Chapter 10
Special Topics in Analytics of Pre-filled Syringes

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Abstract Pre-filled syringes (PFSs) are extensively used as a container closure system offering convenience of administration. PFS features special and unique characteristics, enabling their proper functionality, but which, in some cases, may result in novel and unanticipated challenges. Such challenges are, for example, the potential interaction of the drug product with trace amounts of tungsten, originating from the pins used to form the syringe, or the presence of large concentrations of silicone oil droplets, potentially masking changes in proteinaceous particle load in a PFS product. This chapter discusses some of the analytical approaches used to tackle such challenges in the development of PFS drug products.

Keywords Pre-filled syringe · Silicone oil · Particles · Tungsten
Leachables and extractables · Container closure

10.1 Introduction

The pre-filled syringe (PFS) is a particular container closure system (CCS), presenting specific and often unique physical and functional features (see Fig. 10.1). For example, in contrast to liquid-filled vials, PFSs typically contain very little headspace and have unique product contact surfaces and materials (steel needle, silicone oil covered barrel). PFSs require careful design and development to ensure proper functionality. The latter typically depends on the combination and interplay of various characteristics such as product solution viscosity, needle and syringe dimensions, fill volume, siliconization technology/level. Thus, the development of PFS products requires additional—and often highly specialized—analytical characterization and testing. In this chapter, some of these special analytical topics and requirements will be reviewed.

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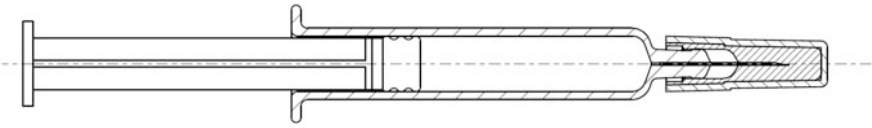

Fig. 10.1 A schematic representation of a staked-in-needle pre-filled syringe, showing the various components: (1) plunger, (2) syringe barrel, (3) needle and (4) rigid needle shield

10.2 Protein Aggregation and Sub-visible Particles

One of the main differences between PFS and vials most commonly used in development of biotech products is the presence of silicone oil (SO; polydimethylsiloxane)[1]. Silicone oil is used as a lubricant of the glass barrel of PFS in order to reduce the frictional force between the rubber stopper/piston and the syringe barrel and in turn ensure proper syringe functionality (break lose and gliding forces allowing unobstructed administration over the shelf-life of the product).

The silicone oil is applied to the barrel of the PFS using different technologies. For example, the silicone oil can be sprayed onto the barrel directly, resulting in a layer of "free" (or loosely bound) silicone oil. Alternatively, the silicone oil can be baked onto the glass surface of the barrel, resulting in a much more tightly bound silicone oil layer. Typically, spray-on processes also result in larger amounts of silicone oil delivered to the PFS barrel surface: typically approximately 1 mg SO/barrel or less versus approximately 0.1 mg SO/barrel or less for baked-on SO processes. Clearly, such apparent differences in both the amount of silicone oil and the mobility of the silicone oil layer on the barrel of the PFS are very important, particularly in the context of the frequently asked question about potential interactions between SO and the protein API in biotech products [1]. Such potential interactions have been discussed in recent studies [2], which have raised the possibility for serious adverse effects of SO on proteins, such as induced protein aggregation, presumably caused due to the very hydrophobic nature of SO. Interestingly, such studies have so far demonstrated measurable effects only in highly exaggerated and unlikely conditions (e.g. at very large SO/protein ratios, far exceeding the ones typically used in biotech products). Furthermore, it has been demonstrated that the presence of a surfactant (a nearly mandatory pharmaceutical excipient for protein therapeutics) obliterates the induction of protein aggregation by SO in such experiments [3, 4]. Nevertheless, testing the compatibility of protein therapeutics with SO remains a very important endpoint of PFS development.

The presence of SO in PFSs contributes to another important facet of the PFS target product profile (TPP), namely the sub-visible particle load of the product. Interestingly, SO present on the barrel surface may migrate to the bulk of the product in the form of microscopic SO droplets, thus contributing to the overall

[1]With some exceptions, liquid-filled vials typically do not feature siliconized glass walls.

particle load of the product. Here it is important to emphasize that the mechanism of migration of SO to the aqueous protein solution is still not well understood. In fact, it is likely that the spontaneous migration of SO to the bulk aqueous solution is a misconception. Clearly, in static conditions and in the absence of external energy added to the system, such migration constituting essentially the creation of a SO micro-suspension would not be favoured thermodynamically due to the hydrophobic effect. Naturally, in cases where PFSs are subjected to external forces, such as agitation for example, one could envision how such external energy may lead to partitioning of SO micro-droplets into the aqueous solution of the product. However, it is arguable to what extent do such effects contribute to the presence of large amounts of SO droplets in the PFS products. The fact is that during the testing of PFSs, large amounts of SO micro-droplets are commonly observed. It should be pointed out that sample preparation practices likely vary widely between different laboratories and testing sites. Nevertheless, in the context of the discussion above, one of the more drastic examples of exertion of force onto PFS (and the barrel-bound silicone oil layer in particular) is the ejection of the PFS content prior to testing. This is a typical and most common practice in order to ensure that the analytical tests are performed on the product solution as administered to the patient (ejected through the needle or at the very least through the tip of the syringe in cases of luer lock configurations). It is easy to imagine the action of the syringe piston moving down the barrel of the syringe and essentially stripping the silicone oil layer. In these circumstances, a relatively large volume of SO likely is mixed with the product solution in the form of micro-suspension during ejection of the syringe contents. This line of thought poses an interesting possibility, i.e. the possibility that the majority of silicone oil droplets observed in PFS product solutions is generated during the sample preparation. This idea is supported by the observation that the faster the contents of PFS is expelled, the larger amounts of SO droplets are observed in the test solution (*unpublished data*). Nevertheless, the amount of SO droplets in solution may increase over the shelf-life of a syringe, owing to various factors, such as the syringe manufacturing process, storage and transportation.

With the caveats mentioned above, the SO droplets still constitute a large (in most cases the dominant) portion of the sub-visible particles in the PFS solutions. Whereas proteinaceous sub-visible particles have been and continue to be a topic of high scientific interest within the biopharmaceutical community, particularly in the context of concerns about potential adverse effects [5, 6], SO droplets are generally thought to be relatively inert and safe if administered to patients parenterally, particularly in the low-microgram quantities contained in solutions expelled from PFS units. Thus, the presence of different types of particles (proteinaceous particles, SO droplets and other particles) effectively poses a challenge to sub-visible particle testing.

The light obscuration sub-visible particle test universally used for quality control of biotech therapeutic products in accordance with the major pharmacopoeias is (in its current technical implementation) unable to differentiate between a SO droplet and a non-SO (e.g. proteinaceous) particle. Thus, SO droplets may effectively "mask" the particle load in a product. This may become particularly

important if the proteinaceous particles in a given product exhibit a tendency to increase over the product's shelf-life—a tendency which may not be readily detectable due to the masking effect of the often more numerous SO droplets. Thus, during the development of a PFS program, it is very important to characterize the sub-visible particle profile of the product and understand the relative contribution of the SO droplets and non-SO particles in the product. Within the last several years, the development of three technologies in particular made possible the relatively detailed characterization of SO/non-SO sub-visible particles.

The first technology which enabled characterization of the SO droplets is the technique known as flow imaging microscopy (aka dynamic imaging microscopy) [7, 8]. Flow imaging microscopy features a flow-cell set-up somewhat similar to the one of light obscuration, but in contrast to the latter, which simply enumerates the particles passing through the flow cell, flow imaging microscopy features imaging capabilities, i.e. provides images of the individual particles collected by a high-speed camera. The ability of this technique to produce the images of individual particles has proven very useful for characterization of SO droplets in PFS. Due to their hydrophobic nature, the SO droplets have a very regular circular appearance, regardless of their size [7, 9]. Furthermore, the refractive properties of SO lead to the particular appearance of larger SO droplets in flow microscopy images (the exact size thresholds depend on the specific instrument set-up)—i.e. darker outer border and lighter interior. This characteristic appearance of SO droplets allows for differentiating them from other microscopic particles, which are commonly present in PFS test solutions, e.g. air bubbles, proteinaceous particles (see Fig. 10.2).

The second advance that we have witnessed within the last decade is the development of algorithms for automatic, machine differentiation of SO and non-SO particles. This operation until recently was performed manually, with operators sorting sometimes through thousands of particles and deciding how to categorize

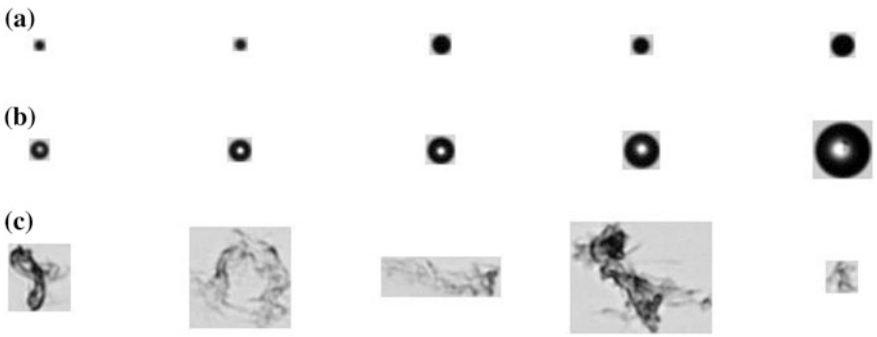

Fig. 10.2 Examples of different types of sub-visible particles commonly present in PFS: **a** air bubbles, **b** silicone oil droplets, **c** proteinaceous particles

them based on their personal experience. Clearly, such manual procedure is extremely cumbersome and prone to error. Thus, the recent invention of several approaches for automatic categorization of particle images ([7, 9, 10, 11], Schnaible and Koulov unpublished) allowed for more efficient accurate and precise differentiation of particles. Further, the latest approaches allow for differentiation of particles of sizes which were previously inaccessible via manual categorization (<5 um).

It needs to be mentioned that in general, due to the different detection principles of flow imaging microscopy and light obscuration, these techniques produce different results (in terms of particle enumeration) when measuring identical samples. Whereas this has been the subject to extensive research and debate in the community and the physical causes have been largely understood ([12, 13], Koulov et al. unpublished), the fact of the matter is that this fact is not that relevant to the characterization of sub-visible particles in PFS. More specifically, LO is likely to remain the tool of choice for quality control and flow imaging microscopy—the method of choice for "extended/additional characterization"—e.g. SO/non-SO particle profile characterization in PFS.

Paradoxically, the presence of different commercial flow imaging microscopy systems which use different optical systems and non-harmonized particle morphology descriptors has effectively stymied the efforts on development of common techniques for data analyses. Recent efforts to develop algorithms for data analysis have overcome these differences and have made major progress in direction of harmonization of these data and in understanding these differences [14].

In practical terms, during the development of PFS biotech therapeutic products, various studies to understand the sub-visible particle profile of the product need to be carried out. Typically, these encompass the analyses of stability samples (long-term storage, accelerated and stressed conditions) from various product lots accompanying the PFS development. Such extended characterization efforts are particularly important to understand the proportion of SO particles in the product as well as the kinetics of the different categories of particles over the shelf-life of the product. This knowledge may prove essential when defining product-specific limits for sub-visible particles (typically required for licensure in the USA) for PFS products and in general—in order to ensure the definition of a sound control system.

As a final note, it may be mentioned that the current practices with regard to sub-visible particle characterization of PFS vary significantly from company to company (and even sometimes—from site to site). Different approaches are applied to not only sample preparation, measurement and instrument settings, but also to the characterization "philosophy" applied during the duration of the lifecycle of a product. Whereas regulators provide certain latitude to sponsors in justifying individual control strategies (based on the specifics of each product), such broad variance in practices and approaches may contribute to larger discrepancies in the characterization of different products than often appreciated.

10.3 Container Closure Integrity

The guarantee of sterility of parenteral dosage forms is a critical facet in their development. In this aspect, PFS presents a more complex challenge simply due to the larger number of closure interfaces (see Fig. 10.3). In addition, some of these interfaces are formed with moveable parts—e.g. the syringe plunger, the syringe needle shield; thus, factors present during storage and shipping (e.g. air pressure differences) may affect the container closure integrity (CCI) of PFS.

It is critical that during the development of PFS products, special attention is paid to the characterization and qualification of the CCS, particularly with regard to the aspects mentioned above. However, such careful characterization requires the availability of analytical tools and approaches to measure the relative contributions of the different container closure interfaces (sealing areas). For example, it is important to carefully select the method for measuring container closure integrity in order to distinguish small differences in tightness of the various sealing areas. Typically, only very sensitive deterministic methods (e.g. the helium leak CCI test method) may be suitable for this purpose. In addition, the methodology for CCS characterization should be developed so the individual contributions of the various sealing areas can be revealed (Pelaez et al., in preparation) and characterized. Also, the CCI testing methodology needs to allow for accurate and precise measurement of the contributions of the movements of the various moveable PFS components mentioned above. For example, one such critical question is the robustness of the tip cap seal. It should be noted that such methodologies to date are not commercially available, but need to be designed for purpose and custom-built (Pelaez et al., in preparation).

10.4 Extractables and Leachables

Similarly to any other parenteral CCS, PFS requires leachable and extractable evaluations. However, this is also an area where PFS presents additional analytical challenges as compared to other CCSs (e.g. vials). In general, the presence of additional (and unique) product contact surfaces in PFS requires that the potential

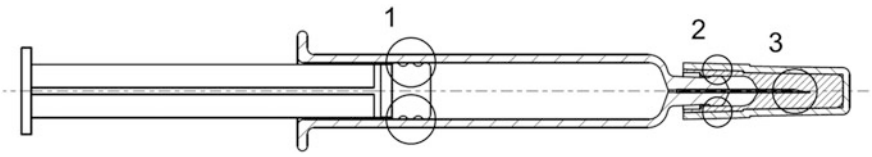

Fig. 10.3 A schematic overview of a rigid needle shield (RNS) staked-in-needle pre-filled syringe showing the main sealing interfaces: (1) ribs of syringe plunger, (2) RNS/tip cone and (3) RNS/needle tip

contributions of these contact surfaces are specifically addressed. An example of such distinct contribution may be the interface of the stainless steel needle and the tip cone of the glass barrel of the syringe. Typically, the syringe needle is fixed to the tip cone using an adhesive (see Fig. 10.1). In most syringe designs, the adhesive comes into direct contact with the product and therefore has the potential of affecting the product stability. Some cases of such occurrences have been reported, where either the incomplete curing or incomplete drying of the needle adhesive has resulted in leaching of compounds from the adhesive into the product with adverse consequences, such as protein oxidation [15, 16].

Another example of unique product contact surface in syringes, which may potentially contribute to the elemental impurity profile of the product, is the metal needle itself. Of course, the elemental impurity profiles of PFS are typically assessed according to ICH Q3D, similarly to other dosage forms. However, it is worth mentioning one interesting aspect of the elemental impurity profile of PFS, namely the potential influence of the syringe manufacturing process. It has been widely reported [17, 18, 19] that the manufacturing process of syringes (formation of the glass tip cone orifice using a hot tungsten pin) may result in the presence of tungsten and a variety of tungsten-compound residues (such as oxides and salts), which may have adverse effects on the stability (and potentially on the safety) of the product. More specifically, it has been demonstrated that such tungsten compounds may induce protein aggregation. Thus, the potential sensitivity of a given product to the presence of tungsten compounds is important to assess during the development of pre-filled syringes. Typically, this is achieved by means of tungsten spiking studies using either pure model tungsten compounds or preferably tungsten pin extracts.

10.5 Closing Remarks

There are several unique aspects in the development of PFS, resulting in distinct quality attributes, which need to be specifically addressed during the development process. The characterization of these special quality attributes in turn often requires the use of specialized analytical approaches. Whereas some of these aspects (e.g. the presence of adhesive in staked-in-needle syringes as a potential source of leachables) are unique in origin, they can be characterized, monitored and controlled using analytical tools that are readily available and applied to other parenteral formats. Others, however, (e.g. assessing the robustness of the tip cap seal or the differentiation of SO and non-SO particles), present unique analytical challenges and require the development of new, custom-built technical solutions, which are not readily available.

Although many of the analytical challenges encountered during product development can be anticipated by following the general logic and rules of product development, there have been cases of surprising challenges, which were difficult to anticipate. One such example is the clogging of syringes due to evaporation of

product in the syringe needle. As the number of market approvals of PFS products is continuously increasing, it is likely that such surprises will decrease in the future. However, the complexities of PFS development will likely still pose analytical challenges for the foreseeable future.

Finally, it is important to remember that the different quality attributes of PFS are often interlinked and interdependent (e.g. the viscosity profile of the drug product as a function of temperature, which determines the syringe functionality parameters, needs to be taken into consideration in developing the protein content specifications for the product). Thus, the development of PFS products in the future may benefit from development approaches such as quality by design (QbD). However, regardless of the development strategy of a given program, proper PFS drug product development will always surely require a holistic analytical approach integrating state-of-the-art analytical tools and advanced scientific knowledge.

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