 shows a schematic layout of a single-wavelength, near-IR laser sensor configuration. This block diagram shows all the major subcomponents of the sensor that are contained within one compact sensor control electronics unit. The diode laser temperature and injection current are controlled by an integrated diode laser controller. The laser may be temperature-tuned over approximately 20 cm^{-1} (~ 5 nm in the near infrared spectral region) around the design wavelength and rapidly current-tuned over approximately 2 cm^{-1} (approximately 0.45 nm) about its nominal central operating wavelength. While monitoring the lyophilization process, the laser frequency is only tuned over ~ 0.13 cm^{-1} (approximately 0.025 nm) to maximize the measurement sensitivity of the instrument.

The output from the laser is fiber-coupled through an optical isolator to eliminate frequency or amplitude instabilities in the diode laser arising from back reflections in the remainder of the optical setup. The fiber-coupled laser output is split using a $1\times M$ (M can vary between 2 up to 32 or more) fused-fiber coupler, an all-solid-state component widely used in multiplexing applications in the telecommunications

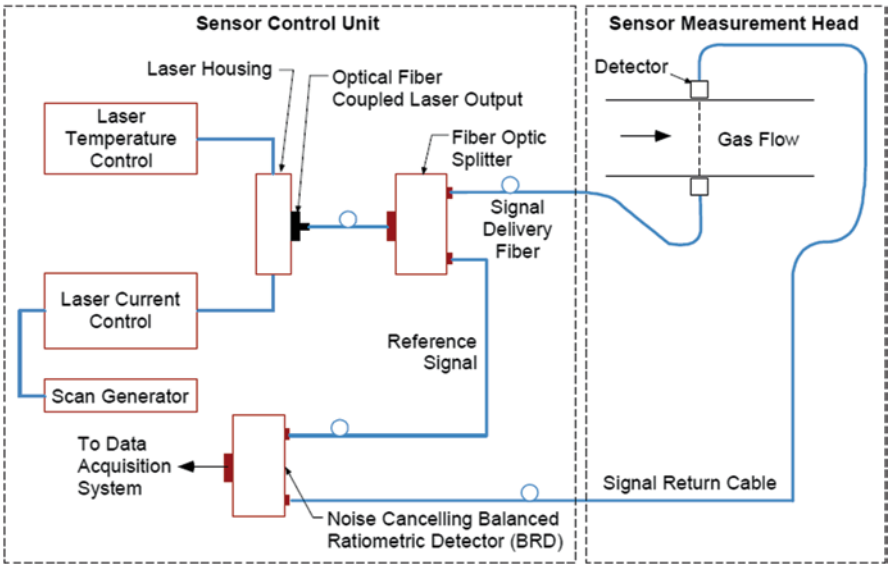

Fig. 25.4 Schematic diagram for a TDLAS sensor. Reprinted with permission from Physical Sciences

industry. The split laser intensity is delivered by the signal delivery fiber to the gas sensor measurement head and is directed across the measurement path. This provides a simple, reliable interface to the measurement volume compatible with operation within laboratory and manufacturing environments.

The transmitted near-IR radiation used to monitor water vapor is detected using a room temperature InGaAs photodiode detector. The electronic signal from the photodiode is transmitted to a balanced ratiometric detection (BRD) circuit which enables ultra-sensitive, near shot-noise limited absorption detection sensitivity (Hobbs 1997). The second leg from the fused-fiber splitter is directed via the reference signal optical fiber for detection by a second InGaAs detector located within the BRD circuitry. The light from this fiber experiences no absorption and provides a reference signal for the BRD circuitry for elimination of the laser intensity ramp and laser intensity noise. In a multiple measurement location setup, a $1 \times M$ coupler is used for $M/2$ measurement locations.

The electrical signals from the BRD circuitry are captured by the computer-controlled data acquisition system for recording and analysis of the water vapor absorption lineshapes.

The water vapor number density can be directly determined from the measured absorption lineshape. Thus, the water vapor monitor is not a transducer type of sensor, but determines the water vapor number density from first principles using known spectroscopic parameters and fixed, measured laser characteristics.

To determine mass flow rate of the water vapor, the velocity of the target gas is also required (Miller et al. 1996). The velocity measurement concept is shown in

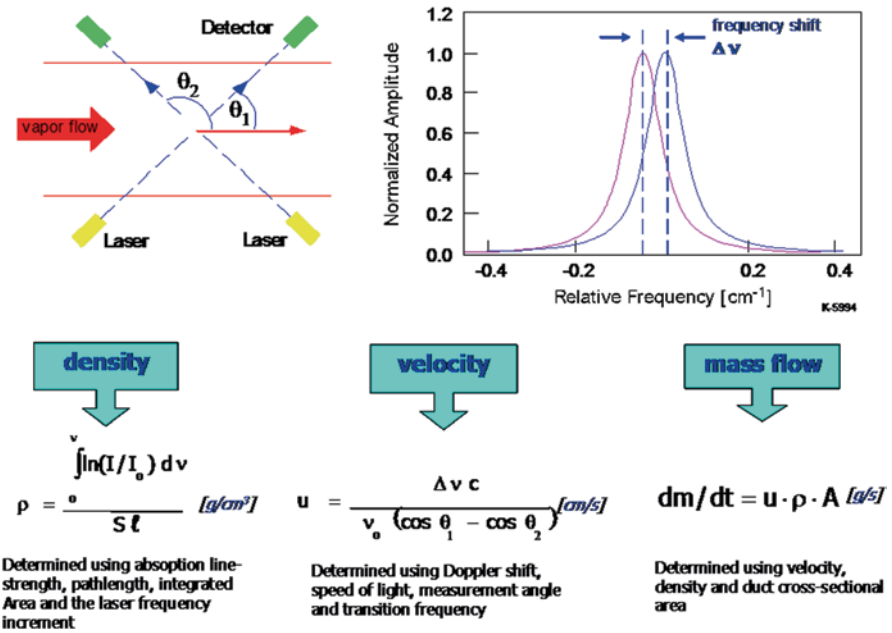

Fig. 25.5 Schematic diagram showing the Doppler-shifted absorption spectroscopy velocity measurement concept

Fig. 25.5. The velocity is determined from the Doppler-shifted absorption spectrum which is shifted in wavelength or frequency with respect to the absorption wavelength of a static gas sample by an amount related to the velocity of the gas, $\boldsymbol{u}$, and the angle, $\boldsymbol{\theta}$, between $\boldsymbol{u}$ and the probe laser beam propagation vector, $\boldsymbol{k}$. Thus, a simultaneous measurement across the vapor path in the lyophilizer and in a sealed low pressure water vapor absorption cell or two counter-propagating measurements across the duct connecting the lyophilizer product chamber and condenser at 45° and 135° using the same wavelength-tunable laser source may be used to determine the water mass flow rate exiting the lyophilizer product chamber.

Equation 25.7 shows the relationship used to determine the gas flow velocity for a single line of sight measurement across the lyophilizer duct (combined with a seal water absorption cell with $\cos\theta_2 = 0$), u. c is the speed of light (3×10^{10} cm/s), Δv is the peak absorption shift from its zero velocity frequency (or wavelength) in cm^{-1}, ν_o (7181 cm^{-1}) is the absorption peak frequency, at zero flow velocity and θ is the angle formed between the laser propagation across the flow and the gas flow vector.

$$u = \frac{c \cdot \Delta v}{\nu_o \cdot \cos\theta} \tag{25.7}$$

The velocity may also be determined using crossed measurement paths within the flow volume, one directed with the gas flow and one directed against the gas flow. In this configuration Eq. (25.7) is replaced by Eq. (25.8) where θ_1 and θ_2 are the

measurement angles with respect to the gas flow. We note that in either case the same laser is used to produce both absorption lineshapes and the frequency shift is determined by the shift in data points between the two absorption profiles and converted to absolute frequency using the diode laser frequency scan rate (cm^{-1}/point). The dual line-of-sight measurement across the lyophilizer duct provides twice the measurement sensitivity.

$$u = \frac{c \cdot \Delta\nu}{\nu_o \cdot (\cos\theta_1 - \cos\theta_2)} \tag{25.8}$$

The mass flow rate, d*m*/d*t*, (g/s) is calculated by the product of the measured number density (N, molecules cm^{-3}), the gas flow velocity (u, cm/s), and the cross-sectional area of the flow duct (A, cm^2; and the appropriate conversion factors). This is shown in Eq. (25.9).

$$\frac{dm}{dt} = N \cdot u \cdot A(\text{g/s}) \tag{25.9}$$

Figure 25.6 shows water vapor absorption data recorded in the spool of an SP Scientific Lyostar II® laboratory-scale freeze-dryer during an ice slab sublimation test. The single line of sight TDLAS measurement across the lyophilizer duct was com-

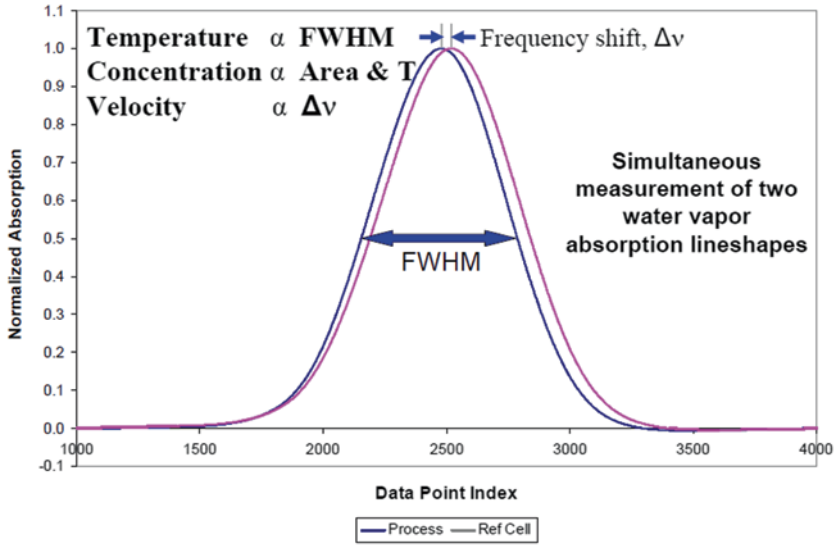

Fig. 25.6 Example water vapor absorption line shapes recorded using a near-IR tunable diode laser absorption spectroscopy mass flow rate monitor. Reprinted with permission from Physical Sciences Inc.

bined with a simultaneous measurement through a ~ 0.5 Torr reference absorption cell for the determination of the water vapor mass flow rate. The water vapor temperature was calculated from the absorption lineshape full width at half maximum (FWHM), converting data points to frequency using the diode laser frequency scan rate calibration (cm^{-1}/point) and frequency to temperature using Eq. (25.6). The temperature was used to calculate the absorption linestrength, $S(T)$, which was used in combination with the integrated peak area, the absorption pathlength and the laser frequency scan rate calibration factor to determine the water vapor density. The peak shift between the two absorption features was determined in data point units and converted to a frequency shift also using the diode laser calibration. The frequency difference was converted to a velocity using Eq. (25.7).

25.5.9 Instrument Requirements

Application of the sensor technology for monitoring water vapor mass flow during lyophilization requires an electronic sensor control unit (SCU) and an optical sensor measurement head (SMH) as indicated in Fig. 25.4. The SCU contains an ultra-stable DC power supply, the near-IR diode laser and diode laser controller, a pair of BRD circuits and reference InGaAs photodiode detectors, a sealed, low pressure reference absorption cell and signal detector. The SCU is controlled by a computer outfitted with a 1.25 MHz data acquisition system. The SCU is connected to the sensor measurement head (SMH) shown in Fig. 25.7, used for measurement application within an SP Scientific Lyostar 3® laboratory-scale lyophilizer. The SMH consists of an optical transmitter and an optical receiver positioned on opposite

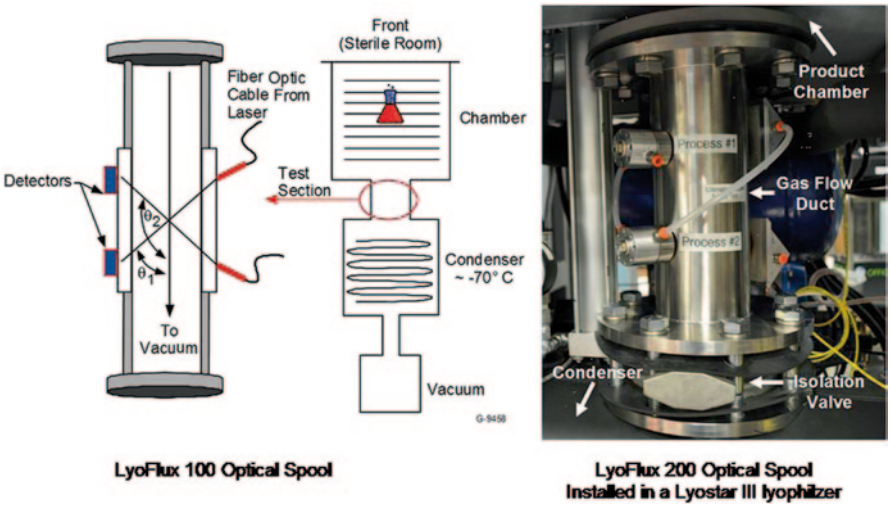

Fig. 25.7 TDLAS water vapor mass flow rate monitor sensor measurement head (SMH) installed in a FTS Lyostar 3® laboratory-scale lyophilizer

sides of the lyophilizer duct. The transmitter uses a fiber optic collimator to transmit the ~ 1–2 mm diameter near-IR laser beam through an antireflection (AR) coated window that forms the vacuum interface with the dryer. The beam is oriented at a 45° angle with respect to the dryer gas flow axis. The transmitted laser light is detected on the opposite side of the duct using a room temperature InGaAs photodiode detector. The detector cap window is also AR-coated and is configured to mount flush with the inner wall of the duct to limit flow perturbations. The detector housing and cap also form a vacuum seal with the dryer duct. The SMH hardware has undergone repeated testing and has demonstrated compatibility with CIP and SIP procedures typically used in production lyophilizers. The SCU is connected to the SMH via a single mode fiber optic patchcord and shielded electronic signal cable.

The installation of the SMH optical spool piece requires physical separation between the lyophilizer chamber and condenser consistent with 45° and 135° measurement angles. The use of smaller measurement angles is possible with the development of a modified SMH and a modified data analysis algorithm. Smaller measurement angles will result in lower velocity measurement sensitivity as described in Eq. (25.7).

Dry nitrogen purge gas is supplied to both the SCU and the SMH. The purge gas supplied to the SCU removes atmospheric pressure water vapor from the BRD reference detectors and the reference absorption cell optical path located outside of the low pressure cell. The presence of atmospheric pressure water vapor in these paths distorts the absorption lineshapes and creates measurement error. The nitrogen purge supplied to the SMH removes atmospheric pressure water vapor in the optical path outside of the low pressure lyophilizer duct. As with the reference absorption cell, atmospheric pressure water vapor in the optical measurement path distorts the absorption lineshape resulting in measurement errors.

The SCU is designed for continuous (24/7) unattended operation. At the start of monitoring for each lyophilization cycle, the user determines the instrument zero velocity offset value. This is accomplished by freezing the product loaded into the chamber to a temperature of approximately −40 °C or lower. The pressure within the product chamber is then reduced and stabilized at the process set point value. The combination of frozen, water-laden product within the low pressure chamber creates a strong water absorption signal that can be used to determine the offset value. The isolation valve between the chamber and the condenser is then closed to ensure zero flow velocity in the lyophilizer duct. The mass flow rate monitor is then used to measure the zero velocity offset which is subtracted from all subsequent velocity determinations. Typical zero velocity offset values are $<\pm 1$ m/s corresponding to a $<\pm 1$ data point shift between the two measured lineshape peaks.

There are a number of different electronic, optical, and spectroscopic-based factors that contribute to the zero velocity offset. The separate BRD circuits each have independent electronic phase shifts. The use of a single, channel-multiplexed data acquisition system results in a phase shift between the two rapidly acquired measurement channels. Reflections within a single optical element (e.g., window) or between optical elements create Fabry–Perot interferometer (etalon) cavities that cause modulation of the transmitted optical beams that are similar to molecular

absorption features that can cause signal phase shifts. The sum of all of these effects is captured with the determination of the zero velocity offset factor which is subtracted from each measured velocity.

25.5.10 Sensor Validation

Validation of the sensor measurement accuracy has been performed through a series of ice slab sublimation tests. These tests have been previously reported by Gieseler et al. (2007) and Schneid et al. (2009). The ice slab sublimation tests provide a direct comparison between the integrated TDLAS water mass flow rate (dm/dt) and the gravimetrically determined amount of water removed. The tests do not provide direct comparison with the three measurements made by the sensor, the average water vapor temperature, water concentration, and gas flow velocity along the optical line of sight through the lyophilizer duct, but do provide a standard method to evaluate the sensor mass flow rate measurement accuracy. The gas concentration measurement can be separately compared to the capacitance manometer-based pressure measurement if the lyophilizer chamber is filled with 100 % water vapor and the pressure is converted to molecular number density through the use of an independent temperature measurement.

Sublimation tests within a SP Scientific Lyostar II® laboratory-scale dryer and an IMA Edwards LyoMax 3® pilot-scale dryer were conducted using "bottomless trays" made from stainless steel frames outfitted with thin plastic bags (0.003 cm thickness) attached to the frame to form the tray bottom (Schneid et al. 2006a). The three laboratory trays were placed on the lyophilizer shelves and each filled with ~1500 g of pure water, while the four pilot shelves were each filled with ~7.5 kg of water, providing an ice thickness of ~1 cm on each dryer shelf. The shelf temperature was lowered to −40 °C and held for 1 h to form the ice slabs. Prior to freezing the ice slab, fine wire thermocouples (Omega, CT) were placed in the middle of the tray water layer to monitor the slab "product" temperature. Following the freezing step the chamber pressure was reduced using the dryer condenser and vacuum pump to the experimental set point pressure (between 65 mTorr and 500 mTorr). The shelf temperature was then ramped (typically 0.5–1 °C/min) to the experimental set point temperature and approximately 50 % of the ice slab product was sublimed under steady state conditions. Once the target of sublimated water mass was reached, the isolation valve located between the chamber and the condenser and downstream of the TDLAS optical measurement station, was closed, ceasing water removal. The TDLAS data collection was simultaneously ended, followed by a ramping of the lyophilizer shelf temperature to melt the remaining ice. The water remaining in the bottomless trays was removed and weighed enabling a gravimetric determination of the total mass of water removed during the sublimation test. The gravimetrically determined mass balance was compared to the integrated TDLAS determined mass balance to validate the measurement accuracy of the instrument. Alternatively the average mass flow rates of each measurement technique may be compared. Figure 25.8 displays the TDLAS measured water vapor concentration and gas temperature and the lyophilizer shelf fluid inlet temperature during a typical sublimation test.

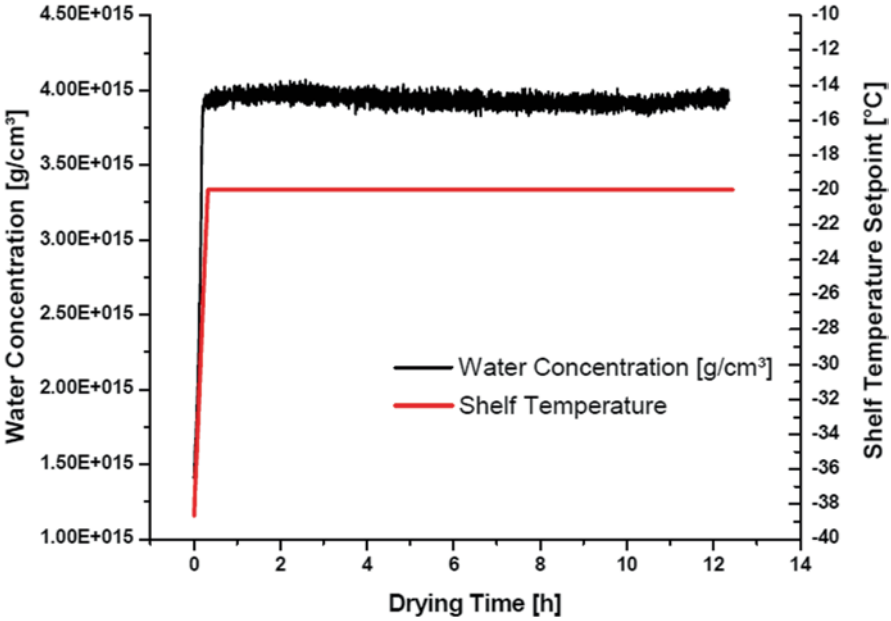

Fig. 25.8 TDLAS-measured water concentration [molecules/cm^3] and lyophilizer shelf temperature temporal profiles during a typical ice slab sublimation test

Figure 25.9 displays the measured velocity and the calculated water vapor mass flow rate. The data in Figs. 25.8 and 25.9 both display the development of steady state ice sublimation conditions following the initial shelf temperature ramp.

Table 25.2 provides a summary of the laboratory-scale dryer measurement conditions and experimental results, including a ratio of TDLAS to gravimetric water sublimation rates. The ratio shows general agreement in mass flow rates with an average error of $<\pm 2\,\%$. The measurement results for the pilot-scale dryer are compiled in Table 25.3 and were not as accurate as for the laboratory scale. This may have been due to the dryer geometry and nonaxisymmetric gas flow within the dryer spool. The standard TDLAS data analysis algorithm is based upon the assumption of axisymmetric gas flow within the dryer spool.

25.5.11 Sensor Applications

TDLAS sensing technology may be applied to a wide variety of lyophilization monitoring needs (Patel and Pikal 2009; Schneid and Gieseler 2009) including lyophilizer operational qualification (OQ) (Patel et al. 2008; Nail and Searles 2008; Hardwick et al. 2008), determination of primary and secondary drying end points (Gieseler et al. 2007), vial heat transfer coefficients (Schneid et al. 2006b; Kuu et al. 2009), product temperature (Kuu and Nail 2009), product residual moisture (Schneid et al. 2007), and freeze-drying cycle optimization (Kuu and Nail 2009). TDLAS sensing

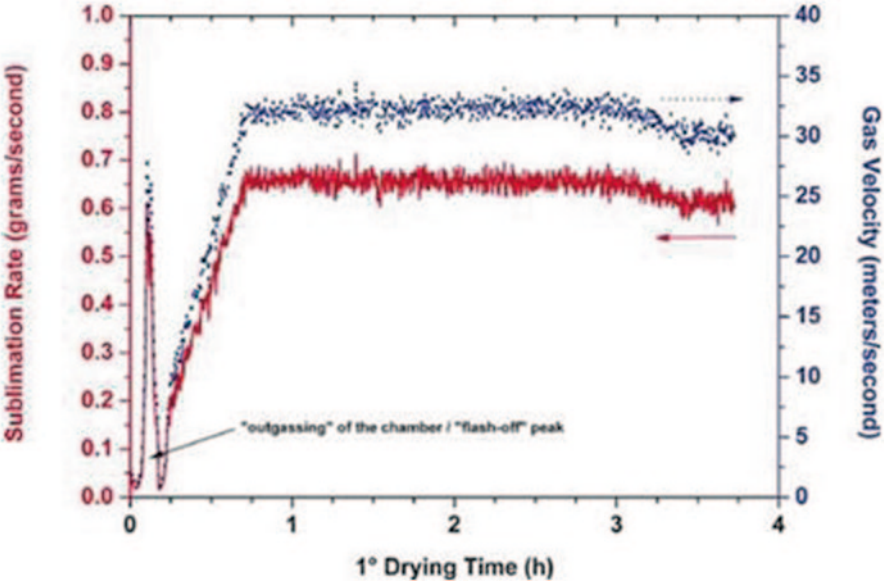

Fig. 25.9 TDLAS-measured gas flow velocity and calculated mass flow rate, dm/dt (g/s) temporal profiles during a typical ice slab sublimation test. Reprinted from Gieseler et al. (2007) with permission from J. Pharm Sci

technology stands out from many other PAT tools applied to lyophilization because of its ability to provide a direct measurement of the gas flow velocity in the duct, which can be used to determine the water vapor mass flow rate. In addition, the technique may be applied to laboratory-, pilot-, and production-scale lyophilizers. Few other technologies have been demonstrated to nonintrusively provide broad measurement capability which can be linked to not only dryer operation, but to critical process parameters such as product temperature, which is linked to final product quality. It is the combination of continuous, real-time mass flow rates (d*m*/d*t*) with established heat and mass transfer models (Pikal 1985; Nail 1980; Rambhatla et al. 2006) that will drive application of the technology.

In the following section of this chapter a brief review of a few of the applications listed above will be provided to demonstrate the value of the measurement technology. This is not a comprehensive review nor is it meant to provide an in-depth analysis of any one of the applications. The reader is referred to the cited publications for additional information.

25.5.12 Lyophilizer OQ

Pharmaceutical companies are highly motivated by economic and regulatory forces to develop robust product formulations and lyophilization processes that maximize product throughput consistent with maintaining product quality. One aspect of

Table 25.2 Summary of laboratory-scale comparison data between TDLAS and gravimetric measurement of total water removed during ice slab sublimation tests

Pressure/shelf temp	Loading/no of trays	Ratio TDLAS/ Gravimetric	Velocity (m/s)	d*m*/d*t* (g/h)
100 mTorr/0 C	3	0.97	110	79.4
150 mTorr/20 C	3	0.97	108	165.8
200 mTorr/40 C	3	0.97	105	185.7
500 mTorr/40 C	3	1.09	51	189.9
60m Torr/−30 C	1	1.02	16	18.8
100 mTorr/−33 C	1	0.96	7	17.3
100 mTorr/−27 C	1	1.00	12	28.5
100 mTorr/-20	1	1.03	17	44.6
100 mTorr/-5 C	3	1.03	95	206.5
100 mTorr/0 C	1	1.03	30	79.6
100 mTorr/40 C	1	1.03	73	152.2
150 mTorr/-30 C	1	1.00	5	19.5
150 mTorr/20 C	1	1.07	39	140.3
150 mTorr/40 C	1	1.05	51	172.8
65 mTorr/−40 C	3	0.95	39	37.8
65 mTorr/−35 C	1	1.00	17	17.0
65 mTorr/−35 C	1	1.03	18	17.9
65 mTorr/−25 C	3	1.06	118	101.9
65 mTorr/−25 C	3	1.06	128	108.0
100 mTorr/−20 C	3	1.05	80	140.8
100 mTorr/−30 C	3	1.03	42	75.2

Table 25.3 Summary of pilot scale comparison data between TDLAS and gravimetric measurement of total water removed during ice slab sublimation tests

Pressure/shelf temp	Loading/no of trays	Ratio TDLAS/ Gravimetric	Velocity (m/s)	d*m*/d*t* (g/h)
100 mTorr/0 C	4	1.00	32	568.1
150 mTorr/20 C	4	1.06	41	1077.7
200 mTorr/40 C	4	1.09	79	1294.9
500 mTorr/40 C	4	1.11	24	1670.8
65 mTorr/−30 C	4	1.03	30	500.4
65 mTorr/−25 C	4	1.00	37	644.4
65 mTorr/−10 C	4	1.02	64	1137.6
65 mTorr/0 C	4	1.05	84	1494.0
100 mTorr/−30 C	4	0.88	17	446.6

maximizing throughput is the development of efficient drying processes that are consistent with the mass flow rate limitations of both the laboratory-scale process development dryer and the manufacturing-scale dryer that will be used to produce the drug product (Chang and Fisher 1995). The development of a process that can be transferred between lyophilizers requires knowledge of the maximum supported rate of mass transfer between the chamber and the condenser and the relationship between the ice sublimation rate (g/s) and the dryer shelf temperature, chamber pressure, and product temperature (Patel et al. 2007; Nail and Searles 2008).

The development of a family of sublimation rate curves as a function of chamber pressure and shelf temperature is needed to define the lyophilizer operational limitations. Traditionally this information was gathered through a series of ice slab sublimation tests with each test providing a single data point at a single shelf temperature and pressure. The gravimetric determination of total water removed would provide the average sublimation rate during one experiment. Thus, a complete family of curves would require numerous experiments and a large investment of time and labor resources.

The TDLAS sensor technology enables the development of the required data set and the determination of choked flow conditions within a few experiments (Patel et al. 2008; Nail and Searles 2008). To accomplish this, ice slabs are formed on the dryer shelves and the chamber pressure is reduced to the set point value (typically starting with the lowest values). The shelf temperature is then ramped to the lowest value of interest. The TDLAS instrument is used to monitor the sublimation rate (g/s) and to determine the establishment of steady-state drying conditions. During steady state operation the TDLAS measured sublimation rate (dm/dt), the ice slab product temperature (T_b), the shelf temperature (T_s), and the chamber pressure are recorded. The chamber pressure is then changed and the sensor is again used to verify the establishment of steady state operation (indicating that both the shelf temperature and the product temperature have stabilized). A new set of measurements is then recorded including dm/dt, T_s, T_b, and chamber pressure. After completing measurements over all pressures of interest the shelf temperature is raised to the next setting of interest and the measurement process is repeated until a complete set of sublimation rates is measured for all pressures and temperatures. This process enables lyophilizer OQ to be completed in days rather than weeks, dramatically saving time and money.

25.5.13 Determination of Primary and Secondary Drying End Points

During the design and development of a lyophilization process it is desirable to build in flexibility in process strategies (process robustness) for efficient process scale-up and technology transfer and improvements that can minimize the process time for economical and sustainable cycles. During commercial manufacturing it is imperative to control and monitor the process as the product value in the

freeze-dryers may exceed millions of dollars. Current practice is to measure and reject the process trajectories rather than responding as the current monitoring techniques are inadequate in providing measurements of the key dependable parameters. With the advancement of the TDLAS technology it has become possible to monitor and potentially control all the critical parameters and apply in-process corrective actions in response to process variations resulting in improved quality and elimination of waste.

Precise determination of the end of the primary drying phase is of utmost importance both during the design of the process as well as monitoring/controlling of the process during commercial manufacturing. The current practice of running lyophilization cycles in commercial manufacturing is by fixed time. Advancement to secondary drying without completion of sublimation of ice jeopardizes the quality attributes of the product. TDLAS sensor technology has been used to monitor drying of numerous "product" drying cycles including mannitol, lactose, trehalose, sucrose, dextran, glycine, PVP, and BSA formulations (Gieseler et al. 2007; Schneid et al. 2009). Measurements and monitoring of water concentration in the drying chamber during the primary drying phase using TDLAS enables the determination of the end point of primary drying. As the sublimation of ice nears completion the composition of the gas in the product chamber changes from nearly all water vapor to nearly all nitrogen and a sharp drop in the TDLAS water concentration curve can be observed. The inflection point of the curve or more conservatively when it plateaus off can be used as the end point of primary drying. This is illustrated in Fig. 25.10, a temporal plot of the TDLAS measured water vapor concentration and the lyophilizer shelf temperature for drying a 5 % w/w sucrose solution contained in 336 vials. The 20 cc vials containing 5 % w/w sucrose solution were freeze dried at a full load at a constant chamber pressure of 65 mTorr using a SP Scientific LyoStar II® laboratory-scale dryer.

As shown in Fig. 25.10 the water concentration remained nearly constant throughout the primary drying. The spikes in concentration data in late primary and secondary drying correspond to withdrawal of samples using a sample extractor unit, which were used to cross-correlate the rate of water removal. Additional information of velocity and mass flow data (sublimation rate) is also obtained using the TDLAS sensor and complement the water vapor concentration data in determining the end points of primary and secondary drying. Careful examination of the velocity and mass flow profiles also reveal differences in product characteristics or potential problems with the drying process in response to the shelf temperature changes as it can be observed near the end of primary drying in Fig. 25.11. Figure. 25.11 displays the temporal profiles of the gas flow velocity measurement and the mass flow rate (dm/dt) determination. The data spikes are due to isolation valve closing events used during MTM pressure rise measurements. The mass flow profile closely follows the velocity profile throughout primary drying, including the obvious response to the shelf temperature adjustments during the early portion of primary drying. The end point determinations made through TDLAS were found to be comparable to determinations made from comparative pressure measurements, mass spectrometry, or cold plasma devices (Milton et al. 1997).

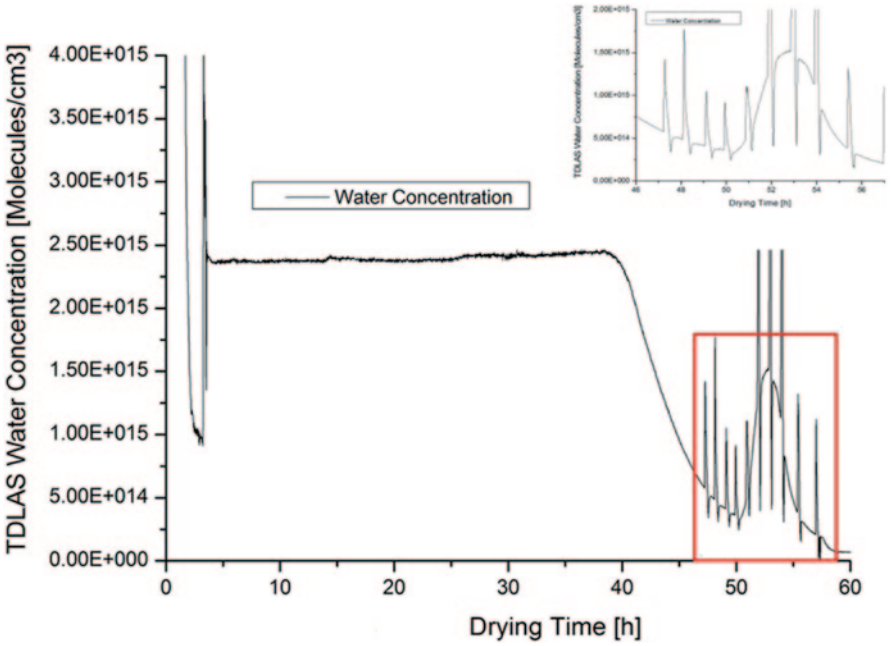

Fig. 25.10 TDLAS water vapor concentration temporal measurement profile during lyophilization of 5% w/w sucrose in a laboratory-scale dryer

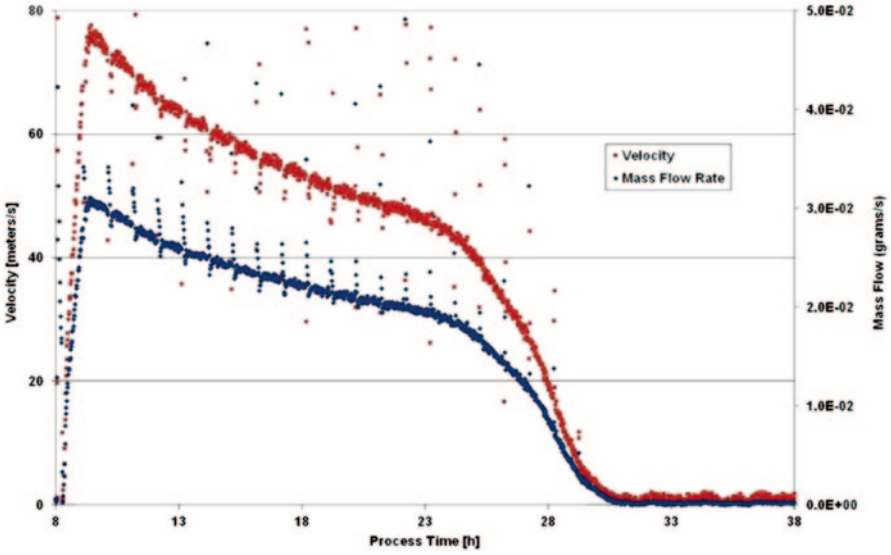

Fig. 25.11 TDLAS vapor flow velocity and water mass flow rate temporal measurement profiles during lyophilization of 10% w/w glycine in a laboratory-scale dryer

25.5.14 Determination of Vial Heat Transfer Coefficients and Product Temperature

During lyophilization, product temperature history is a critical process parameter that cannot be directly controlled, but is influenced by shelf temperature, chamber pressure, and product resistance to drying. The standard laboratory approach is to place temperature sensors, usually thermocouples, directly in the product in a few selected vials. Product temperature determined by thermocouples represents the temperature at the bottom center of a vial, but does not directly measure the temperature of the product at the continuously moving sublimation interface. The temperature at the sublimation interface, however, governs the product quality. If the product temperature at the interface exceeds a critical temperature for the matrix, the product will undergo collapse, compromising product quality. Product temperature during drying directly affects cake appearance, residual moisture content, and reconstitution time and it may affect product stability and shelf life. Thermocouples are not typically used during manufacturing-scale freeze-drying due to the need for hand placement of the sensors which is not feasible when using automatic loading systems and due to sterility concerns. Thus, the development of a widely applicable, robust measurement solution that can be used in both laboratory- and production-scale dryers is an important industry goal. Accurate temperature measurements during a process abnormality may prevent the loss of millions of dollars of product.

The previously described MTM pressure rise technique has been used to provide batch average product temperature during the first two thirds of primary drying. Due to the requirement of a quick-closing isolation valve, this technique is generally only applied to laboratory-scale lyophilizers and does not provide a solution for production-scale temperature monitoring. In contrast, the TDLAS-based measurement technique may provide the needed measurement capability for all scales of freeze-dryers.

Recently it has been demonstrated that TDLAS-based mass flow rate measurements (dm/dt) may be combined with a steady-state heat and mass transfer model (Pikal 1985; Nail 1980; Rambhatla 2006; Milton et al. 1997; Tang et al. 2005) to provide continuous, real-time determinations of batch average product temperature in a laboratory-scale dryer (Schneid et al. 2009). Due to the wide applicability of the TDLAS sensor technology, it is anticipated that this approach may also be applied to pilot- and production-scale dryers, providing a nonintrusive measurement solution that may be applied from process development through production.

As previously described, heat transfer during vial-based lyophilization can be described in terms of thermal barriers and temperature gradients. Heat is supplied to the frozen product from the drying chamber shelves through the bottom of the glass vials to compensate for the heat removed by sublimation. Heat flow from the shelves to the product is described by Eq. (25.10).

$$dQ/dt = A_v \cdot K_v \cdot (T_S - T_b) \tag{25.10}$$

where dQ/dt is the heat flow (cal/s or J/s) from the shelves to the product; A_v is the cross sectional area of the vial calculated from the vial outer diameter; K_v is the vial heat transfer coefficient (for a specific vial type at a specific pressure); T_s is the temperature of the shelf surface and T_b is the temperature of the frozen product at the bottom center of the vial.

During steady state, the heat flow (dQ/dt) can be related to mass flow (dm/dt) by using the heat of ice sublimation, ΔH_s (Eq. (25.11)):

$$\frac{dQ}{\mathrm{d}t} = \Delta H_S \cdot \frac{\mathrm{d}m}{\mathrm{d}t} \tag{25.11}$$

where ΔH_S is given in the literature (~650 cal/g) (Pikal 1985). Eqs. (25.10) and (25.11) can be combined and rearranged to provide the product temperature in the bottom of the vial as shown in Eq. (25.12):

$$T_b = T_S - \left[\frac{(\Delta H_S \cdot (dm/dt))}{A_v \cdot K_v} \right] \tag{25.12}$$

In the laboratory, K_v can be separately determined using Eq. (25.13) and by performing sublimation tests with pure water filled into vials.

$$K_v = \frac{dm/dt \cdot \Delta H_3}{A_v \cdot (T_s - T_b)} \tag{25.13}$$

Here, the average temperature difference, $(T_s - T_b)$, can be determined using thermocouples in selected vials (bottom center) as well as adhesive thermocouples on the shelf surface during the experiments. Note that in the laboratory, temperature bias between vials containing thermocouples and vials not containing thermocouples is usually very small due to particulate contamination in the product fluid used to fill the vials. A_v is easily determined by measurement.

Mass flow can be determined either gravimetrically from the known initial mass of water and the remaining mass of water after a predefined time interval in primary drying (Tang et al. 2005) or by the TDLAS sensor. Figure 25.12 displays experimentally determined K_v from both gravimetric and TDLAS mass flow rate determinations as a function of chamber pressure (Schneid et al. 2009, 2006; Kuu et al. 2009). As anticipated, increasing chamber pressure results in larger K_v values as the contribution from gas conduction to the vial heat transfer coefficient dominates over the shelf conduction and radiative heat transfer contributions. Fig. 25.13 shows good agreement between the TDLAS and gravimetric K_v determinations throughout the pressure range relevant during lyophilization.

Following the determination of the weighted average vial heat transfer coefficient, experiments were performed with product-filled vials to demonstrate the use of TDLAS dm/dt measurements for the determination of batch average product temperature (Schneid et al. 2009). The dm/dt measurements were combined with thermocouple-based shelf temperature measurements, the vial cross sectional area, and the water heat of sublimation to determine the batch average product

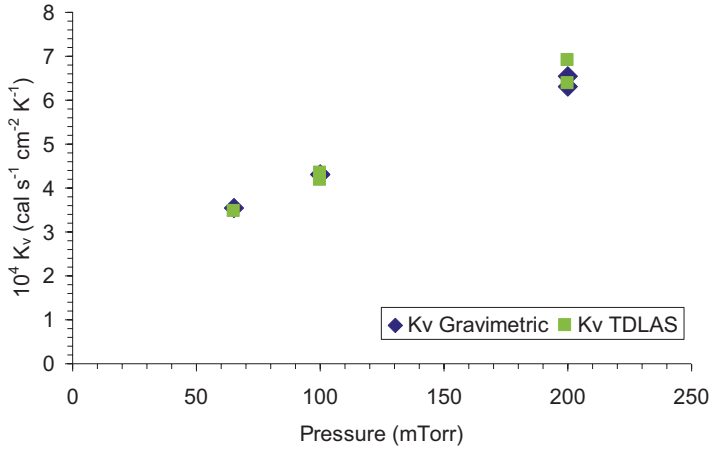

Fig. 25.12 Gravimetric and TDLAS-based determinations of vial heat transfer coefficient, K_v, as a function of the laboratory-scale lyophilizer chamber pressure

temperature using Eq. (25.12). The TDLAS-determined bottom center temperature was compared to thermocouple-based product temperature measurements to assess the accuracy of the measurement technique. Experiments were performed using sucrose, glycine, and mannitol product formulations with the results of the 10% glycine drying experiment for primary drying shown in Fig. 25.13. The plot shows a

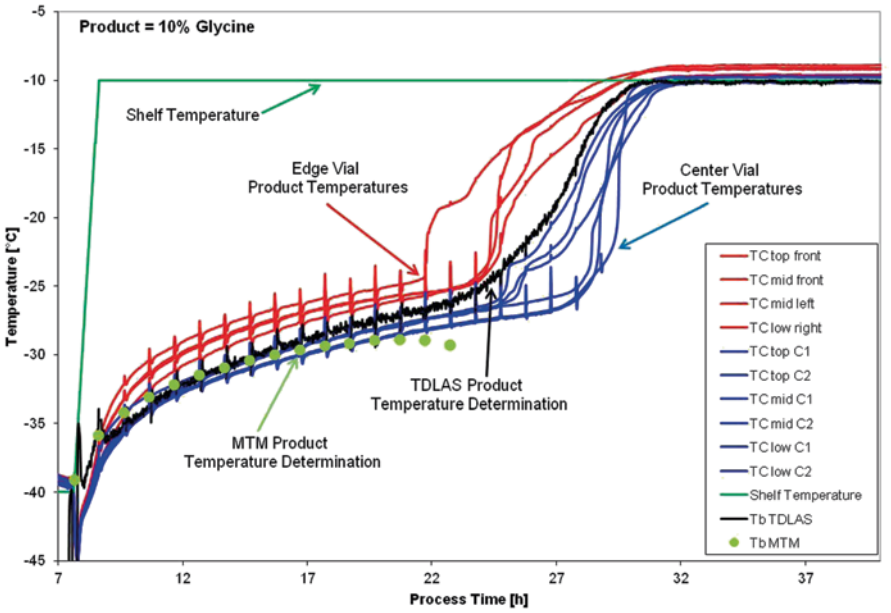

Fig. 25.13 Product temperature temporal profile during 10% glycine primary drying as determined using thermocouples, TDLAS, and MTM measurement techniques—dryer. Reprinted from Jameel and Kessler (2011) with permission from CRC Press

clear difference between the center vial and edge vial thermocouple-based temperature measurements, with the edge vial product temperatures higher than the center vials due to radiative heat loading from the warm dryer walls and door. The TDLAS determined batch average product temperature is initially biased to the center vial thermocouple-based measurements during early primary drying and then provided an average determination between edge and center vials in the later stages of primary drying. In addition to the TDLAS and thermocouple-based temperature measurements, the batch average product temperature was also determined using the MTM technique which is generally more representative for the coldest vials in the batch.

Figure 25.13 shows that the MTM and TDLAS techniques agree very well during the first half of primary drying before the MTM measurement techniques fails due to insufficient pressure rise.

Additional analysis may enable the determination of the product temperature at the sublimation interface, $T_{p,}$ by using Eq. (25.14):

$$T_p = T_b - \left[\frac{dQ/dt \cdot L_{ice}}{(A_v \cdot 20.52)} \right] \tag{25.14}$$

where dQ/dt is the heat flow, L_{ice} is the ice thickness and A_v is the cross-sectional area of the vial. The value 20.52 in Eq. (25.14) represents the thermal conductivity of ice (cal/hrcm²K). L_{ice} may be instantaneously calculated from TDLAS mass flow rate measurements and the knowledge of the initial fill depth (Tang et al. 2005). This procedure needs to be verified through experimental investigations.

25.5.15 Determination of Product Resistance

Knowledge of product resistance to drying is important to aid in the design of the product formulation, drying process, and scale-up and transfer to commercial manufacturing. Knowledge of product resistance during the selection of the excipients and their weight ratios will aid in the identification of excipient(s) and the solid content that will exhibit low product resistance and enable the development of an efficient drying process (short cycle time). While monitoring and controlling of the product resistance during scale-up, transfer and commercial manufacturing will help to eliminate heterogeneity/variability in the physical characteristics of the lyophilized cake such as residual moisture content, reconstitution time, and appearance within a batch and from batch to batch (Rambhatla et al. 2004). This variability is believed to arise from heterogeneity in pore size and drying times. The morphology of ice/pore size is dependent on the degree of supercooling which in turn is dependent upon the nucleation temperature. Increased supercoiling results in smaller crystal/pore sizes and higher product resistance to mass transfer through the dry cake. The degree of supercooling is a scale-up issue and is commonly observed in GMP manufacturing due to the low particle loading in a class 100 environment.

TDLAS can be utilized for formulation and process development as well as for the inline characterization and monitoring of the degree of supercooling in the GMP manufacturing setting through the estimation of the product resistance using Eq. (25.15) and the measured mass flow rate as described above.

$$R_p = \frac{A_p \cdot (P_{ice} - P_c)}{dm/dt_{vial}} \tag{25.15}$$

where dm/dt is the mass flow rate (g/h per vial), P_{ice} is the vapor pressure of ice at the sublimation interface (mTorr), P_c is the chamber pressure (mTorr), R_p is the product resistance ($cm^2 \times Torr \times h/g$), R_s is the stopper resistance ($cm^2 \times Torr \times h/g$), and A_p is the inner cross-sectional area of the vial (cm^2), i.e., the surface area of the product. P_{ice} can be directly calculated from the TDLAS product temperature using Eq. (25.2) and dm/dt_{vial} can be directly measured by TDLAS.

Schneid et al. studied the effect of various excipients on the product resistance as a function of primary drying and compared the results with the MTM data. A typical R_p profile obtained using TDLAS overlaid with an MTM-based determination is displayed in Fig. 25.14. Schneid et al. concluded that the general behavior and the values during early primary drying are in good agreement with the MTM data. Subsequent unpublished results from The University of Connecticut (Sharma et al.) demonstrated that the nonphysical rise in the R_p curve near the end of primary drying was due to early completion of primary drying of some vials, thus having inaccurate knowledge of the surface area of subliming ice within the lyophilizer as

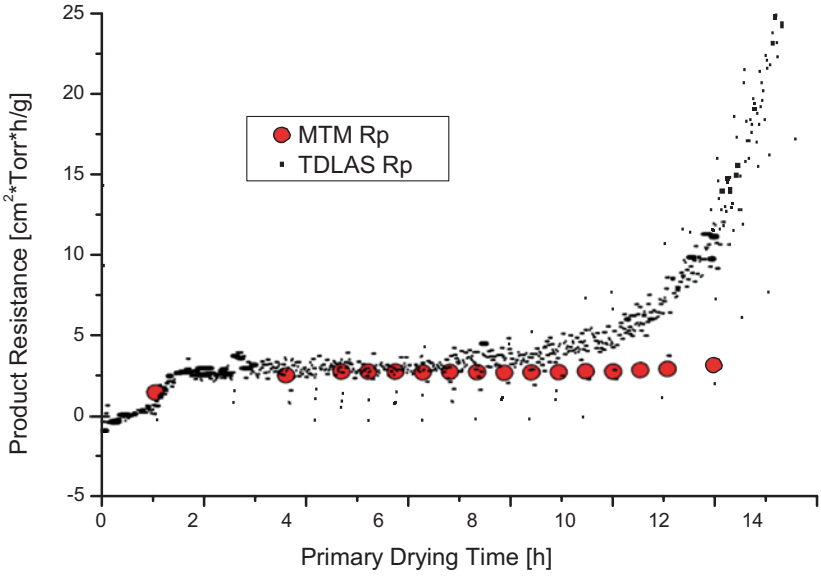

Fig. 25.14 Product resistance (Rp-TDLAS) data calculated for a 50 mg/mL sucrose run compared to MTM data

a model input for calculating R_p. A linear extrapolation of the R_p curve near the end of primary drying was used to provide an estimate of the number of vials that had already completed primary drying and thus provided an indication of drying heterogeneity. Another study was conducted by Awotwe-Otoo et al. (2014) where they used TDLAS for in-line measurement of mass flow rate to obtain product resistance to study the correlation between the degree of supercooling and product resistance to control the onset of ice nucleation of the freezing step of a model mAb formulation, using ControLyo™ Nucleation on-demand technology. Fig. 25.15 displays the product resistance as a function of dried layer thickness for sucrose-based formulations under controlled [C] and uncontrolled [UNC] nucleation cycles. The TDLAS data was overlaid with MTM data and some differences in the product resistance values were observed.

A number of recent publications by Nail et al. (Nail and Searles 2008; Hardwick et al. 2008; Kuu et al. 2009; Kuu and Nail 2009; Nail and Kessler 2010; Wegiel et al. 2014.) have combined many of the previously discussed applications of the TDLAS water vapor mass flow rate sensor into a fundamental tool in QbD-based freeze-drying process development. This body of work describes an approach in which the sensor is used to rapidly establish the relationship between process variables that can be controlled (such as shelf temperature and chamber pressure) and those that cannot be directly controlled (such as product temperature). Once this information is known, a design space can be constructed through the calculation of shelf temperature and product temperature isotherms. In addition, the sensor is used to measure the limit of mass transfer within the lyophilizer to define an upper limit of the freeze-drying equipment. The combination of the design space and the equipment limitation enables the development of an efficient and robust drying process. This approach has been demonstrated using a laboratory-scale freeze-dryer and has the potential to be applied to production scale dryers, enabling the determination of process parameters that leverage the full capability of the manufacturing equipment (as opposed to the laboratory equipment) while maintaining product quality.

The QbD-based approach to freeze-drying cycle development identifies the optimal processing conditions based on a thorough understanding and knowledge of the edges of failure through execution of five steps:

1. Measure the thermal response characteristics of the product formulation to determine the upper product temperature limit for primary drying.
2. Establish the relationship between the process variables that can be directly controlled (shelf temperature and chamber pressure) and those that cannot be directly controlled (product temperature).
3. Calculate the design space by constructing shelf temperature and product temperature isotherms.
4. Define equipment limitations.
5. Establish optimum process conditions within an acceptable zone.

This approach was first reported by Chang and Fisher (1995) in 1995 as a method of efficiently developing freeze-drying processes for protein formulations. The process variable relationships were developed through extensive experiments which

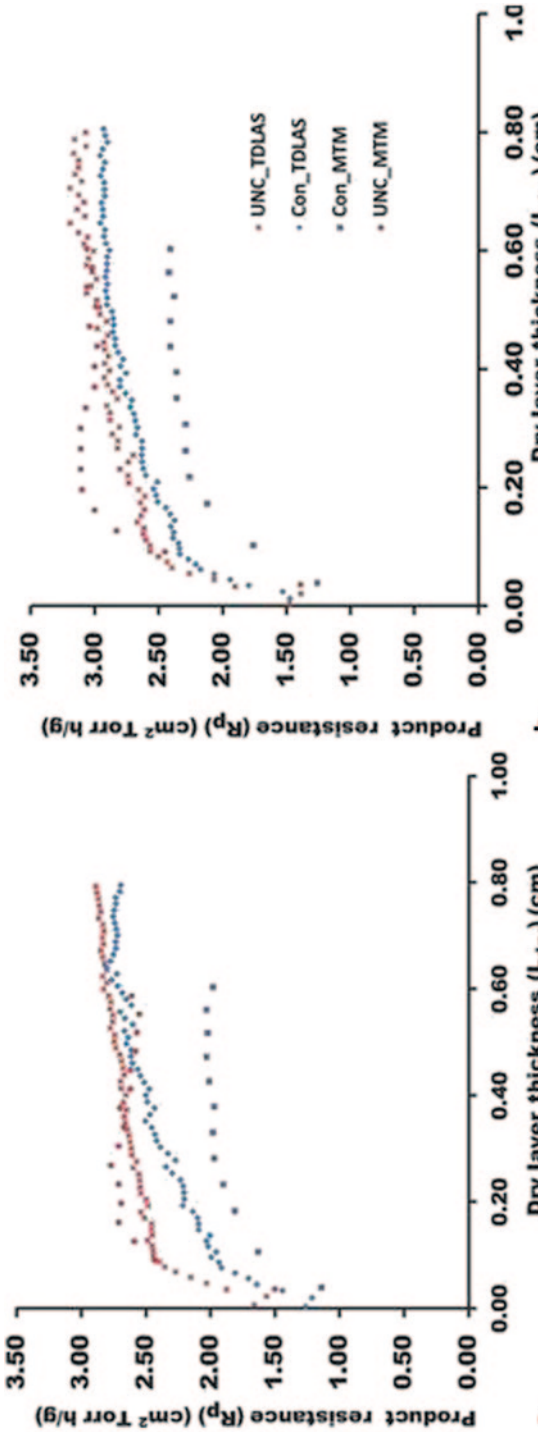

Fig. 25.15 Product resistance (R_p) as a function of dry layer thickness (L_{dry}) for uncontrolled and controlled nucleation cycles during primary drying for **a** 1 mg/mL and **b** 20 mg/mL mAb formulations. TDLAS data for R_p were compared with R_p values determined by MTM. Although for controlled nucleation cycles, MTM values were lower than TDLAS, for uncontrolled nucleation, MTM values were initially higher at L_{dry} = 0.20 cm before leveling off with TDLAS values. Reprinted from Awotwe-Otoo et al. 2014, with permission from J Pharm Sci

included gravimetric analysis of water removed to enable calculation of the product temperature isotherms. Nail and Searles (2008) recognized that the TDLAS sensor technology could be used to dramatically reduce the number of experiments required to acquire the data which is combined with a model of vial-based freeze-drying to construct shelf-temperature and product temperature isotherms. In addition, the TDLAS sensor is also used for equipment qualification through ice slab tests for the determination of the freeze-dryer choke point and operational limits. This QbD-based approach was described for a model formulation, sodium ethacrynate, during the 2010 conference on Freeze-Drying of Pharmaceuticals and Biologicals (Nail and Kessler 2010). The advantages of the approach include the development of an optimum drying cycle, maximizing process and product understanding through a minimum number of experiments and the creation of a body of information that facilitates handling of process deviations.

During the 2014 Freeze-Drying of Pharmaceuticals and Biologicals Wegiel et al. 2014 described an extension of the prior work with the development of a three-dimensional QbD design space. This approach was developed to demonstrate the potential to dramatically improve drying efficiency through the knowledge of the constantly changing value of R_p throughout primary drying. At the start of primary drying product dry-layer resistance is low enabling an increase in shelf temperature with a corresponding increase in the sublimation rate, sublimative cooling, and an ability to maintain the product temperature below the collapse temperature under more aggressive drying conditions. As R_p increases, the shelf temperature was reduced in a stepwise manner to ensure that the product temperature was maintained below the collapse temperature. Knowledge of the equipment capability was also used to ensure that the drying operation was maintained within the range of the equipment capability. The development of the three-dimensional (time being the third dimension) design space and resulting cycle decreased the primary drying time by 30% as compared to a cycle developed using the previously described two-dimensional design space. Future development of this approach, partially enabled by the TDLAS sensor, should result in optimized cycles and a reduction in lyophilization costs while maintaining robust process cycles and product quality.

25.5.16 TDLAS Summary

The TDLAS technique enables continuous, real-time, nonintrusive measurements of gas temperature, water concentration, and gas flow velocity based upon fundamental principles of absorption spectroscopy. These measurements are combined with computational fluid dynamic (CFD) modeling of the gas flow within the lyophilizer to interpret the line of sight measurement data and provide the mass flow or sublimation rate of water throughout both the primary and secondary drying phases of lyophilization. A number of measurement applications have been reviewed, including lyophilizer operational qualification, determination of primary and secondary drying end points, the determination of vial heat transfer coefficients and finally the real-time, nonintrusive determination of batch average product temperature. These final two applications combine the TDLAS sublimation rate measurements with a

steady-state drying heat and mass transfer model to provide temperature information that may be directly linked to product quality. TDLAS measurement technology is a powerful tool for monitoring and future control of lyophilization processes and for linking measurements to product quality.

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Chapter 26
Product Homogeneity Assessment During Validation of Biopharmaceutical Drug Product Manufacturing Processes

Fuat Doymaz, Frank Ye and Richard K. Burdick

26.1 Introduction

Drug product (DP) homogeneity (uniformity) refers to the sameness of quality attribute(s) across the units that make up a batch. Manufacturing of biological parenteral DPs involves the use of multiple interconnected and complex unit operations during formulation, fill, and finish steps. Depending on the nature of DPs, these steps may include thawing, formulation buffer preparation and mixing, sterile filtration, filling, and/or lyophilization (Agalloco and Carleton 2008). From DP quality and regulatory perspective, homogeneity within a batch and consistency between manufactured batches are key to guard public health against various sources of variability (FDA 2011). Furthermore, quality control (QC) testing performed at release and during stability studies necessitates that homogeneity assessment of DP batches be performed for the justification of sample size used. Results obtained from validation batches included in the homogeneity study can then be rationalized and used in regulatory filings for biological license applications.

In this chapter, we use a stepwise approach to generate a sound data package for assessing batch homogeneity during process validation activities. These activities can be grouped under three main steps: protocol phase, protocol execution phase, and report phase. Now we will describe these steps in detail.

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26.1.1 Protocol Phase

1. DP process steps at which homogeneity testing will be conducted are first identified. Depending on the product nature, these steps may vary. For example, for the post-formulation step and during filling operation, one may want to check if formulated DP is homogeneous prior to filling into final containers (e.g., vials or syringes)
2. To assess the impact of any process steps of the formulation, DP fill and finish operation, orthogonal and sensitive analytical test methods should be identified. Methods that measure protein concentration, aggregation, and subvisible particle counts should be used to determine the impact on product quality.
3. For each selected product attribute at each identified process step, one should establish homogeneity acceptance criteria (HAC). Setting an appropriate HAC requires understanding of how much variability is due to analytical method and how much is due to the variation among filled DP units. HAC is generally linked to uncontrolled common cause variation and can be established at a certain magnitude of the intermediate precision of the analytical method.
4. Proper application of the statistical approach for assessing batch homogeneity requires determination of the required sample size. One of the statistical methodologies appropriate for assessing batch homogeneity is average equivalence (Limentani et al. 2005). We recommend the use of this method for assessing homogeneity because it provides customer protection at preset level against incorrectly concluding homogeneity when in fact the batches are not homogeneous. The preset level is usually fixed at 5%.
5. For sampling a formulated DP from the hold vessel or during filling of DP into final containers (vials or syringes), one can choose to conceptually divide the filling period into three intervals: Beginning, Middle, and End. Samples are then selected from each interval to ensure a representative sample of the entire batch (Fig. 26.1).

Fig. 26.1 Sampling locations recommended during the filling of a DP batch

6. The sample size needed to ensure adequate power is determined after considering the distribution of the product attribute, HAC, number of intervals that will be sampled from each batch, and confidence level in the statistical equivalency test.
7. Clear instructions on the collection, handling, and transfer of these samples to QC laboratories should be provided to operation personnel.

26.1.2 Protocol Execution Phase

Two critical steps are involved in this phase.

1. Handling (storage and transfer of) representative batch samples collected for each method at each process step during validation runs.
2. Creating a randomized testing schematic for QC testing of these samples via the respective analytical method and data verification of the test results for data analysis.

26.1.3 Report Phase

1. Reporting deviations from the executed protocol.
2. Analysis of the collected test results per statistical methodology used in the study design.
3. Comparison of the analysis results with each performance parameter(s) HAC in each process step.

Next, we provide more details of the statistical methodologies mentioned above and then illustrate the framework with an example.

26.2 Methodology

This section focuses on selection of the sampling design, data analysis, and sample size determination.

Sampling Design As discussed in Sect. 1.1 (point 5 of protocol phase), sampling from filling line can be viewed as a design with one factor having three positions: Beginning, Middle, and End. Samples withdrawn from these positions are assumed to be independent samples and will be later tested in a randomized way in QC laboratory by the specified analytical method. The sample size per sampling interval (n) will later be determined with consideration of the HAC. Hence, for a batch there will be a total of $3 \times n$ independent measurements that represent a batch in the particular product quality attribute during the filling process. These data will be analyzed by the statistical model described next.

Statistical Model The statistical model assumes that we have three fixed positions from which we sample DP during the fill—Beginning, Middle, and End. The appropriate statistical model is

$$Y_{ij} = \mu_i + E_{ij}, \tag{26.1}$$

where Y_{ij} is the measured response for position i ($i=1, 2, 3$) and replicate j ($j=1, \ldots, n$), μ_i is the average for position i, and E_{ij} is a normal random measurement error with mean 0 and variance σ_E^2.

26.2.1 Equivalence Testing and the Homogeneity Acceptance Criterion (HAC)

To provide the strongest statistical evidence of homogeneity across the fill, it is necessary to develop a statistical test of equivalence. General references on statistical equivalence can be found in (Limentani et al. 2005, Chambers et al. 2005, Richter and Richter 2002). The US Food and Drug Administration (FDA) recommends the use of average equivalence testing for demonstrating average bioequivalence (see, Guidance for Industry 2001, p. 10).

Consider a demonstration of equivalence for the average of a product quality characteristic (suppose that the response is protein concentration) between the beginning and middle positions. The parameter of interest is $\theta = \mu_1 - \mu_2$, where μ_1 and μ_2 are the average protein concentrations for position 1 (beginning) and position 2 (middle), respectively. To demonstrate equivalence (or homogeneity in this scenario), it is necessary to show θ is less than some value deemed to be practically important. The definition of "practically important" is provided by the HAC.

Once HAC has been defined, equivalency assessment is performed via a two one-sided t-test (TOST) with 95% confidence. If the lower one-sided 95% confidence limit on θ is greater than −HAC and the upper one-sided 95% confidence limit on θ is less than+HAC, then equivalence between two positions is demonstrated (see scenarios 1 and 4 in Fig. 26.2). Whereas failure to meet the established HAC either partially (as in scenario 2 in Fig. 26.2) or completely (as in scenario 3 in Fig. 26.2) requires further investigation.

For setting HAC, variability in the analytical method along with fill weight variability should be considered. During method development of a quantitative response, intermediate precision (σ_M) is one of the studied variance terms. It is also common to conduct studies for gauging fill weight variability (σ_F) prior to validation. It is best to base the HAC on the sum of these two variability components under normal operating conditions. This provides a basis for establishing a "practically important" threshold for assessing the level of variability in quality attributes during filling of validation batches. The standard deviation of this total variance is

$$\sigma_E = \sqrt{\sigma_M^2 + \sigma_F^2}. \tag{26.2}$$

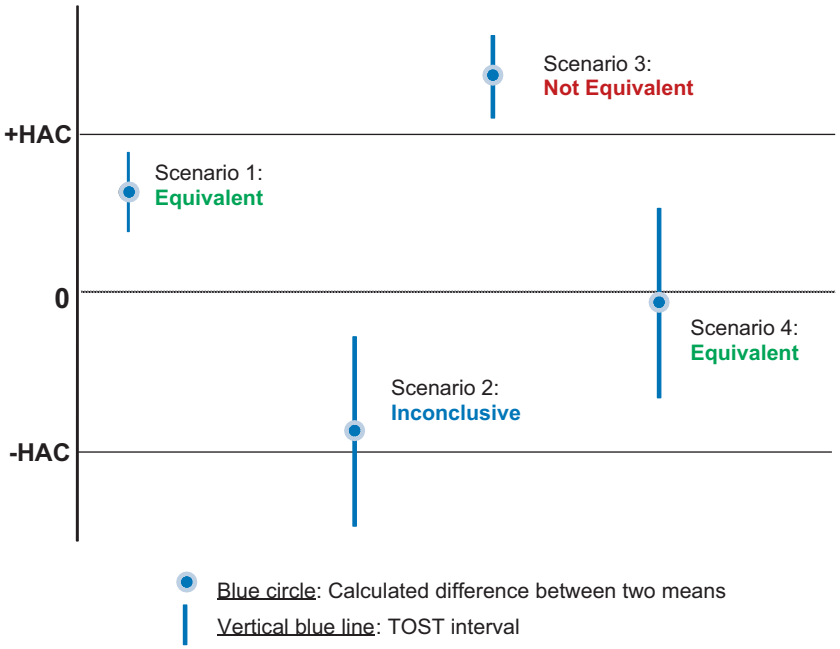

Fig. 26.2 Possible outcomes of a statistical test of equivalence

It should be noted that $\sigma_F \ll \sigma_M$, hence, in the absence of σ_F, σ_M is still a good source to calculate the HAC per Eq. 26.3. Given how much one can sample from a manufactured batch, the level chosen in Eq. 26.3 was found to be working well in practice; however, study owner may find the use of other levels cited in (Burdick and Sidor 2013), more appropriate for their application.

$$HAC = 3\sigma_E \tag{26.3}$$

26.2.2 Confidence Intervals

Equivalence tests are performed by computing two one-sided 95% confidence intervals for the three following parameter functions:

$$\mu_1 - \mu_2,$$

$$\mu_1 - \mu_3, \text{ and}$$

$$\mu_2 - \mu_3,$$

where μ_1, μ_2, and μ_3 are the means defined in Eq. (26.1).

Table 26.1 Comparisons of mean differences among sample positions against HAC. LCL and UCL denote lower and upper one-sided confidence limit, respectively

Compared positions	Comparisons
Beginning (1)–Middle (2)	$-\text{HAC} < \text{LCL}(\mu_1 - \mu_2)$ and $\text{UCL}(\mu_1 - \mu_2) < \text{HAC}$
Beginning (1)–End (3)	$-\text{HAC} < \text{LCL}(\mu_1 - \mu_3)$ and $\text{UCL}(\mu_1 - \mu_3) < \text{HAC}$
Middle (2)–End (3)	$-\text{HAC} < \text{LCL}(\mu_2 - \mu_3)$ and $\text{UCL}(\mu_2 - \mu_3) < \text{HAC}$

In order to demonstrate average equivalence, one must pass the comparison for all three differences against ± HAC. Using the proposed HAC, the set of six comparisons reported in Table 26.1 must be simultaneously satisfied to demonstrate DP batch homogeneity for product quality responses included in the study. Here, we propose using 90 % two-sided confidence intervals to conduct all three pair-wise comparisons.

26.2.3 Statistical Power and Sample Size Computation

A simulation-based statistical power calculation is performed to determine the probability of demonstrating homogeneity for various mean values. In particular, a power calculation was computed where

$$\begin{aligned} \mu_2 &= \mu_1 + \tfrac{1}{2}\text{C}, \text{ and} \\ \mu_3 &= \mu_1 + \text{C}, \end{aligned} \tag{26.4}$$

where C is a constant. The value of C in Eq. (26.4) denotes a possible linear trend in the fill. Only values with positive C are considered in the simulation since negative values of the same magnitude will provide the same results. The power computation is used to establish the sample size (n) needed to ensure adequate power in the statistical equivalence test.

26.3 Results and Discussion

In this section, we demonstrate the proposed DP homogeneity assessment during validation using a simulated data for a product quality response. We will follow steps described in the introduction section.

Example During DP process validation, batch homogeneity needs to be demonstrated. The following steps describe this process.

26.3.1 Protocol Phase

1. DP process steps: filling
   Filler used in filling formulated and filtered DP into vials will be selected to asses whether any impact due to the filling operation is beyond a threshold.
2. Analytical method: protein concentration
   Homogeneity of the batch will be tested by means of protein concentration method.
3. Establish HAC for protein concentration.
   Intermediate precision reported in the method development for protein concentration is $\%CV = 2$. Intermediate precision (σ_M) at target protein concentration 50 mcg/mL of the filled DP is then

$$\begin{aligned}\sigma_M &= 0.02 \times 50 \\ &= 1.0 \text{ mcg/mL}\end{aligned}$$

Fill variability (σ_F) is not available and assumed to be $\sigma_F \ll \sigma_M$, hence, $\sigma_E = \sigma_M$. This leads to

$$\begin{aligned}\text{HAC} &= \pm 3 \times \sigma_E \\ &= \pm 3 \times 1.0 \\ &= \pm 3 \text{ mcg/mL}\end{aligned}$$

4. Statistical approach appropriate for assessing batch homogeneity: Average equivalence approach described in Sect. 26.2 will be used.
5. Sampling intervals: During filling, the DP batch will be sampled from three fill positions of Beginning, Middle, and End as shown in Fig. 26.1).
6. Sample size and power calculation: The protein concentration attribute is assumed to have a normal distribution. Sample size is chosen such that it provides a high percentage of passing ($\geq 90\%$) when the difference among compared group means is small (e.g., when $C = 0$ in Eq. 26.4), and low percentage of passing ($\leq 5\%$) when difference among compared group means is large (e.g., when $C = \text{HAC}$). Figure 26.3 illustrates the probability of passing the average equivalence test as a function of C. According to this assessment, sample size (n) is chosen to be 7 per interval and 21 samples for the batch. It should be noted that this sample size represents the number reportable values that should be generated for homogeneity assessment. Therefore, if the product volume in a DP container does not meet the test method's sample volume requirement, then sufficient number of DP containers should be pooled to generate stated number of reportable values.

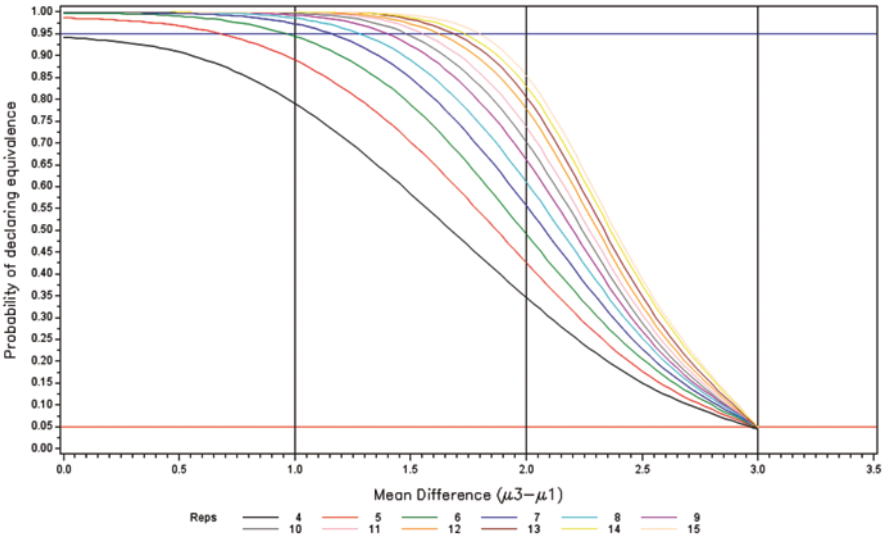

Fig. 26.3 Power curves with HAC = ±3 mcg/mL

Based on the computed probabilities, $n = 7$ is a reasonable sample size. The probability of passing equivalence with $C = 1$ is 97% and the probability of passing equivalence when means are all equal ($C = 0$) is greater than 99%. There does not appear to be much gained by using a greater sample size. The risk of falsely stating homogeneity is controlled at 4.8% based on a simulation of 100,000 iterations. The total sample size is $3 \times 7 = 21$ reportable values.

1. Samples are collected from each of the designated position (stage) of the fill and handled per procedure to preserve sample integrity.

26.3.2 Protocol Execution Phase

1. Collected samples from the batch are transferred to QC laboratory for testing.
2. Randomized testing schematic for testing the samples shown in Table 26.2 was applied created and test results were readied for data analysis.

Table 26.2 Example of original and randomized test order of samples

Original sample order		Randomized test order	
Sample ID	Sampling position	Sample ID	Sampling position
B-1	Beginning	E-6	End
B-2	Beginning	M-4	Middle
B-3	Beginning	B-5	Beginning
B-4	Beginning	B-3	Beginning
B-5	Beginning	E-2	End
B-6	Beginning	E-5	End
B-7	Beginning	B-6	Beginning
M-1	Middle	E-3	End
M-2	Middle	M-3	Middle
M-3	Middle	E-1	End
M-4	Middle	M-2	Middle
M-5	Middle	B-7	Beginning
M-6	Middle	E-4	End
M-7	Middle	B-4	Beginning
E-1	End	M-6	Middle
E-2	End	M-7	Middle
E-3	End	B-1	Beginning
E-4	End	M-5	Middle
E-5	End	B-2	Beginning
E-6	End	M-1	Middle
E-7	End	E-7	End

26.3.3 Report Phase

1. Protocol was executed without any deviations noted.
2. Protein concentration test results were analyzed using average equivalency approach. Figure 26.4 depicts test results from three sampling positions. Tables 26.3 and 26.4 tabulate summary statistics and the results from equivalency assessment between pair sample positions, respectively (Fig. 26.4).

1. Comparison of analysis results against HAC of protein concentration shows that the confidence intervals for the difference in means among sample positions are within the homogeneity AC.

These results demonstrate that the filling process is capable of producing homogeneous batches.

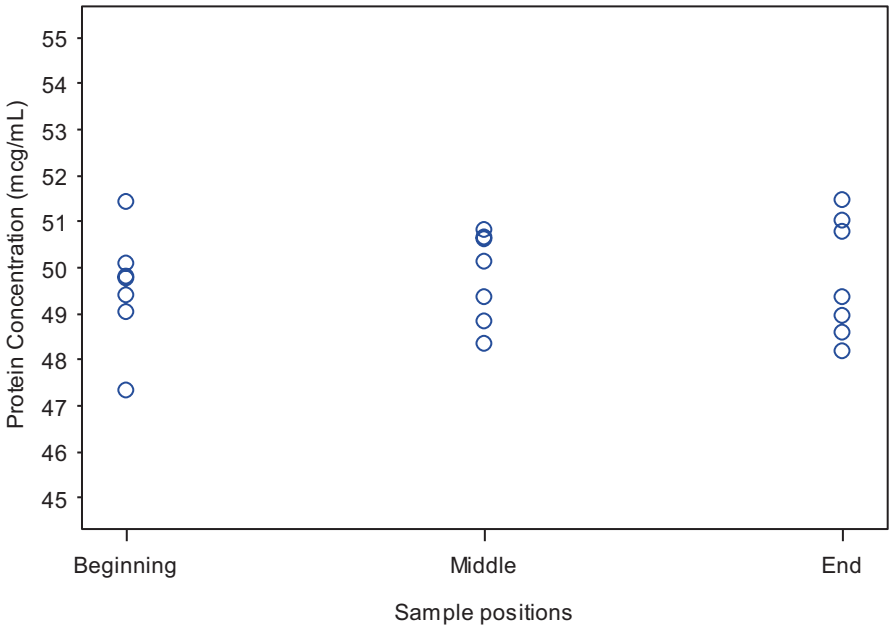

Fig. 26.4 Plot of simulated protein concentration test results

Table 26.3 Summary statistics of simulated protein concentration test results

Position	Number of observations	Minimum	Maximum	Mean	Standard deviation
Beginning	7	47.3406	51.4422	49.5495	1.2371
Middle	7	48.3572	50.7992	49.8212	0.9774
End	7	48.1729	51.4799	49.7644	1.3120

Table 26.4 Equivalency assessment among sample positions for the simulated protein concentration test results. Homogeneity acceptance criteria = ± 3.0 mcg/mL

Position	− Position	Average difference	Lower one-sided 95 % confidence limit of mean difference	Upper one-sided 95 % confidence limit of mean difference	Equivalency demonstrated? (Yes or no)
Beginning	Middle	−0.27170	−1.36933	0.82593	Yes
Beginning	End	−0.21486	−1.31249	0.88277	Yes
Middle	End	0.05684	−1.04079	1.15447	Yes

26.4 Summary

A stepwise approach for assessing homogeneity of DP batches during validation of a commercial biomanufacturing process was detailed with an illustration on a simulated dataset. Results clearly demonstrate the utility of the framework for robust assessment of the process capability to manufacture homogeneous DP and for generating a robust data package for regulatory filing purposes.

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Chapter 27
Application of Quality by Design Principles to the Drug Product Technology Transfer Process

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27.1 Introduction

The transfer of a manufacturing process from an existing site to another site is referred to as a technology transfer (TT). In the pharmaceutical industry, these transfers may occur between two clinical manufacturing sites (e.g., from early- to late-stage development); from a clinical manufacturing site to a commercial site (for product launch); or between two commercial sites. The transfer may be of the same scale (e.g., typical commercial to commercial site transfer) or an increase in scale (e.g., clinical to commercial scale). The receiving site may be an established facility or a greenfield facility, which may require additional process development. In a recent article (Thomas 2012), it was mentioned that the Roche/Genentech network conducted 10–28 TTs per year during 2010–2012. The reasons cited for the large number of transfers included retirement of older facilities to newly commissioned in-house manufacturing plants; cross-licensing for business continuity and flexibility; leveraging contract manufacturing operation (CMO) expertise; and for risk- and cost-mitigation purposes. In addition, with the rise in the global demand for pharmaceutical products, the number of TTs to emerging markets is increasing due to decreased costs and the desire for local control.

The level of complexity of the TT depends on the project phase. Transfer of a commercial product is subjected to more stringent requirements than an early-stage

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or research-product transfer. In this chapter, we focus on the transfer of a marketed sterile antibody drug product (DP) in a vial, from a licensed fill/finish commercial site to a second commercial site. We illustrate how a risk- and science-based approach incorporating elements of quality by design (QbD) can be applied to support the DP TT process. A general description of the different phases of the TT process is provided, and we describe the application of risk- and science-based approach during the phases of the TT process. We present case studies to illustrate the various components of the approach, and we discuss strategies to assess product comparability between lots produced at the donor and receiving sites.

27.2 Drug Product (DP) Process Flow

A typical DP process flowchart is shown in Fig. 27.1. The DP process begins with the receipt of the filtered drug substance (DS) bulk for storage (FBS). FBS is typically frozen to extend DS shelf life. The frozen FBS is thawed in a controlled manner and mixed to homogeneity. The bulk is then filtered into a tank where it may be pooled or diluted (if needed). Excess thawed bulk may be refrozen. The pooled bulk is sterile filtered and filled into sterilized and depyrogenated vials. For a liquid product, the filled vials are then fully stoppered. For a lyophilized product, the vials are partially stoppered and then inserted into the lyophilizer. After completion of the lyophilization cycle, the vials are fully stoppered in the lyophilizer. The stoppered vials are capped and sent for 100% inspection. Inspection may be done manually or through an automated system. The vials are then stored in bulk form at an appropriate temperature before being sent for labeling and secondary packaging.

The goal of the technology transfer activities is to transfer product and process knowledge between development and manufacturing, and between manufacturing sites to achieve product realization. This knowledge forms the basis for the manufacturing process, control strategy, process validation approach, and on-going continual improvement. The transfer should demonstrate that the transferred process is reproducible and will consistently produce a product that meets all in-process, release, and stability specifications, and is comparable to the product from the donor site. Traditionally, successful production of a statistically significant number of consecutive process validation (PV) lots (typically three) has been used to demonstrate control. Supportive studies and activities are needed to ensure that the validation lots are successful and to demonstrate process robustness (US Food and Drug Administration 2011).

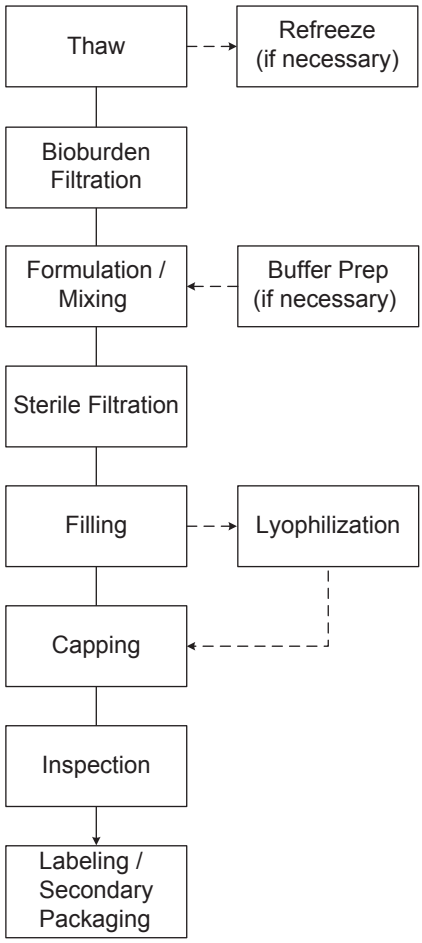


Fig. 27.1 Drug product process flow for large molecule in a vial

27.3 Technology Transfer (TT) Phases

Many important factors must be considered during a TT. For the receiving site, introduction of a new product may require new practices or equipment changes/additions. For multiproduct facilities, the impact of these changes to the existing product portfolio and the risk of cross-contamination must be determined. The potential impact to quality attributes of the transferred product due to equipment or process differences between the sites must to be understood, and mitigating studies must be performed prior to PV lots. Some of these issues may be addressed by leveraging information from the donor site. An assessment of the quality/analytical systems and resources is also required. To deal with these types of issues, a comprehensive TT plan must be established. The typical plan involves several phases, which are described as follows.

27.3.1 Initiation and Planning

A transfer-working team, which comprises members from both the donor and receiving sites, is established during the initial phase of the TT. A steering committee should be assigned to oversee the transfer and settle disputes. The functions represented in the transfer working team typically include process development, manufacturing, quality control, quality assurance, analytical, supply chain, validation, and regulatory. This ensures that all aspects critical to the successful licensure of the product (at the receiving site) and a successful lifecycle are adequately involved from the first to the last stages of the process.

During this initial phase, a master transfer plan (MTP) is created. The MTP outlines the critical milestones and activities for the project, identifies responsibilities among different functional groups, and defines success criteria for the project.

27.3.2 Documentation and Transfer

During this phase, knowledge transfer takes place between the donor and receiving sites. The donor site typically shares documentation relating to product and process specifications, prior regulatory submissions, validation information, raw material specifications, manufacturing recipes, and relevant standard operating procedures (SOPs). This ensures that all critical information related to the manufacture and licensure of the product is provided in the early phases of the transfer to enable the successful design of the process at the receiving site.

27.3.3 Quality Risk Management (QRM)

QRM is integrated into the TT process to evaluate the impact of transfer activities on the quality of both the product to be transferred and the existing products at the receiving site. The analysis includes the following elements:

1. Multiproduct control
2. Introduction of new equipment and changes to the donor site equipment or facility
3. Analysis of product-quality impact risks based on process and equipment differences for the product to be transferred (equipment differences may be associated with site practices or due to a change in scale between the donor and receiving sites)

The application of QRM during the TT, which will be discussed in greater detail later in this chapter, identifies high-risk items that require appropriate mitigation activities, including process studies, to ensure that risks are minimized.

27.3.4 Production of Batches

Process studies and other risk mitigation steps should be completed prior to the production of the process-validation (PV) batches. Risk mitigation may include production of at-scale technical or engineering batches. These batches provide an opportunity to make final adjustments to the process, check the accuracy of batch tickets, and to assess comparability by accelerated stability with product filled at the donor site.

Successful production of PV batches is a critical goal of the TT process. The PV batches demonstrate that the receiving site manufacturing process consistently maintains process parameters within established ranges, and produce DP that meets predefined acceptance criteria. The PV batches should span the entire range of batch sizes. A more extensive sampling plan should also be incorporated to demonstrate dose uniformity and consistency of composition and quality of the DP from the beginning to the end of the batch. These requirements are captured in the PV protocol. In addition, comparability and stability protocols are needed to demonstrate comparability between the products produced at the donor and receiving sites. Comparability testing strategies are discussed in the final section of this chapter.

27.3.5 Reports and Change Control

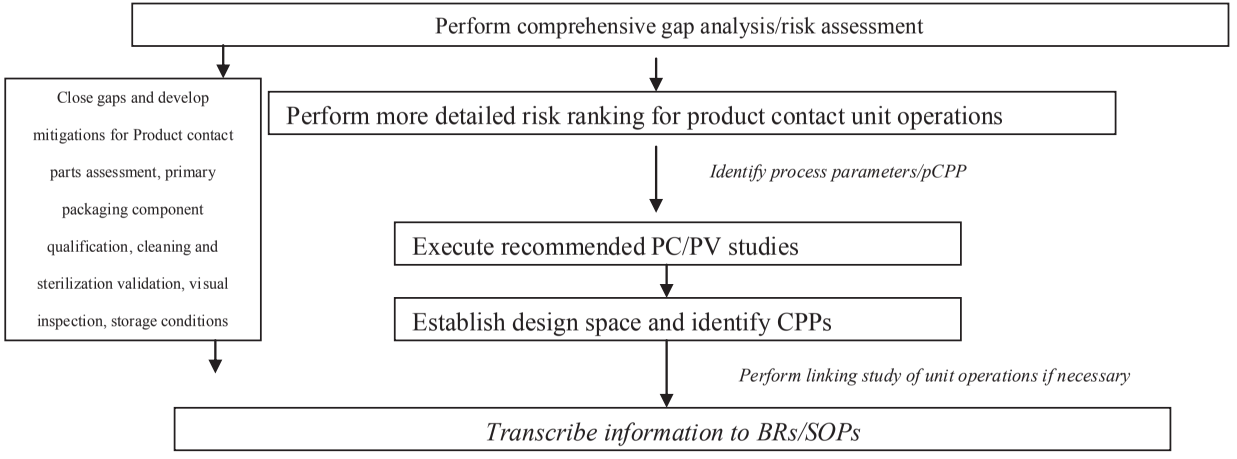
The transfer is considered complete when all the activities and deliverables defined in the MTP are accomplished and approved by the receiving site. These include the master batch records, risk-assessment reports, transfer-summary report, and manufacturing parameter specifications.

27.4 QRM Approach

To ingrain the concept of QbD into a TT, due diligence must be exercised during the different phases of the transfer in order to understand the risks and develop mitigations. The purpose of integrating quality risk management during TT is to ensure appropriate assessment, analysis, evaluation, control, review, and communication of quality-related risks associated with a process transfer. Figure 27.2 is a schematic diagram outlining the different risk management activities and the outcomes**.**

27.4.1 Risk Assessment

Risk identification is performed in several steps that recognize the complexity of process and/or facility changes that can occur during a process transfer. To ensure comprehensive risk identification, the following steps should be included:



pCPP – potential Critical Process Parameter

PC/PV – Process Characterization / Process Validation

CPP-- Critical Process Parameters

BRs/ SOPs – Batch Records / Standard Operating Procedures

Fig. 27.2 Schematic diagram of the phases of the risk assessment and gap analysis

1. Development of a process flow identifying each step of the process that will be assessed for risks
2. Develop a gap analysis that identifies process differences and changes between the donor site and the receiving site for each step defined in the process flow (process changes include location- and facility-driven process modifications based on current facility design, such as isolators, filtration process changes, filler types, freeze/thaw cycles, etc.)
3. Identification of potential hazards associated with each of the identified gaps, and the given process and/or facility operations (such as utilities, equipment, automation, and procedural changes) and the possible consequences of those hazards (harm) or failure modes to product quality

27.4.2 Gap Analysis

A detailed gap analysis is performed to identify process and manufacturing differences in operations between the donor and receiving sites. The gap analysis is comprehensive, and includes the following actions:

1. Address primary packaging components: ensuring the procurement of components from supplier sites which have been already certified by the donor site to ensure component quality; component fit to the filling line and ensure procurement of any change parts necessary to ensure smooth vial flow, minimal glass particulate formation; qualification of stopper wash procedures to ensure particle free stoppers; vial sealing and ensuring of container closure integrity.
2. Evaluate product contact materials: a thorough evaluation of product contact materials at the receiving site may be done to ensure that the materials are (a) compatible with the product and (b) do not generate leachables/extractables that affect the safety and quality of the final DP. The evaluation must focus on tubing, hoses, gaskets, and valve diaphragms. In some cases, a change may be necessary to a material with a more suitable extractable profile (e.g., switch from sulfur-cured EPDM to peroxide-cured EPDM, or moving to a Teflon®-EPDM type gaskets where the product contact side is Teflon). In cases where a change cannot be made, a risk-based evaluation may be performed to estimate the potential cumulative (throughout the entire process) extractable levels in the final dosage configuration. Such a risk-based assessment may also be supplemented by evaluating first-filled DP vials for leachable levels from engineering runs. Situations where the leachable level is deemed to be high may require additional flushing of solution or a toxicological assessment of the specific extractables to ensure safety of the DP. Finally, consideration must be given to the impact of differences in cleaning and sterilization conditions of these product contact parts on contamination levels (e.g., leachables) or part performance.
3. Assess storage procedures and conditions for cryovessels and compounding tanks; light exposure conditions, as compared with the donor site; hold times (at different temperatures) at different steps of the process.

4. Evaluate sampling points and limits to ensure the highest quality of the final DP. For example, bioburden and endotoxin sampling must be done at various stages of the process and appropriate limits established to ensure low probability of microbial contamination in the final product. Such sampling may also be performed during engineering runs to ensure that there are no parts of the manufacturing process that contribute to microbial contamination.
5. Visual inspection alignment and particle assessment. A comprehensive visual inspection program must be initiated in the initial phases of the filling transfer process. A defect library (either physical or pictures) may be shared with the receiving site and a review of the inspection station (e.g., light intensity, background) performed. On-site training may be provided with trained inspectors from the donor site. During engineering runs, samples must also be evaluated for particle load. In cases where the particle load is deemed high, appropriate mitigation activities must be put in place to ensure particle levels are brought down to acceptable levels. In some cases, particle characterization may be necessary to identify the source of the particle and aid in the troubleshooting of the particles resulting in the final vial.
6. Perform a detailed analysis of the gaps between the sites for process aspects (freeze, thaw, mixing, filtration, filling, lyophilization). The analysis provides the normal operating ranges for each unit operation parameter. Based on this information, a second risk assessment is performed to determine the necessary studies to characterize the unit operations. These studies are used to develop a robust design space for each unit operations and may include studies to support manufacturing excursion scenarios. The additional risk assessment and studies are described later in this chapter.

For identified gaps with high risk, recommended mitigations to close the gaps are proposed. The mitigations may include additional studies, improvements to detection and control systems at the receiving site, and modification of manufacturing procedures. Prior to the launch of the commercial process, all risks must be reduced to as low as reasonably practicable, and a robust understanding of the process at the receiving site must be demonstrated.

27.5 Quality by Design (QbD) Approach

The QRM activities identify high-risk items for the TT. Implementation of a QbD approach is useful in addressing process-related risks resulting from the TT. Application of QbD to biological manufacturing has been discussed in several review articles (Rathore and Winkle 2009; Martin-Moe et al. 2011) and presentations (Lim 2010; McKnight 2010). In this chapter, the approach is applied to the primary product contact unit operations only (see Fig. 27.1). Secondary operations and equipment are typically validated at the receiving site, and no additional work is necessary. These secondary operations (see Fig. 27.3) include primary compo-

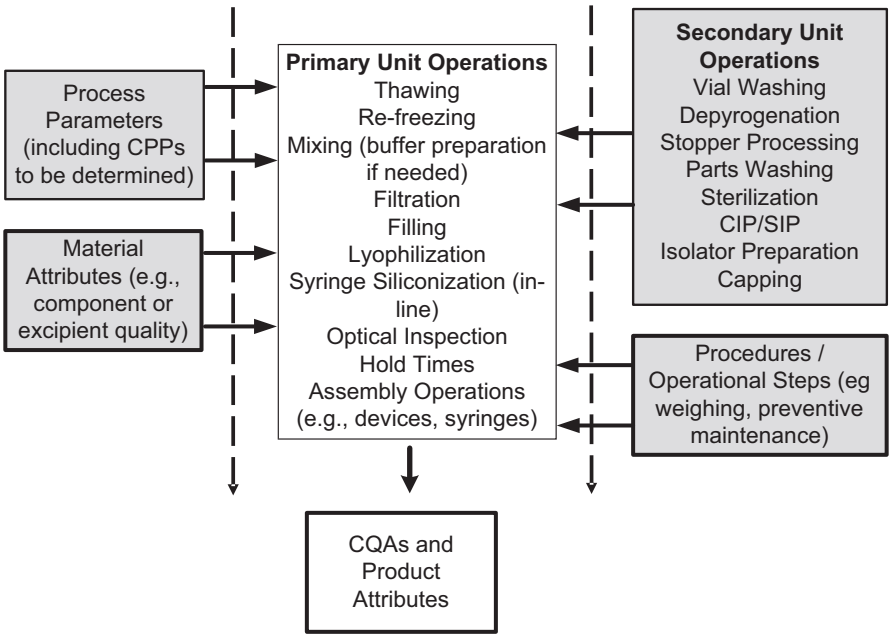


Fig. 27.3 Process boundaries for primary and secondary unit operations

nent and fill equipment preparation, cleaning and sterilization operations, vial handling operations, and plant utilities such as water for injection (WFI) and heating, ventilation and air conditioning (HVAC) systems, and media fills. Additionally, these unit operations are also generally product independent and instead are driven by specific equipment and component considerations. We also assume that the transfer of analytical procedures for in-process or release testing has been completed.

The impact of secondary unit operations conditions on product quality should also be considered. For example, stopper-processing conditions and filter-sterilization conditions should be assessed for their potential impact on product leachables and stopper moisture levels for lyophilized products. If isolator technology with vaporized hydrogen peroxide for decontamination is employed, then the impact of residual peroxide in the atmosphere should be studied, as it may lead to oxidation of the protein and/or excipients.

The basic approach is as follows. For each primary unit operation, a risk ranking and filtering (RRF) exercise is initially performed to identify potential critical process parameters (pCPPs) and guide the study design. pCPPs are unit operation parameters that must be studied to determine whether they are critical. A list of all of the unit operation process parameters is compiled. A risk assessment is performed and rationale is provided to identify those parameters that when varied across a

defined range, could impact CQAs or key performance indicators (KPIs). These recommended process studies are used to determine acceptable univariate and/or multivariate process parameter operating ranges for each unit operation. Operation within this defined space should result in acceptable product quality attributes. This information is also used to identify critical process parameters (CPPs), based on the quantitative assessment of the impact on the CQAs. Finally, strategies are provided to determine whether further studies are needed to link multiple unit operations or to link formulations with unit operations to ensure robustness of the end-to-end process.

27.5.1 Tool to Identify Potential Critical Process Parameters (pCPPs)

Leveraging previous knowledge from the donating site, scientific understanding, platform knowledge, and experiences from the receiving site, RRF is performed for each unit operation to determine pCPPs that may have an impact on CQAs and KPIs. Since each parameter may eventually impact CQAs at an extreme range, the risk assessment should be made based on a predefined characterization operating range for that parameter. This characterization range should be at least as wide as the normal manufacturing operating range. When setting the characterized range, consideration should be given to typical excursions and to the operating space at the receiving site and potentially, other sites in the company's DP manufacturing network. The results of the assessment are formally documented in a report.

Appropriate subject matter experts from both donor and receiving sites identify a list of process parameters associated with each unit operation. Using a RRF tool (McKnight 2010), each process parameter is assessed for its (1) main effect and (2) interaction effect with other process parameters against predefined responses. Two types of responses are considered: (1) product quality attribute responses, which include CQAs that can impact product safety, or efficacy, and (2) other quality attributes and process attributes, which generally relate to process performance. The main effect score assesses the degree of impact of a given process parameter on all of the responses, independent of the other process parameters. The interaction effect score assesses the potential that the interaction of two or more factors that are simultaneously varied results in a greater (or lesser) response than the sum of each factor varied individually. Score values are assigned for the main and interaction effects based on set criteria (see Table 27.1 and 27.2). The CQAs are more heavily weighted than noncritical or process attributes. The product of the two scores is used to identify the pCPPs and the study approach. There are three possibilities for types of studies: (1) no study required, (2) univariate study, or (3) multivariate study as depicted in Table 27.3 and Fig. 27.4. Process parameters may be upgraded to a higher level study (i.e., univariate to multivariate) if appropriate. A product specific interaction matrix can then be generated (see Fig. 27.5) that summarizes the linkage between unit operations and the potentially impacted CQAs. This matrix is useful when preparing study protocols.

Table 27.1 pCPP risk ranking and filtering (RRF) impact scoring

Impact description	Rank	
	Critical quality attribute (CQA)	Noncritical product quality attribute or process Attribute
No impact	1	1
Minor impact	4	2
Major impact	8	4

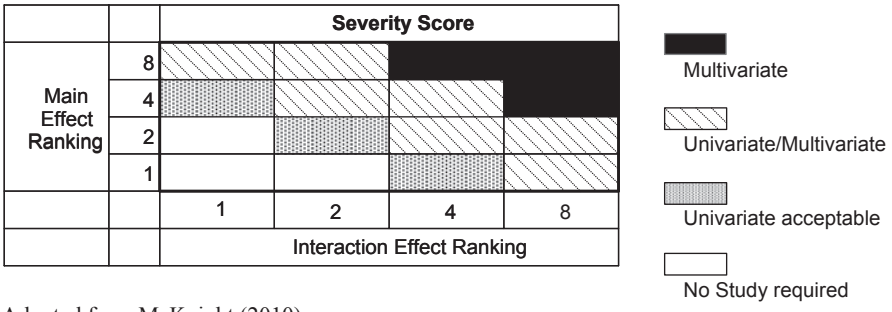
Table 27.2 pCPP risk ranking and filtering impact scoring

Impact Description	Definition
No impact	Effect causes variation in process output which is not expected to be detectable (e.g., no effect or within assay variability)
Minor impact	Effect causes variation in process output which is expected to be within acceptable range
Major impact	Effect causes variation in process output which is expected to be outside acceptable range

Effect is considered for variation of parameter across a proposed design space range

Table 27.3 pCPP risk ranking filtering decision matrix

Severity Score	Experimental strategy
≥32	Multivariate study
8–16	Multivariate, or univariate with justification
4	Univariate acceptable
≤2	No additional study required



Adopted from McKnight (2010)

Fig. 27.4 Risk ranking and filtering (RRF) tool to determine potential critical process parameters (pCPPs)

	Unit Operation / Hold Steps							
CQA	Formulation	Freeze / Thaw	Mixing / Dilution	Filtration	Filling	Inspection	Storage	Shipping
Aggregates								
Fragments								
Deamidation								
Oxidation								
Particles (Visible/ SubVisible)								
Composition								
Process Impurities								
Sterility / Endotoxin								

Interaction

No Interaction

Fig. 27.5 Product interaction matrix indicating critical quality attributes potentially impacted by each unit operation

27.5.2 Process Studies to Define Acceptable Operating Ranges

After identification of the pCPPs, univariate or multivariate studies are conducted to determine the impact of the process parameters on output variables. Univariate studies may be performed for process parameters that do not interact with each other, or to capture typical manufacturing deviations where a single parameter value is outside of the established multivariate range. An example would be to support transient pressure deviations during lyophilization. Both small and at-scale studies may be used. DP processing is designed to maintain the quality attributes of the active pharmaceutical ingredient. The process steps involve mainly physical manipulation rather than chemically or biologically modification of the molecule. Optimization of the process parameters is often unnecessary, with many process steps employing generic operating conditions that are product independent. Therefore, for these operations, exact scale-down models are not required when conducting small-scale studies. Instead, models that mimic the type of stresses that occurs at-scale can be used.

The DP process is generally well understood, with known worst-case operating conditions. Therefore, a design of experiment approach is not needed, which reduces the number of studies required to characterize the manufacturing acceptable range. However, there may be different worst-case conditions depending on the impacted quality attribute. For example, for the mixing/dilution unit operation, the worst-case condition for attributes related to homogeneity is the largest bulk volume, mixed at the lowest mixing speed for the shortest time. However, the worst-case condition for product variant attributes is the opposite extreme condition of smallest bulk volume mixed at the fastest mixing speed for the longest time. The entire characterization range can then be established for this unit operation just by conducting these two studies.

Table 27.4 Examples of unit operation supportive studies

Unit Operation	Study	Scale/Material
Freeze/thaw	Cycle verification for completion of F/T	At-scale/buffer
	Number of freeze/Thaw cycles	At scale and small/active
	Impact of freeze rate	Small/active
	Impact of thaw recirculation stresses and thaw rate	Small/active
Dilution/pooling	Mixing homogeneity	At-scale/surrogate
	Impact of mixing stresses	Small/active
Filtration	Microbial retention	Small/active
	Filter compatibility	Small/active
	Filter capacity	Small/active
	Number of refiltrations	At-scale/active
Filling	Fill weight validation	At-scale/active or surrogate
	Filler compatibility	Small/active
Isolator	VHP spiking studies	Small/active
	VHP aeration and uptake studies	At-scale/active
Lyophilization	Impact of freeze rate	Small/active
	Impact of drying conditions	At-scale/active
Automated inspection	Impact of light exposure	Small/active

Table 27.4 lists typical studies that are conducted for each of the primary unit operations. A mixture of small-scale and at-scale studies is often employed. Some of the at-scale studies may be performed with an appropriate surrogate. For example, for many protein formulations, the corresponding buffer takes longer to freeze or thaw than the active solution. Therefore, buffer rather than active bulk may be used to verify that the freeze or thaw cycles are adequate to ensure complete freeze and thaw. Appropriate use of surrogates minimizes the demand for active material required for the studies.

Other supportive studies that should be considered are as follows:

1. Ambient temperature hold studies using containers with the same material of construction as the process vessels
2. Light studies, which subject unprotected product (in disposable bags or clear vials) to light exposure at maximum intensity and time encountered in the manufacturing facility
3. Elevated frozen temperature studies to address a common deviation of exposure of the frozen bulk substance (FBS) to conditions above the maximum frozen storage temperature
4. Low temperature DP studies to address a common deviation of partial freezing of liquid DP during storage or transport
5. Primary container leachable studies if this primary container component or stopper sterilization conditions are changed

These studies may already be completed, but should be evaluated to determine if they apply to the receiving site.

27.5.3 Identification of Critical Process Parameters (CPP)

The results of the process studies are used to support the identification of CPPs, which are process parameters whose variability have an impact on a CQA and therefore should be monitored or controlled to ensure the process produces the desired quality (International Conference on Harmonisation, Q8 Pharmaceutical Development 2009). The process for identifying CPPs can be based on a modified failure modes and effects analysis (FMEA). (see ICH Guidance for Industry, Q9, Quality Risk Management 2006). For each potential failure mode, three components are rated: severity (S), occurrence (O), and detection (D). Criteria are defined to set a numerical ranking for each category. The identification of a parameter as a CPP is based on the severity score resulting from the assessment of deviations of the parameter from the defined operating or characterized ranges on product quality. Additionally, manufacturing experience and existing controls is used as a filter to ensure that the severity analysis is performed only for those failure modes which fall in the realm of reasonable manufacturing excursions. This ensures that the CPP determination focuses on those parameters that have the most potential to affect the product. Product studies, along with previous knowledge of plant operations, are used to determine the scores.

FMEA participants should include subject matter experts from manufacturing, quality assurance, quality control, and pharmaceutical development. Focus should be placed on failure modes associated with potential deviations of process parameters. Occurrence scoring is initially performed for each failure mode. For CPP determination, only failure modes that have moderate occurrence (score of 4 or 6 in Table 27.7) are considered. The risk assessment (RA) participants should consider the experiences of the entire DP network when rating the occurrence score to minimize the potential of site specific CPPs for unit operations that use identical equipment and manufacturing parameter ranges. Additionally, occurrence scores for many drug product unit operations can be pooled across different products since these are common across the drug products. Severity scoring is performed for the failure modes with moderate occurrence. For this example, parameters with a severity score of 8 or greater would be considered CPPs. A noted exception is for sterility impacting filtration parameters (pressure/flow rate, time) which are considered default CPPs since exceeding the validated ranges for these could typically cause lot rejection. An example of the modified FMEA for the thaw unit operation is shown in Table 27.5. The criteria for the S, O, and D scores are provided in Table 27.6, 27.7 and 27.8.

Table 27.5 Failure mode effects analysis for refreeze unit operation (Thaw FMEA)

Process parameter	Potential failure mode	Potential failure effect(s)	Severity	Rationale for severity score	Potential cause(s)	Occurrence	Current controls	Detection
Maximum number of freezes	Exceeded validated number of freeze/thaw cycles	Bulk product degradation	N/A	Additional stability testing may need to be performed to assess impact. Failure mode is the result of an unplanned event	Human error	2	Tag on tank lists number of freeze/thaw cycles, controls in ticket	2
	At limit of freeze-thaw cycles	Bulk product degradation	8	At-scale freeze-thaw studies have shown product impact	N/A	8	Tag on tank lists number of freeze/thaw cycles, controls in ticket	2
Recirculation duration	More than characterized range	Shear/Aggregation	4	Longer recirculation times have not shown impact on product quality	Human error equipment Equipment malfunction	2	Operator/verifier step	6
Recirculation duration	Less than time of 5.5 h for 120 L tank or 8.5 h for 300 L tank	Incomplete thaw and/or mixing	6	May necessiate additional recirculation time in order to achieve complete thaw/homogeneity (pH, concentration)	Human error quipment malfunction-pump not ready Equipment malfunction-pump breaks down	8	Verification of thaw completeness/homogeneity via spec scan Operator/verifier step	6
Silicone oil temperature	Higher than acceptable range	Thermal Degradation due to exposure to higher temperature	4	Product data with ambient thaw at 25 C (for SEC, IEC) and due to controls, product unlikely to exceed 25 C by more than 1 degree	Equipment/instrument malfunction	6	HiHi alarm at 26 C which causes valve to shut off HTF supply to tank	2
Silicone oil temperature	Lower than acceptable range	Inefficient heat transfer and Insufficient mixing resulting in incomplete thaw and/or nonhomogeneous solution	6	thaw will not be complete in time and extended recirc times will be needed	Equipment/instrument malfunction	2	System controls (alarms), hourly monitoring of product temperature	2

Table 27.6 Severity evaluation criteria

Effect	Criteria	Rank
Very high	Effect of parameter deviation causes definite impact to product quality (e.g., exceeding validated ranges for sterility impacting filtration parameters)	10
High	Effect of parameter deviation will probably cause impact on product quality (e.g., impact ratio is greater than 0.1). One of the following or both occur:	8
	Discrepancy is initiated and product may be assessed after supplemental testing, which may include accelerated stability	
	Significant procedural interventions may be required (e.g., clearance of vials from filling line when critical parameter on filling is violated)	
Moderate	Effect of parameter deviation potentially causes impact to product quality. One of the following or both occurs:	6
	Discrepancy is initiated and product may be assessed after supplemental t = 0 testing. In some cases, testing is not required if technical justification is available	
	Minor procedural adjustments may be required (e.g., extending recirculation time during thaw)	
Slight	Effect of parameter deviation is unlikely to impact product quality. Both of the following occur:	4
	No supplemental testing is required, but a memo may be issued to address the discrepancy and release the lot	
	No procedural adjustments are required	
Low/none	Effect of parameter deviation has no impact to product quality	2

Table 27.7 Occurrence evaluation criteria

Occurrence[a]	Effect	Rank
Very high	The parameter failure occurs a few (≥3) times a month	10
High	The parameter deviation/ failure occurs a few (≥3) times a year	8
Moderate	The parameter deviation/failure occurs ~ once every 1–2 years	6
Low	The parameter deviation/failure occurs ~ once every 2–5 years	4
Minimal	The parameter deviation/failure occurs less than once every 5 years	2

[a] Frequency of occurrence can be pooled across products because of generic unit operations for drug product manufacturing

An alternate method for determining CPPs may be used if the worst-case multivariate process studies outlined in Sect. 1.5.2 are performed. In this method, the change in the values of the impacted CQAs from the worst-case process studies is compared with the allowed variation of that CQA. An "impact ratio" is defined as follows (McKnight 2010):

$$\text{Impact Ratio} = \frac{\text{Effect Magnitude}}{\text{Allowed CQA Variation}} = \frac{\left|CQA_{\text{Stress}} - CQA_{\text{Control}}\right|}{CQA_{\text{Mean}} - CQA_{\text{Spec}}}$$

Table 27.8 Detection evaluation criteria

Detection	Criteria	Rank
None	This failure will not be detected with in-process or CofA testing	10
Low	In-process testing controls or monitoring will not detect this failure, but CofA testing will catch this failure	8
Moderate	In-process testing or monitoring will not catch this failure during the unit operation, and detection is delayed several downstream unit operations, but prior to CofA testing	6
High	The failure may or may not be detected by in-process controls or monitoring, but would definitely be detected in the next downstream operation	4
Very high	The failure can be immediately and readily detected by inspection, in-process testing or monitoring controls, prior to downstream unit operation	2

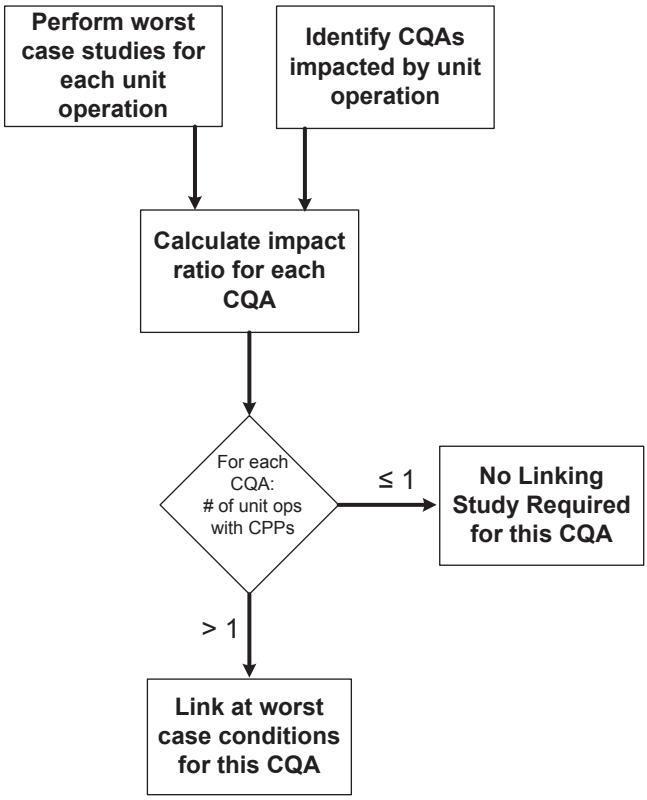
CofA Certificate of Analysis

where CQA_{Stress} is the resulting CQA test result for the stressed sample; CQA_{Control} is the resulting CQA test result for the un-stressed or control sample; CQA_{Mean} is the average value for the CQA from commercial batches; and CQA_{Spec} is the specification limit for the CQA. If desired, the specification can be narrowed to provide a safety margin.

If the impact ratio is greater than a prescribed value for any of the potentially impacted CQAs, then the parameter is a CPP. If all of the CQA impact ratios are less than the prescribed value, or the effect magnitudes are smaller than assay precision, then the parameter is not a CPP. A conservative value is 0.1, which represents an allowed variation of only 10% of the acceptable limit. The final value selected will need to be properly justified. Benchmarking tests may help with the value-selection process to ensure that the correct parameters are being identified. For attributes that are qualitative (e.g., sterility or appearance), parameters are considered CPPs if there is in an observed change to the CQA. Otherwise, the parameters are considered non-CPPs.

This approach provides a quantitative method to determine CPPs across the characterization range of the parameters based only on how severely the parameter impacts CQAs. Occurrence or detection is not considered.

The identified CPPs must have alarm limits and operating ranges within the established multivariate acceptable range. Any excursions outside this range must be tracked on a per batch basis through an established quality review process to ensure to reduce product impact or risk to patient safety. Additionally, a subset of relevant CPPs may also be tracked across batches as part of a process monitoring program to ensure batch–batch consistency. The extended process monitoring program also includes tracking and trending of CQAs (through Certificate of Analysis (CofA) testing) and applicable KPIs (e.g., filling yield, inspection defects) on a periodic basis. Trend limits can be identified for the relevant CPPs, CQAs, and KPIs based on appropriate statistical analysis of the historical data, and used to gauge the consistency and process performance aspects well before any anomalous trends could lead to an excursion outside established trend limits and characterized ranges.



CQAs – Critical Quality Attributes

CPPs – Critical process parameters

Fig. 27.6 Unit operation linkage decision tree

27.5.4 Unit Operation Linking Strategy

The unit operation development studies investigate the impact of individual unit operations on CQAs. However, an overall assessment is needed to determine the impact of all of the unit operations on CQAs. A conservative approach is to link together worst-case conditions for each unit operation. Several linking studies may be needed since worst-case conditions may differ depending on the CQA. Alternatively, an assessment can be made to determine if linkage studies are needed. We propose to conduct only linkage studies if multiple DP unit operations are found to have CPPs that impact the same CQA as determined by the impact ratio calculation. For CQAs that are impacted by only a single unit operation, the worst-case unit operation study is sufficient to define the characterized range, and no additional work is needed. Figure 27.6 illustrates the decision process.

The linkage studies are conducted with identified CPPs at their worst-case settings in the characterized range for the particular pCQA. Other parameters may be run at target. Unit operations that do not impact the CQA are run at the target parameter conditions or, if feasible, not included in the linkage study. If more than one CQA is impacted by multiple unit operations, then multiple linkage studies may be needed unless the worst case settings parameter settings for the particular CQA are the same. With proper justification, a theoretical calculation of the cumulative impact of multiple unit operations may be performed in lieu of actual linkage studies.

After the linkage study is conducted, the measured value of the CQA from the linkage study must be within the CQA acceptance criteria. If the resulting value is outside the acceptance criteria, then the characterized range for the impacted unit operations will need to be reduced or restricted.

27.6 Case Studies

In this section, we present case studies to illustrate the implementation of the risk and science-based approach to the TT of a commercial product from a donor site to a receiving site. These examples will illustrate the various steps from the gap analysis to CPP determination. We will not consider overall risk assessments including secondary unit operations and cleaning validation. We will also present case studies around the filling and lyophilization unit operations.

27.6.1 Example of Gap Analysis and Risk Assessment

As discussed in the section above, the following operations/components are in scope for the gap analysis: primary unit operations, primary packaging components, product contact materials, sampling, cleaning/sterilization procedures that may impact leachables, storage conditions and hold times, and visual inspection assessment. An example of a gap analysis encompassing the thaw, pooling and filling primary unit operations is provided in Table 27.9.

Following the completion of the gap analysis, an assessment of the risks associated with the gaps is made and associated mitigation plans are developed (see Table 27.9). In the example shown in Table 27.9, the donor site employs a different thaw skid, mixing impeller system and filling system than the receiving site. The thaw cycle at the donor site employed product temperature feedback to control the delivery of heat transfer fluid to the tank jacket and fins. At the receiving site, no feedback was used. Hence, cycle development/characterization studies had to be performed to develop a thaw cycle for the receiving site skid.

For the dilution operation, a top-mounted mixer was used at the donor site, which is generally known to impart a low shear stress to the antibody solutions, while a bottom mounted mixer was used at the receiving site. Protein solutions are susceptible to particulate formation when mixed for extended times with bottom mounted mixers (Ishikawa and Kobayashi 2010). Homogeneity studies, air entrainment studies, and

Table 27.9 Example of risk and gap analysis, and remediation Plan for several primary unit operations

Unit operation gap assessment	Donor site	Expected parameter at receiving site	Gap description and remediation plan
Bulk thaw of cryovessel			
Environmental conditions	Class D	Cold room	Perform media fill/bioburden study
Thaw skid	Custom skid	Custom skid	Skids are different. Skid qualification needed. Perform thaw completion study
Thaw cycle time			No gap
HTF temperature			No gap
Thaw cycle environment temperature conditions	Ambient	2–8°C	Different temperature conditions, but should not impact thaw—since HTF is controlled at similar temperatures at both sites
Recirculation time (hrs)			No gap
Recirculation flow rate (L/min)			No gap
Postthaw storage			No gap
Bulk thaw in-process testing			No gap
Pooling			
Environmental conditions for pooling tank	Class C	Class C	No gap
Gas used for tank overlay during transfer? (Y/N)	N	N	No gap
Motive force	Pressure	Pressure	No gap
Receiving vessel size	600 L	300 L	No impact except for batch size
Receiving vessel prep	CIP, SIP	CIP, SIP	No gap
Max number of tanks pooled	3	3	No impact except for batch size
Mixer type and Size	A310 top mounted, 10”	Bottom mounted mixer	Perform mixing studies to characterize mixing speed and time ranges. The studies need to be conducted to assess a) Prevent air entrainment at low batch size b) Ensure homogeneity at maximum batch size c) Assess impact of mixing conditions on chemical and physical stability
Mixing Speed Range	125–135 rpm	TBD	
Mixing Time Range	30–90 min	TBD	
Min–max batch size	150–600	60–250	
Filtration during transfer from F/T to pooling vessel?	0.22 µm Millidisk 40	0.22 µm Millidisk 40	No gap
Bulk pooling in-process testing			No gap

Table 27.9 (continued)

Unit operation gap assessment	Donor site	Expected parameter at receiving site	Gap description and remediation plan
Filling			
Environmental requirements	Grade A classified area	Grade A classified area	No gap
Gas used for tank overlay during transfer	Nitrogen	Nitrogen	No gap
Holding tank process temperature	2–8 C	2–8 C	No gap
Holding/filling vessel size and MOC	300 L, 600 L; 316 L SS	300 L, 316 L SS	No gap
Expiration time in holding tank	48 h from beginning of filtration	TBD	Media hold study for microbial control must be established through media fills
Target fill weight	Filed fill weight and alert/action limits	TBD	Lower and upper limits must be preserved since lyophilized product to ensure correct dosage
IPC	sterility samples; fill weight once every 10 min	sterility samples; in-process weight checker (5% of vials)	Receiving site has higher degree of fill weight checking
Maximum fill time	18 h	48 h	Microbial retention testing of filter must support longer fill time. Media fills must support longer fill time
Holding tank process temperature during filling	2–8 C	2–8 C	No gap
Batch size—min–max weight	60–275 kg	60–250 kg	Receiving site must validate min/max batch size during validation runs
Max batch size (no of units)	14,924	13,860	Receiving site must validate min/max batch size during validation runs
Filler type	8 head-rolling diaphragms	4 head time–pressure fillers	Perform engineering run studies to determine whether established fill weight target and ranges are feasible
Max fill speed	114 vpm	TBD	Will be determined during fill line PQ

product impact development/characterization studies are required. Finally, the receiving site uses a time–pressure (T/P) type filler as opposed to a rolling diaphragm filler at the donor site (see example in 1.7.1). The time–pressure filler is gentler on the product but may have a different fill weight precision than the rolling diaphragm filler. Fill weight and product impact characterization/validation studies would be needed.

27.7 Case Studies for Unit Operations

27.7.1 Filling Example

We discuss a scenario where the process at the donor site used a rolling diaphragm filler, while at the receiving site, a T/P filling system was used. A rolling diaphragm filler accomplishes filling by the mechanical movement of a piston (which is attached to a diaphragm) inside a cylinder, while a T/P filling system accomplishes the task of filling containers by using pressurized nitrogen and gravity as a motive force to push formulated DP through a precision-metering orifice, tubing, and a dispensing needle.

In a T/P filling system, a surge tank is employed to isolate the bulk storage tank from the filling system and to provide a small volume to which precise pressure control can be asserted. This surge tank pressure is controlled to a partial atmosphere of nitrogen pressure. Each outlet from the surge tank has a dedicated precision orifice that serves to provide backpressure during filling and to minimize the effects of the tubing on flow rate. A pinch valve located after the orifice starts and stops product flow. Temperature and pressure sensors are used to provide data to the control system for temperature and pressure compensation.

Several process parameters, relevant to T/P technology that could impact product quality attributes were identified: deviations in product temperature or surge tank pressure that could affect fill weight (dose accuracy) or any process parameters that could affect quality attributes traceable to the stresses of pressure and flow. The forming of bubbles and splashing upon delivery to the vial are optimized during screening and selection of the tubing kit (orifice, nozzle, tubing) and is typically not subject to variation during normal production. Machine speed, which is typically considered a process parameter affecting vial filler performance, was excluded from the responses because in a T/P filler, product-flow rate through the filling system is a function of the tubing kit and surge tank nitrogen pressure, and it is not linked to machine speed.

To evaluate the type of studies that need to be performed to characterize the filling of the product in the T/P filling system, a RRF assessment was performed on each process parameter. Table 27.10 shows the assessment for one of the process parameters (surge tank pressure). The identified pCPPs for T/P filling are shown in Table 27.11.

Table 27.10 Time pressure filler risk ranking and filtering (RRF)

Process Parameter	Process Outputs Affected	Rationale	Potential Interaction Partners	Rationale for interaction parameters	Recommended Characterization Study
Surge tank nitrogen pressure	Fill weight	The impact is very minimal if compensation is adequate but, some impact should be anticipated	Product temperature at orifice	Temperature change may have a moderate additive effect on fill weight	Multivariate study of temperature and pressure design space
		The impact is minimal if compensation is adequate but, some impact should be anticipated	Interruption time	Interruption time alone has minor impact since protein solution is low concentration and is not susceptible to nozzle clogging. Temperature after interruption is scored separately	None required
Surge tank nitrogen pressure	Product variants	Stress on product will increase slightly with increasing pressure	Product temperature at orifice	No expected interaction	Univariate stress test of surge tank pressure variation and/or multiple recirculation tests
		Stress on product will increase slightly with increasing pressure	Interruption time	No expected interaction	None required

Small-scale product impact studies and at-scale fill weight studies were performed to determine the impact of the pCPPs on the CQAs listed in Table 27.10. The impact ratio method was used to determine CPPs (see 1.5.3). For the product impact study, bulk solution was subjected to 20 passes at the highest surge tank pressure. Aggregation due to shear stresses from the T/P filler was not observed compared to a control. However surge tank pressure, interruption time, and orifice temperature were determined to be CPPs because of the significant impact on fill

Table 27.11 Potential critical process parameters (pCPPs) and impacted critical quality attributes for filling operation with time/pressure fillers

PCPPs	Affected CQA
Surge tank pressure	Size variants, fill weight
Orifice temperature	Charge variants, size variants, fill weight
Interruption time	Charge variants, size variants, fill weight

weight. Controls were put in place (via automation and SOP) to ensure that these parameters were strictly monitored during manufacturing and any excursions outside the design space range resulted in the appropriate action to reject a certain quantity of vials.

During another TT, both the donor and receiving site used piston pump fillers, but from different suppliers. Piston pumps have a tendency to cause shear and particulate problems with certain protein products. A risk-based approach was taken where the piston-body clearance was compared and the number of strokes per fill were compared across the two piston pumps; the risk was determined to be low–medium. To supplement this evaluation, product quality and particulates were also examined during engineering runs with DP prior to proceeding for validation batches.

27.7.2 Lyophilization Example

During a TT of a lyophilized DP, differences in freeze-dryer equipment must be evaluated for impact on the DP. A review of the freeze-dryer's operational qualification documentation including shelf fluid heating/cooling rates, shelf temperature uniformity, and condenser load tests is performed to ensure that this matches or exceeds the requirements of the process. In the following sections, we discuss a few additional studies that are executed as part of a TT of a lyophilized DP to ensure that quality is built into the lyophilization process at the receiving site.

27.7.2.1 Lyophilization Equipment Runs

Prior to performing development runs with product, initial studies were performed with vials filled with water running the proposed product lyophilization cycle in order to assess lyophilizer capability. In general, water will sublimate more quickly than an active product and will serve as a worst case test for lyophilization capacity. Some of the important considerations are listed below:

- Verify the uniformity of ice on the condenser coils after the run
- Verify vials for residual water
- Ensure that the shelf inlet temperature (controlling resistance temperature detector (RTD)) is within a prespecified range of the setpoint during the phases of peak drying and cooling

- Ensure that the pressure is within a prespecified range of the set point during primary and secondary drying
- Ensure that the maximum difference between shelf inlet and outlet temperatures is within product allowable limits during the phase of peak cooling demand (typically during freeze ramp when vials undergo nucleation)
- All ramps must hold linear for the duration (i.e., ramp to freeze and ramp to drying)
- Evaluate Pirani gauge/pressure rise measurement data to ensure that these can be used during characterization runs
- The highest condenser temperature recorded should be at least 5 °C lower than the dew point during drying to ensure that even under aggressive sublimation conditions, the refrigeration circuit is able to maintain temperatures in the condenser that are low enough to maintain pressure control and far from the edge of failure
- Visual verification during unloading confirms complete stoppering without vial breakage
- Additionally, power outage simulation studies must be conducted to ensure that appropriate pressure and temperature control is maintained, and that the correct valve sequencing occurs during a power transition

27.7.2.2 Product Characterization Runs

To evaluate the type of studies that need to be performed to characterize the lyophilization process, a RRF assessment is performed on each process parameter. Table 27.12 shows the assessment for one of the process parameters (primary drying pressure). The identified pCPPs for lyophilization are shown in Table 27.13.

For lyophilization, at-scale characterization studies using the actual commercial lyophilizer are recommended. The studies are used to understand the effect of varying process parameters (shelf temperature, chamber pressure, drying durations) on product attributes and to develop acceptable operating ranges around the target cycle.

During these runs, extensive mapping of the lyophilizer shelves is performed with product vials to ensure that the critical attribute of residual moisture is uniform throughout the chamber and meets specifications. The product is also placed on stability, and compared to DP from the donor site to demonstrate comparability (see Sect. 1.8). During the at-scale characterization runs, nonroutine sampling is performed during different unit operations, for example during the thawing, mixing/pooling process, and samples from filling.

In addition, small-scale studies are performed to assess the impact of potential manufacturing excursion on product attributes. For example, a temporary loss of pressure control leading to transient pressure spikes due to equipment issues such as inadequate condenser capacity or vacuum pump failure is a typical excursion. A worst-case small-scale study is performed. where pressure spikes are introduced at the beginning of the drying phase (when product is still susceptible to collapse due to the presence of significant water as ice, and lower collapse temperature dur-

Table 27.12 Lyophilization risk ranking and filtering (RRF)

Process parameter	Process outputs affected	Rationale	Potential interaction partners	Rationale for interaction parameters	Recommended characterization study
Chamber pressure during primary drying	Moisture Content	High chamber pressure may cause elevated cake temperatures leading to partial collapse and higher moisture content. Low pressure may result in adequate drying rate during primary drying which may affect secondary drying performance	Primary drying shelf temperature and duration and secondary drying parameters	Primary drying temperature impacts sublimation performance. Secondary drying conditions impact final moisture	Perform multivariate characterization with primary drying shelf temperature (high pressure/temperature, low pressure/temperature) and secondary drying parameters at full-scale Optional: Perform similar study at small-scale, univariate study to support higher pressure variations at small-scale
	Appearance and reconstitution time	High chamber pressure may cause elevated cake temperatures leading to partial collapse that may impact appearance. Reconstitution time may be longer if partial collapse	Primary drying shelf temperature and duration and secondary drying parameters		
	Product variant attributes	Product variant attributes may be dependent on final moisture content especially on stability	Primary drying shelf temperature and duration and secondary drying parameters		

Table 27.13 Potential critical process parameters (pCPPs) and impacted critical quality attributes for lyophilization unit operation

Process parameters	Impacted quality attribute
Primary drying temperature	Residual moisture, stability, charge and size variants
Primary drying chamber pressure	
Secondary drying chamber pressure	
primary drying chamber pressure	
Freeze ramp rate	
Freeze hold duration	
Primary drying duration	
Secondary drying duration	
Preaeration pressure	Reconstitution time
Stoppering force	Container closure

ing this phase) as well as when the product temperatures started ramping up to the secondary drying temperature (when the cake resistance is highest resulting in a lower rate of sublimation cooling, and the drying front is close to the heating surface). This effectively brackets the drying duration and exposes the product to aggressive heat-transfer conditions during points in the process when the product is most susceptible to failure. During this small-scale study, the product temperatures are monitored to ensure that they are still below the collapse temperature during the temporary pressure spikes. The DP is subjected to extensive moisture testing at the initial timepoint. Stability testing is also performed to ensure all shelf life specifications are met.

27.8 Comparability Testing During Biologics Drug Product Technology Transfers

The previous section described the risk mitigation activities to ensure that successful PV batches will be made with DP that meet all product quality specifications and is comparable to DP from the donor site. In this section, we discuss comparability strategies for a clinical to commercial transfer and a commercial to commercial transfer.

27.8.1 Clinical to Commercial Technology Transfers

Data from pivotal clinical studies have to be submitted during licensure applications (either BLA or MAA) to health authorities for marketing approval. These applications require that the sponsor use material that has been generated using the final locked commercial process (DS and DP) and that the material is representative of

the commercial lots that would be marketed. Since there are significant challenges to introduce changes during late-stage development, it is highly recommended that material from potential launch sites be used to support on-going pivotal trials depending on feasibility of such an approach in the manufacturing network. Typically, however, clinical material is made at a representative scale that may or may not be the final commercial scale or plant. During late-stage development, it becomes important to initiate a TT for both DS (out of scope for this chapter) and DP (discussed below) between clinical sites and commercial facilities. A comparability testing strategy is put in place that is not just an analytical exercise, but also contains a comprehensive data package including risk assessments that are now an essential part of the TT strategy. Representative analytical characterization includes methods that are based on a thorough understanding of protein instability during the DP fill/finish operation and usually include ion exchange and size exclusion high-performance liquid chromatography (IE-HPLC and SE-HPLC) to characterize charge variants and aggregates or fragments. However, additional testing such as oxidation using peptide mapping via liquid chromatography-mass spectrometry analysis, subvisible particle analysis using light obscuration or other methods are also important.

Subvisible particle analysis has recently been a topic of strong debate given the increasing concern over potential immunogenicity to the particles (Carpenter 2009). Data from particle analysis, of not only > 10- and 25-μm particles, but also > 2- and 5-μm particles, are valuable and should be considered during TTs.

Samples from the clinical production as well as from commercial scale are also characterized using potency assays to determine biochemical similarities.

27.8.2 Case Study for a Monoclonal Antibody

During late-stage development of a product in clinical development, a transfer-working team looked at the stability of a monoclonal antibody, mAb-1 (liquid fill in a glass vial). The stability was assessed after thermally stressed storage conditions (40 °C) for 7, 14, 21, and 30 days. Product quality was evaluated using IE-HPLC for charge variants and SE-HPLC for monomer, low-molecular weight species (LMWS), and high-molecular weight species (HMWS) content using validated methods. A linear regression analysis was done on this data set. Results from this study are summarized in Table 27.14. A statistical approach using homogeneity of slopes can also be applied and will be discussed in the next section.

Decrease in percent monomer (as determined by SE-HPLC) which corresponded to increases in both percent HMWS and percent LMWS, as well as a decrease in percent main peak (as determined by IE-HPLC), which corresponded to an increase in percent acidic peaks, were detected in DP filled at both clinical and commercial sites after storage at 40 °C for up to 30 days. Additionally, the rate of change in percent main peak and percent monomer for these samples was similar as illustrated in Fig. 27.7 and 27.8. Based on these preliminary stability results, mAb-1 DP manufactured at clinical and commercial sites were deemed comparable.

Table 27.14 Comparison of mAb-1 drug product quality from lots filled at clinical and potential commercial site after 30 days at 40°C

Filling Site	Temp (°C)	Time (days)	SE-HPLC			IE-HPLC		
			% HMWS	% Monomer	% LMWS	% Acidic peak	% Main peak	% Basic peak
Clinical site	NA	0	0.2	99.7	0.1	19.7	68.5	11.8
	40	7	0.2	99.6	0.2	24.4	63.5	12.1
	40	14	0.2	99.5	0.3	29.0	58.9	12.1
	40	21	0.3	99.3	0.4	33.3	54.7	12.0
	40	30	0.4	99	0.6	38.7	49.4	11.9
Potential commercial site	NA	0	0.2	99.8	0	17.9	69.7	12.4
	40	7	0.2	99.7	0.1	22.4	64.6	13.0
	40	14	0.2	99.6	0.2	27.2	59.8	13.0
	40	21	0.3	99.4	0.4	32.7	54.5	12.9
	40	30	0.5	98.9	0.6	38.0	49.1	12.9

SE-HPLC size exclusion high performance liquid chromatography, *IE-HPLC* ion-exchange high performance liquid chromatography, *HMWS* high-molecular weight species, *LWMS* low-molecular weight species, *NA* not applicable

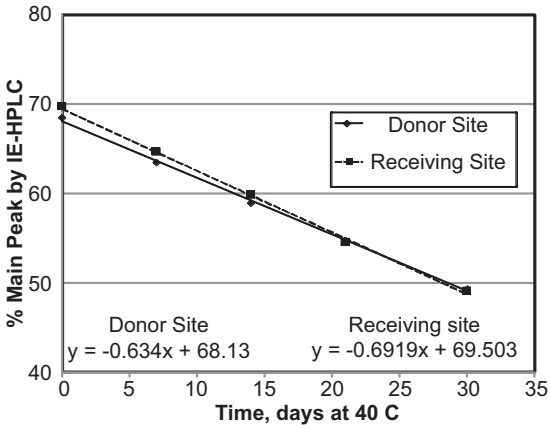


Fig. 27.7 Change in percent main peak (as determined by IE-HPLC) over time at 40 °C for mAb-1 drug product filled at different filling sites

Long-term stability studies at 25 °C and 2–8 °C were also conducted from mAb-1 DPs filled at the two sites and were found to be comparable (data not shown).

27.8.3 Commercial to Commercial Site Technology Transfers

The purpose of the comparability assessment at this point of commercial development is to determine if the site transfer and associated process/equipment changes related to the receiving site produce material that is comparable or different as de-

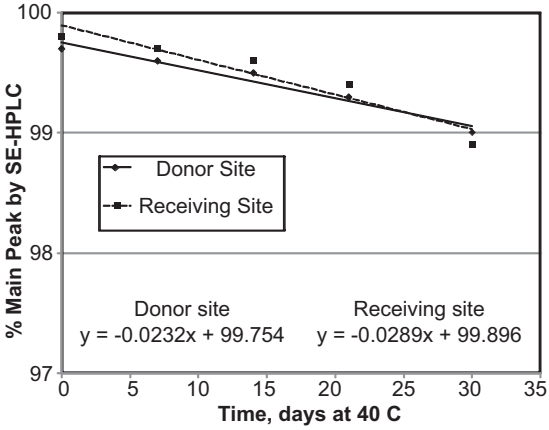


Fig. 27.8 Change in percent main peak (as determined by SE-HPLC) over time at 40 °C for mAb-1 drug product filled at different filling sites

termined using an objective, statistically based evaluation, and predefined comparability criteria.

To demonstrate product comparability with respect to stability, a statistical comparison of the degradation rates under stress conditions for key stability, indicating assays using the following two-stage approach, is usually performed on three lots. In the first stage, a "homogeneity of slopes" analysis is employed to test the hypothesis that the two degradation rates are equal. This analysis will detect a significant difference between slopes but can fail because of statistical differences that are not practically significant. If the slopes of the qualification/validation and control lots are not found to be equal by this analysis, a second-stage analysis is performed. In the second stage, the ratio of the degradation rates is examined to estimate the magnitude of difference between the two variants. A 90% confidence interval on the ratio of the degradation rates is constructed. If the confidence interval falls entirely within the region [0.80, 1.25], the rates are considered comparable. In addition to statistical analysis, it is also important to review chromatographic data to indicate that no new peaks are being generated using the manufacturing process at the receiving site. Furthermore, one lot from the receiving manufacturing site is typically placed on real-time stability for annual review.

Another strategy to support commercial drug product technical transfers is the use of 95/99 tolerance intervals (TI) using batches made traditionally at the donor site. Potentially, release data of three qualification lots from the receiving site can be compared to the data generated previously at site 1 using this TI. To set quantitative acceptance criteria for the comparability assessment, tolerance intervals (TI, 95% confidence/99% probability) can be applied because they provide a range that incorporates the uncertainty in estimates of the population mean and standard deviations based on the sample size. This statistical treatment characterizes expected process variability while maintaining sufficient sensitivity to detect deviations from historical manufacturing experience.

27.8.4 Other Case Studies

Several case studies have recently been published for comparability assessments of process and product changes during development (Lubiniecki et al. 2011). Specifically, comparability exercises during DP changes have been addressed to indicate that manufacturing changes are critical parameters that need to be implemented as early as possible. Any product quality issues that are identified should be carefully considered as a part of clinical studies if necessary. However, DP configuration changes (e.g., lyophilized to liquid formulation, vials to prefilled syringes, etc.) should be avoided in the midst of late-stage development as these may cause additional clinical studies to be undertaken that may affect timelines.

27.9 Conclusion

A TT of a pharmaceutical product includes many different phases and aspects to consider and requires comprehensive planning to ensure the integrity of the transferred product. We have described the different stages of the transfer process and the outcomes from each phase. A risk and science based approach may be applied to the risk mitigation phase of the transfer. Risk-based tools are used to design necessary process studies to reduce risks. Recommended process studies are performed to prospectively define robust process parameter ranges where product quality is maintained, and to identify CPPs. This approach demonstrates process robustness and provides confidence that PV lots will be successful. These PV lots provide final verification. Strategies for comparability are also proposed.

This approach may result in additional studies and risk assessments, but the benefits are substantial:

1. Greater understanding of process and demonstration of process robustness
2. Solid data behind parameter target set points and predefined process ranges which may be used to deal with discrepancies
3. Direct identification of CPPs that may be monitored and tracked during production

In addition, by demonstrating rosbustness and control, there may be an opportunity to discuss regulatory relief with the health authorities. This relief may be in the form of a downgraded regulatory filing (e.g., CBE30 instead of PAS), elimination of testing for certain quality attributes that have been shown to be unaffected by the process, or reducing the amount of stability testing for the annual review.

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Chapter 28
Regulatory Considerations for Implementation of the QbD Paradigm for Biologics: Laying the Foundation for Product and Process Lifecycle Management

Lynne Krummen

28.1 Introduction

The ultimate goal of the quality by design (QbD) paradigm is to demonstrate that the defined process parameters and controlled inputs provide robust control of the expected product quality. ICH Q8R1 (2009a), provides that when a company chooses to apply enhanced approaches to process development and quality risk management (ICH Q9, 2006) in the context of an appropriate pharmaceutical quality system (ICH Q10 2009b), opportunities may arise for enhanced science- and risk-based regulatory approaches. These approaches should be based on the concept that it is possible to define the important attributes of the product and link the demonstrated ability of the process to control product quality attributes with the logical design of the Attribute Testing Strategy (ATS), creating a robust, risk-based overall control strategy. Attribute testing, whether at lot release or at limit of shelf life, is a risk mitigation measure that assures attributes of the highest criticality, or for which tight process control has not been demonstrated, are confirmed either during lot release or stability testing or through continued monitoring at some frequency. Application of the QbD approach also creates the basis to enable risk-based lifecycle management. ICH Q8 (2009a) suggests that if enhanced process knowledge is provided in a regulatory application, approval of a design space, within whose acceptable ranges changes are allowed without preapproval by health authorities, is possible. As such, the design space concept offers one path to lifecycle management of process change while continuing to assure the defined product quality. However, the overall QbD approach of using systematic assessments of process and product understanding to design comprehensive risk-based control strategies and postapproval lifecycle management plans provides a foundation for risk-based management of change well beyond the design space.

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In practice, full implementation of the QbD concept has proven challenging for both regulators and applicants. Regulators essentially must agree that the applicant's data provide a high degree of assurance that self-management of change within the approved design space is of acceptable risk, and that reliance on the applicant's quality management system (QMS) and routine inspections provide sufficient oversight for changes that may have previously required preapproval. Both parties have struggled with what constitutes "a high degree of assurance," and how to come to a common view of how to manage any residual risk remaining at approval with an effective postapproval risk management strategy. This involves developing an agreed perception of the residual risk, and a way that confidence in the company's QMS can be assessed by providing transparent understanding to reviewers of how postapproval change is managed and will be reviewed for compliance upon inspection.

In the A–MAb case—study (CMC Biotech Working Group 2009), possible strategies for application of QbD to a hypothetical monoclonal antibody were presented and a number of proposals related to the regulatory implications of implementation of QbD were suggested and are shown in Fig. 28.1.

1. Understanding of CQAs and their linkage to critical process parameters and the design space allows clear identification of the parameters that may effect product safety or effectiveness, and thus require regulatory approval and oversight (i.e., are considered "regulatory commitments"). Other parameters not associated with CQAs are controlled and monitored in the Quality system to ensure process and product consistency, but are not considered regulatory commitments.
2. The design space is based on development data generated from small scale lots up to commercial scale lots. This data in its entirety can form the basis for process qualification and validation when coupled with a program of continued process verification.
3. An iterative, risk based approach for managing changes to the manufacturing process can be implemented by leveraging the original approach for creating a design space by linking process parameters to critical quality attributes.
4. Movement within a design space based on the lack of documented effect on critical quality attributes can be managed within the Quality system.
5. For movement outside of a design space, the outcome of the risk assessment exercise will facilitate determination of the data required to support the change. The level of regulatory oversight required for the change should be proportional to the level of risk identified

Fig. 28.1 Possible strategies for application of QbD for A—Mab

These concepts apply equally to design of drug substance (DS) and drug product (DP) control strategies and their postapproval management. Many of these proposals have now been fully explored through discussions on actual submissions undertaken with health authorities as a part of the FDA pilot program for biotechnology products and through consultation and review by other major global health authorities. The outcome of these experiences has allowed the development of a deeper understanding of health authority expectations and concerns regarding the realization

of QbD as initially envisioned by Industry. The pilot program experiences provided the opportunity to focus on key areas where additional understanding and alignment were needed to eventually achieve approval of both a risk-based control strategy as well as design space for a biotech product in 2013. The key challenges and resolutions are outlined below.

28.2 Definition of Critical Quality Attributes (CQAs) and Development of a Risk-Based Control Strategy

28.2.1 Identification of CQAs

In the QbD paradigm, an overall control strategy is based on a scientific understanding of the linkage between product quality attributes and the control of each attribute offered by parametric control of the process and material inputs. To create such a linkage it is necessary that CQAs are first comprehensively identified based on their potential impact on clinical performance using all available relevant product knowledge. It is clear that developing a direct causal understanding of quality attributes and clinical performance is not always possible; however, the *potential* for criticality is possible to evaluate. Available information regarding possible mechanisms of action, general understanding regarding safety and immunogenicity concerns for some attributes, and mechanistic understanding of how specific attributes may impact potency or PK can be used in addition to product-specific clinical experience and experience with similar products as a basis for risk ranking. For products where a significant knowledge base exists regarding structure/function, such mechanistic understanding can be based on historical information and risk-based assessments of the position of certain product-related variants within the areas of the molecule known to be required for potency, activity or pharmacokinetic properties.

In general, feedback from most health authorities indicates a general acceptance of the risk ranking and filtering (RRF) approach that was developed at Genentech and later incorporated into the A–Mab case study (CMC Biotech Working Group 2009). The RRF approach assesses attributes for criticality based on four distinct categories: safety, immunogenicity, biological activities, and pharmacokinetic impacts. It takes into consideration the degree of certainty the market application holder (MAH) has about the potential impact of the attribute but does not allow for attribute criticality to be diminished because the attribute is well-controlled by the process. This approach appears to be acceptable to health authorities for several reasons. First, it results in attributes being classified within a "continuum" of criticality that assures that in future risk assessments, no attributes will be overlooked because they were judged to be "non-critical" or "well controlled" and therefore removed from the general product knowledge that is carried forward. Second, the uncertainty score increases the potential criticality of an attribute if the information known regarding impact is taken from less direct sources, for example, literature data versus

in-house in vitro knowledge or nonclinical studies, or direct clinical experience. Extremely low uncertainty can only be achieved by direct clinical studies and would be available for specific product-related variants only in unusual circumstances. Thus, the uncertainty score also assures attributes with potential critical impacts are studied further during development.

The quality attribute criticality continuum should be assessed throughout process development and during commercial lifecycle management of the control strategy. A key lesson learned in the pilot program was that it is particularly important that the MAH's view of mechanism of action/toxicity and their assessment of quality attribute criticality is reviewed with health authorities before registration-enabling process characterization/validation and registration-enabling stability studies are undertaken so that agreement of what quality attributes will be studied during those activities can be reached. Missing information on certain CQAs or on the stability behavior of attributes can theoretically be managed at the time of licensure by adding testing of that attribute during lot release or on stability. However, since the attribute was not studied during process characterization/validation, the missing information on process control will likely undermine the ability of the health authority to approve a design space proposal. In addition, the need to add a CQA late in the approval process may result in approval delay or postapproval requirements for additional process characterization and method validation. If a suitable test method is available these may be avoided.

28.2.2 CQA Acceptance Criteria

Once the list of CQAs has been identified and ranked, assigning acceptance criteria (AC) to each is a highly important and challenging activity. Experience during the pilot program suggests that the CQA–AC, and the justification thereof should be previewed with the health authorities at the time CQAs are reviewed prior to onset of PC/PV (process characterization/process validation) studies. While the final CQA–AC is clearly a review issue, it is important to get HA input on the strategy that is planned.

How to set CQA–AC with appropriate ranges is a key area of debate with health authorities. On one hand, our experience in the pilot program indicates that Health Authorities are open to justification of CQA–AC beyond clinical experience for several attributes. Justifications based on previous experiences that provided broader exposure to similar attributes on similar molecules studied in relevant patient populations and indications, or based on knowledge from studies that show the attribute is either present commonly on endogenous molecules (e.g., prevalent on Human IgGs), or is removed or significantly modified after administration to patients were useful. However, for attributes potentially related to safety or immunogenicity, the strength of arguments needs to be quite high, and health authorities still understandably lean heavily towards demonstrated product-specific clinical experience in

these cases (leverage of data from higher exposures in early clinical dose-ranging studies can be useful to justify somewhat expanded ranges).

For example, in our experience a conservative widening of ranges from product-specific clinical experience for an attribute like aggregate, for which health authorities have some general safety and immunogenicity concerns, was possible using historical knowledge from other selected products and general concepts of the predominance and relative safety of dimers in the aggregate population. However, health authorities asked that we consider the broader available evidence of possible impact in addition to our own safety data to propose an AC. In general, it is likely, and logical, that attributes that may potentially impact safety will have the narrowest AC relative to clinical experience, and will likely be the ones that are most closely linked to parameter criticality.

For attributes that impact biological activities (as measured by relevant in vitro methods) or pharmacokinetics (PK), health authorities were amenable to setting of AC based on a threshold that would translate into likely clinical impact rather than on clinical experience alone. In the A–MAb case study, thresholds of ±20% for potency or 80–125% for PK impact were proposed. In practice, these thresholds were accepted, but health authorities also suggested several improvements to ensure consideration of cumulative impacts of several CQAs on potency or PK and avoid unintended interactions.

If multiple CQAs were allowed to vary to the threshold limits listed above, it could result in significant impacts on dose exposure and efficacy at the patient level. Therefore, a practical solution to set a cumulative limit and distribute the allowable variability amongst the CQAs categorized as impacting either PK or potency across this limit was chosen. In this case, the CQA–AC for individual attributes was not equally distributed. For example, if there were four CQAs that impacted potency, each was not assigned a 5% share of the allowable range. Rather, the allowable limit for each was selected after evaluating the process performance data so that allowed variation for each attribute was tied to the process outcomes, while still resulting in an overall acceptable result. Health authorities also suggested that we not use the entire allowable range (i.e., 80–120%) if a narrower range was possible based on the desired design space and proposed DS and DP shelf life claim.

Concerns regarding attribute interactions were focused on the potential unintended impact that setting CQA–AC for some attributes beyond historical manufacturing experience might have on other attributes. An example of this might be the potential impact of setting a "wider than experience" limit for host cell protein content on product fragmentation by trace levels of host cell proteases. Health authorities also voiced concerns over the possibility that an attribute thought only to impact potency or PK could have unintended impacts on an attribute that could impact safety if limits for the first attribute were set wider than clinical experience. In some public discussions, health authorities have questioned whether it is possible to create a multivariate attribute space to examine impacts of extreme combinations of attributes or their interactions. While the question raised is valid, it must be appreciated that it is not really possible to study all conceivable combinations of attributes, and that several more practical ways are available to manage the risk presented by

this possibility that allow unintended consequences to be identified and avoided. For example,

1) Extended biological and analytical characterization of materials exposed to conditions meant to produce excessive levels of attributes, such as oxidations or deamidations can be presented in the marketing authorization to increase confidence that no unintended consequences on other attributes, or net potency impacts are likely to occur.
2) The CQA–AC proposed should not be excessively broad; a reasonable balance between historical experience and a widening that enables flexible process design as well as an optimized control system can help to minimize the extent of the potential risk.
3) A clear lifecycle risk management strategy can be proposed to help manage any residual risk and unintended consequences. If the process targets are moved in the design space postapproval, lots associated with that change should be evaluated by appropriate extended characterization methods in addition to lot release methods, including relevant functional tests, to ensure that no unexpected analytic changes were encountered.
4) In the instance where the postchange process contains levels of process-related impurities beyond those previously encountered in development, a postapproval lifecycle management plan containing a commitment to add those materials to the stability program can be leveraged to minimize risk.

A third challenge is the lifecycle management of CQA–AC. It must be appreciated that the proven acceptable ranges for process parameters detailed in the license are specifically designed to deliver a product that consistently meets the CQA–AC. To ensure the integrity of the overall control system designed using the QbD approach throughout the lifecycle, adjustments to CQA–AC, and by extension the AC for the specified attributes in the lot release and stability testing program, should only be undertaken based on new data related to clinical relevance (including new considerations if route of administration or significant changes in patient populations occur) rather than on the traditional approach of adjusting AC based on statistical analysis of manufacturing performance at target process conditions. In the traditional approach, as process control capabilities become known and sources of variability are eliminated, the quality outputs and thus specification limits may become tighter. This traditional approach ensures that process performance and quality outputs are consistent, but fail to consider clinical relevance and the linkage of the specifications to the process design or approved design space. Narrowing either the CQA–AC or the attendant proven acceptable ranges (PARs) without new clinical justification undermines the ability of QbD to set up a lifecycle process management strategy that enables continuous improvement—one of its central goals. Clearly continued assurance of consistent process performance and identification of potential out-of-trend results is of high importance. However, such assurance of process and product consistency should be realizable by attribute and process monitoring performed as part of continued process verification during the lifecycle, managed through the quality system, without the need to undermine the design of overall control strategy.

28.3 Identification and Regulatory Considerations for Critical Process Parameter (CPP) Identification and Design Space Definition

In the context of the A–MAb case study, the "Regulatory Commitments" were considered the elements of the file that could not change without health authority preapproval. In practice, these were envisioned as the PARs for the CPPs and critical material controls related to the process description provided in Sect. S. 3.2.2, the control of critical materials in Sect. S. 2.3, and critical controls in Sect S. 2.4.

The rationale for this conclusion was that clear identification of all process parameters and material inputs that have an impact on the identified CQAs provides the necessary framework for limiting the regulatory commitments to only those items. In other words, in addition to the DS and DP specifications for lot release and end-of-shelf life (including method descriptions), only the CPPs and critical material controls would be viewed as "regulatory commitments" which would require health authority preapproval for change. By extension, the A–MAb case study proposed that the design space should be comprised solely of acceptable ranges for the CPPs and acceptable controls over critical raw materials. Other non-CPPs would be controlled, and oversight of change would be managed within the quality system and subject to health authority evaluation during inspection.

This design space concept has proven to be one of the biggest challenges to practical implementation. First, the conclusion that a design space can be defined solely as the combination of CPPs and critical materials controls puts quite a bit of focus on the definition of parameter criticality. Shortly after the case study was released, FDA commented at a public conference that "D(esign) S(pace) should include all relevant parameters required for assurance of product quality…If you include some control of non-CPPs—or include them somehow into the DS—then data requirements may be lower. If the DS includes CPPs only, then a thorough data package will be needed to convince regulators that you can ignore controls or inclusion of non-CPPs" (CMC Strategy Forum 2010). A key challenge during the pilot program dialogue was coming to agreement on how to separate critical from non-critical process parameters. There were dilemmas that needed to be overcome to achieve alignment and ultimately agreement on this topic.

The first dilemma is that it is almost always impractical for manufacturers to create the multivariate data to assess parameter criticality, or to prospectively confirm the design space at full commercial manufacturing scale. Therefore, qualified, scaled down models of each unit operation are used to generate the data upon which parameter criticality is based. Such models have been in use to design and develop bioprocesses for many decades, and have a proven track record in predicting the directionality and magnitude of parameter impacts on process performance and product quality. Indeed, these models have historically been used as an important part of the process validation exercise. Data generated using these models have historically been used to justify the PARs presented and approved in license applications for biological process and information related to the "qualification" of these models has

been presented in "traditional" filings. However, these models are not in all cases perfect quantitative predictors of outcomes at manufacturing scale. In some cases it is observed that there is a reproducible offset in the performance of the full-scale process versus the model scale due to a known or unknown factor. For example using a scaled-down version of an affinity column may result in a systematic offset in the amount of a CQA present in the postcolumn pool compared to results in the same pool resulting from full-scale manufacturing. This offset can be confirmed to be due to the scale of the model by showing than upstream process material from both the scaled-down process and the manufacturing-scale process give similar results when processed through the model scale column (no longer offset). In other cases, the cause of the offset may be less well known, but if shown consistently to occur, the small-scale offset can be applied to the predicted effect of the process parameters at scale when the PARs are proposed for the involved parameters. For almost all unit operations and CQAs, the currently available scale-down models can be shown to provide equivalent results or results that are equivalent with the application of a well-justified offset. However, there are instances when it is observed that the scaled down model is insufficient to predict the behavior of a specific CQA at scale. This is not to say that the directional impact of process parameters on the CQA in question is not observable and similar at both scales, but in these cases a factor imparting variability to the full-scale results could be present that is not accounted for in the scaled down model.

Second, it has been well recognized that the width of the range over which the process parameters are varied while studying their criticality has a direct influence on the ability to observe CQA impact and hence, identify CPPs. The dilemma here is that it is not practical or an efficient prioritization of resources to characterize the edges of failure for all parameters, or study ranges that are some arbitrary factor wider than the intended practical operating range, in order to assure that parameters have no potential critical impact.

The fact that both the identification of CPPs and the setting of the quantitative limits for the design space parameters depend on data derived from scaled-down models and experimental designs that include some degree of residual uncertainty with regard to the performance of the process at scale has been the central question challenging reviewers. This is because these data determine what is proposed to be inside versus outside of the design space and which parameters will be subject to health authority preapproval oversight during lifecycle management of their acceptable ranges.

While these are clearly central considerations, it is not practical to imagine that it will be possible that all residual uncertainty with respect to criticality or limits will be eliminated prior to approval. Therefore, it is extremely important that industry and health authorities develop credible strategies for postapproval risk management. This concept is central to the concept of continued verification, improvement, and lifecycle management. Therefore, practical approaches to minimizing the risk that regulators feel in agreeing to design space proposals had to be developed to allow a viable path forward. Based on the learning during the pilot program, further steps were taken to assure that the residual risk was either minimized or that there was a

clear risk management strategy in place. Key examples of possible risk mitigation strategies are detailed below.

First, the applicant's definition of CPP, based on the scaled down data can be more or less objective, and more or less conservative. We chose to create an objective definition of CPP based on a definition of the practical significance of parameter impact in addition to statistical significance. The definition was termed the "Impact Ratio" and was a measure of the impact of the parameter variability on the CQA in question. To be considered "practically significant," and therefore critical, a parameter, at its worst-case setting, had to move a CQA ≥ 10 % towards the allowable limit of a target range (CQA–TR) that was derived from a narrowing (~ 5 %) of the CQA–AC. The CQA–TR was implemented to remove some amount of the risk that the scaled-down model results would not accurately predict an at-scale result within the CQA–AC if the process were run at the edges of the design space. The use of a CQA–TR or similar narrowing of the CQA–AC range for process design helps to ensure a result within the CQA–AC is achieved. The definition of criticality based on an impact ratio of ≥ 10 % was selected to avoid classifying parameters with only minor effects as critical, since it would take multiple such parameters, all impacting the same CQA to be simultaneously operating at the worst-case limit of their range to cause a failure in that CQA. In practice, this is highly unlikely.

To describe how any residual risk that might lead to unexpected outcomes could be identified and managed, a moderately detailed document termed as postapproval lifecycle management plan (or PALM plan), was also included in the regional section of the dossier. This purpose of this document was to describe how changes to CPP operating targets, both within and outside of the design space, and changes to non-CPPs would be monitored within the QMS. While this document did not provide detailed information on the sponsor's internal quality documentation, it did provide a commitment that changes within the design space would be verified at scale before implementation, and that any unexpected outcomes and any required modifications to the design space (i.e., identification of a new CPP) would be reported. The PALM plan has been quite helpful in providing confidence to health authorities that internal QMS procedures can effectively manage change. However, the PALM did not provide sufficient weight to convince health authorities that non-CPPs, even when conservatively defined, could be fully managed within the QMS. Rather a design space definition that includes relevant non-CPPs appears to be necessary to provide assurance that all of the process knowledge that contributed to process performance stays linked throughout the product lifecycle, and that changes to non-CPPs far from the experience used when establishing the design space cannot occur without review and preapproval by health authorities. Thus, the current concept of design space is more closely aligned with the overall process descriptions provided in S. 3.2 and P.3.3. While on one hand this does not afford the full vision for regulatory flexibility initially envisioned by industry, it does create a clear picture of what can be considered regulatory change relevant, and it is a step forward in terms of creating the ability to enable continuous improvements with reduced regulatory oversight while maintaining a high degree of sustained product quality.

28.4 Design of a Risk-based Control Strategy

The full analysis of multivariate behavior of the process relative to the full list of product CQAs and a thorough analysis of CQA stability behavior also allow the rationale design of the attribute testing strategy (ATS). In the biotech pilot program, the multivariate assessments of the impact of parameters and stability behavior on the CQA–AC were used to develop a risk ranking for "process impact" and "stability impact" at the DS and DP level. Such process and stability impact scores can then be considered, along with the impact and occurrence of each CQA to create a rationale, risk-based proposal for which attributes should be tested at lot release or on stability, which attributes should be monitored, either continuously or at intervals to verify continued process performance. In practice, the ATS risk assessment tool assesses the need for "detection" as a risk mitigation measure after assessing the "severity" associated with the quality attribute and the probability that the process provides tight control (i.e., the potential "occurrence" of the failure mode). Combined with an evaluation of the robustness of the method selected for testing or monitoring, this tool was well-received by health authorities and resulted in reducing redundant or non-value added testing from certificate of analysis and stability testing. For example, attributes that are wholly formed or removed from the process at the DS level do not need to be re-tested at the DP level.

The practical result of implementation of the ATS is a risk-based overall control strategy (Fig. 28.2). Any attribute of high criticality that is demonstrated to be formed at a level of potential interest (> 1.0 % for most attributes, > 0.1 % for safety related attributes) is either tested in the quality control system or monitored at the

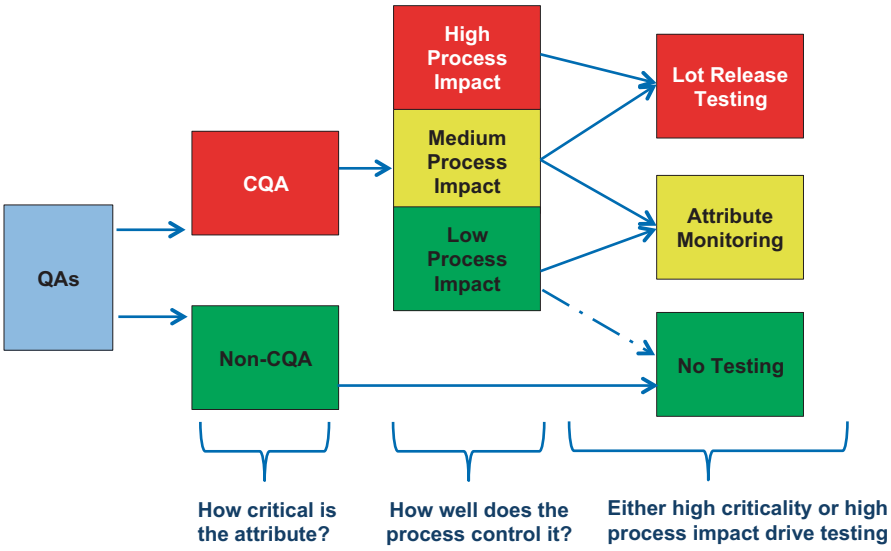

Fig. 28.2 Attribute testing strategy

appropriate level (DS or DP release or stability testing). In addition, any attribute for which tight process control is not demonstrated in the multivariate process characterization design of experiments (DOEs), or for which there is not a satisfactory scale-down model is tested as part of the quality lot release program since poor process control or lack of a "sufficient" model results in a high "process impact" score. Likewise any attribute with a high rate of degradation observed during development and registrational stability studies receives a high stability impact score and is thus included on the annual stability program. So, in combination with various other measures described above to reduce residual uncertainty in translating the small-scale results to define a process-wide design space at the manufacturing scale, the ATS further reduces risk by providing a risk-based approach to attribute monitoring.

The PALM plan provided in the regional section of the MA contained further information regarding how the ATS would be used throughout the lifecycle of the product to ensure that the attribute testing provided continued verification of the understood degree of process performance and that the ATS would remain linked to both attribute impact and process knowledge as more information of both types was learned throughout the lifecycle. For example, the PALM discussed the frequency with which the ATS assessment would be repeated during the lifecycle to incorporate any relevant new information on potential attributes or new information relevant to potential clinical performance. In the case of change in understanding of potential CQA impact, an attribute's testing category might need to be increased, and in the case of a new attribute, some process characterization or monitoring data may need to be collected to assess the need for revised testing. The PALM indicated that such changes would be filed in appropriate regulatory submissions according to local regulation. A description and commitment to ongoing attribute monitoring and data trending was also made in the PALM to ensure continued verification of consistent process performance. Should ongoing monitoring reveal that the "process impact" assumed from development data was not translated to the manufacturing scale, the outcome could be a reassessment that elevated the attribute's testing category. Importantly, the PALM committed that no attribute could be removed from testing or downgraded from the quality system testing to product monitoring without health authority preapproval.

28.5 Risk-Based Regulatory Management of Change During the Product and Process Lifecycle

In the QbD paradigm, manufacturers strive to reduce residual risk as much as possible by developing an enhanced understanding of both their product and process. However, it is not possible or practical to foresee or mitigate all known risks at the time of product approval. The overall results of the combined risk assessments developed during the pilot program, and fine-tuned based on health authority inputs, allow for the implementation of an overall risk-based control strategy that can be leveraged to form the basis for ongoing lifecycle management of both known and

unknown risks. Self-management of changes within the design space allows for some amount of risk-management in the context of reduced regulatory reporting. Additional regulatory flexibility can also be accomplished by using the same types of structured risk assessments to develop expanded postapproval change protocols (comparability protocols in the USA and change management protocols in EU) to manage changes not foreseen by the design space. Such protocols may be used to describe how certain individual or groups of similar changes would be assessed by the manufacturer, including description of specific AC for attributes and the performance indicators that would be tested, and to request preapproval of a reduced regulatory reporting category if all criteria are met. Such postapproval change protocols can be used to manage changes to CPPs and Non-CPPs outside the design space or other commonly occurring lifecycle events, such as site transfers, supplier, raw or intermediate material changes or reporting of control system updates that are meant to improve process and product controls. For the most part such protocols are based on the principles of comparability set out in ICH Q5E and build on the legal frameworks put in place in the USA and EU by the postapproval change and variations guidelines, respectively. Such changes must be fully assessable at the analytical level without the need for non-clinical or clinical studies. Successful application of such concepts in regions that already have frameworks for these approaches can eventually lay the foundation for similar frameworks to be developed in other regions, providing huge benefit and predictability to the global implementation of process and supply chain improvements. As global markets expand it is absolutely necessary that coordinated, globally aligned, and streamlined regulatory change management procedures and principles be developed to ensure robust global supply of important products to patients everywhere.

An example of such an expanded comparability protocol was included in the FDA QbD Pilot for Biologics. This multiproduct, postapproval supplement (PAS) proposed criteria by which a number of well-characterized products could be transferred to a defined list of DS manufacturing sites. Upon approval of the protocol by a health authority (6 months), the approval for any subsequent successfully completed product transfer could be obtained through a CBE–30 submission. The key to the success of this submission was to carefully define the scope of the products and sites involved and the specific analytic accceptance criteria and test methods that would be used to demonstrate comparability. A risk-based approach to assessing facilty compliance at the time of transfer was also critical as the 30-day timeframe for the individual approvals required a mechanism to waive the preapproval inspection requirement. In concept, a similar approach for DP transfers, which would have more immediate impacts on product supply chain, could be envisioned. Likewise a similar concept where a predefined protocol that outlines the level of preapproval verification for required changes outside the design space could be developed to link the postapproval regulatory reporting category to the level of risk involved by leveraging the parameter's criticality defined by the original process risk assessments, combined with specific attribute comparability strategies.

In summary, much progress has been made in developing systematic processes for assessment of parameter and attribute criticality, and using this information to

justify risk-based control and postapproval lifecycle management strategies that are appropriate to assure the quality of each individual product and process. We are beginning to realize the usefulness of these tools in communicating risk to health authorities and using them to calibrate the amount of resources and oversight necessary to mitigate or manage that risk. As implementation becomes more routine and globally understood, these processes can provide the basis for driving convergence among global health authorities with respect to the regulatory decision-making and risk management that will enable both streamlining and consistency of postapproval product management that will benefit industry, regulators, and patients.

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