Sarwar Beg *Editor*

Design of Experiments for Pharmaceutical Product Development

Volume II : Applications and Practical Case studies

Springer

Design of Experiments for Pharmaceutical Product Development

Sarwar Beg
Editor

Design of Experiments for Pharmaceutical Product Development

Volume II : Applications and Practical Case studies

Springer

Editor
Sarwar Beg
Department of Pharmaceutics and
Biopharmaceutics
School of Pharmaceutical Education and
Research, Jamia Hamdard
New Delhi, Delhi, India

ISBN 978-981-33-4350-4 ISBN 978-981-33-4351-1 (eBook)
https://doi.org/10.1007/978-981-33-4351-1

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

About the Editor

Sarwar Beg is currently serving as Assistant Professor of Pharmaceutics & Biopharmaceutics at School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, Dr Sarwar has expertise in implementation of Quality by Design (QbD) in formulation development and analytical development of generic products. He has over a decade of experience in systematic development and characterization of novel and nanostructured drug delivery systems using QbD paradigms including Design of Experiments (DoE), Quality Risk Management (QRM), Multivariate Chemometric Approaches, Advanced Biopharmaceutics and Pharmacokinetic. Besides, Dr Sarwar has acquired know-how of applying advanced release kinetic modeling, pharmacokinetic modeling and in vitro/in vivo correlation (IVIVC) for efficient development of drug products. Till date, he has authored over 170 publications including research and review papers in high impact peer-reviewed journals, 10 journal special issues, 13 books, 45 book chapters and 3 Indian patent applications. He currently serves as the Regional Editor-Asia of the journal Current Nanomedicine (Bentham Science) and is an editorial board member of several other journals. He has been awarded with prestigious "Sun Pharma Science Foundation Award 2017" by Hon'ble Health Minister of India, "Innovative Pharma Researcher Award 2016" by SIPRA Lab (Hyderabad), "Eudragit® Award 2014" in South-Asia by M/s Evonik (Germany), "Budding QbD Scientist Award 2014" and "Budding ADME Scientist Award 2013" by M/s Select Biosciences (UK) and "Novartis Biocamp Award 2012" (Hyderabad). Besides, Dr Sarwar is a visiting researcher to many academic institutes/universities and pharma industrial houses for delivering expert lectures and hands-on training seminars.

1 Introduction to the Application of Experimental Designs in Pharmaceutical Product Development

Sarwar Beg and Suryakanta Swain

Abstract

For decades, pharmaceutical drug product development has been carried out using the traditional hit and trial approaches, which are considered to be less efficient and reliable for attaining desired quality and batch-to-batch consistency. Moreover, such issues create product recalls and rejects, often shortage of medicines. In order to avoid such issues, the federal agencies in the twenty-first century adopted a pharmaceutical quality assessment system (PQAS) for the holistic development of the drug products. Quality by Design (QbD) and design of experiments (DoE) are considered as two pivotal elements of the PQAS for maintaining product quality consistency. DoE analyzes the cause-and-effect relationship among the factors and responses, thus considered as an ultimate resource saving tool for amalgamating the science and risk-based development into the practice. The present chapter provides an overview account on the evolution, principles, and various applications of DoE for pharmaceutical product manufacturing with high robustness and performance.

Keywords

Drug delivery · Experimental designs · Product variability · Product performance · Process performance

S. Beg (✉)
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India

S. Swain
Department of Pharmaceutical Sciences, School of Health Sciences, The Assam Kaziranga University, Jorhat, Assam, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_1

1.1 Introduction

Design of experiments (DoE) or also referred to as the experimental designs are the systematic tools used for decades by the technology-driven industries for rationally optimizing the product and process performance and robustness during manufacturing. As any scientific experiment tends to involve a series of factors which encounter high variability in the end results, thus use of systematic tools has now become a routine practice [1]. DoE particularly emphasizes on rational planning and execution on the basis of a planned set of experiments and utilizes the mathematical and statistical principles to suggest an optimal solution. Experimental designs are very helpful in establishing the relationship between the input and output variables and furnishes the end results in the form of output and reduces the variability [2]. In another way, the experimental designs are very efficient for their predictive ability to detect and plug the error and to reduce the experimental variability to attain batch-to-batch consistency. However, the experimental designs are unable to reduce the impact due to the uncontrollable or noise factors [3, 4].

1.2 Early History and Evolution of Experimental Designs

The concept of experimental design was first envisioned by Sir Ronald A. Fisher in 1920s with his work pioneering to deal with agricultural models implementing the statistical methods. After Fisher, Rothamsted also put forward stepping stones for the application of systematic mathematical models in the agricultural experiments [3]. Later, George E.P. Box and K.B. Wilson in 1951 worked on the response surface mapping (RSM) technique for implementation in the manufacturing sector to improve the product and process robustness. Additionally, Kiefer and Wolfowitz in 1959 worked on multifactorial optimization techniques using factorial designs, while Genichi Taguchi in 1987 proposed the concept of Taguchi experimental designs for robustness testing and optimization [2].

1.3 Experimental Design Principles

Every experimental design relies on three key principles such as replication, randomization, and blocking (or error control), which should be carefully adhered for meaningful execution of the experimental conditions and rational prediction of the optimum response variables [1]. The details regarding the aforesaid principles of experimental designs are provided below.

1.3.1 Replication

It is ideal to use a good number of replicates on any point of an experimental design for reducing the experimental error, if any. There is no fixed rule for selection of the

number of replicates, but it is better to use at least three or more replicates for any design.

1.3.2 Randomization

It is ideal to randomize all the experimental runs and perform them in a random manner. This helps in avoiding the experimental bias which may be encountered owing to performing the experiments in a serial manner. Use of randomization helps in reducing the effect of extraneous factors that may present during experimental practice.

1.3.3 Blocking or Error Control

Blocking is used to reduce or eliminate the variability transmitted from nuisance factors, which may influence the experimental response. Block represents a set of relatively homogeneous experimental conditions and helps in classifying the experiments performed in identical set of conditions.

1.4 General Considerations in Performing DoE

The application of DoE into actual practice involves a multistep process, thus requires defining the experimental objectives, identification of the critical factors and responses, mathematical modeling and statistical validity analysis, and selection of the optimum solutions [5–7]. Figure 1.1 illustrates the typical five-step approach used for implementation of DoE in an experimental setup.

1.4.1 Defining the Problem

Before execution of an experimental design, the study objectives are defined to prepare a list of specific problems that are to be addressed by the experiment. It is also important to keep all the objectives of the experiment in mind before execution of an experimental design.

1.4.2 Establishment of Cause-and-Effect Relationship

The cause-and-effect relationship is established by drawing rational relationship between the input and output variables based on the prior knowledge, experience, and expertise on the similar type of experiments. The cause-and-effect diagram is drawn, which is also known as fishbone diagram by considering the various causes of variability such as men, materials, machine, methods, milieu, and management.

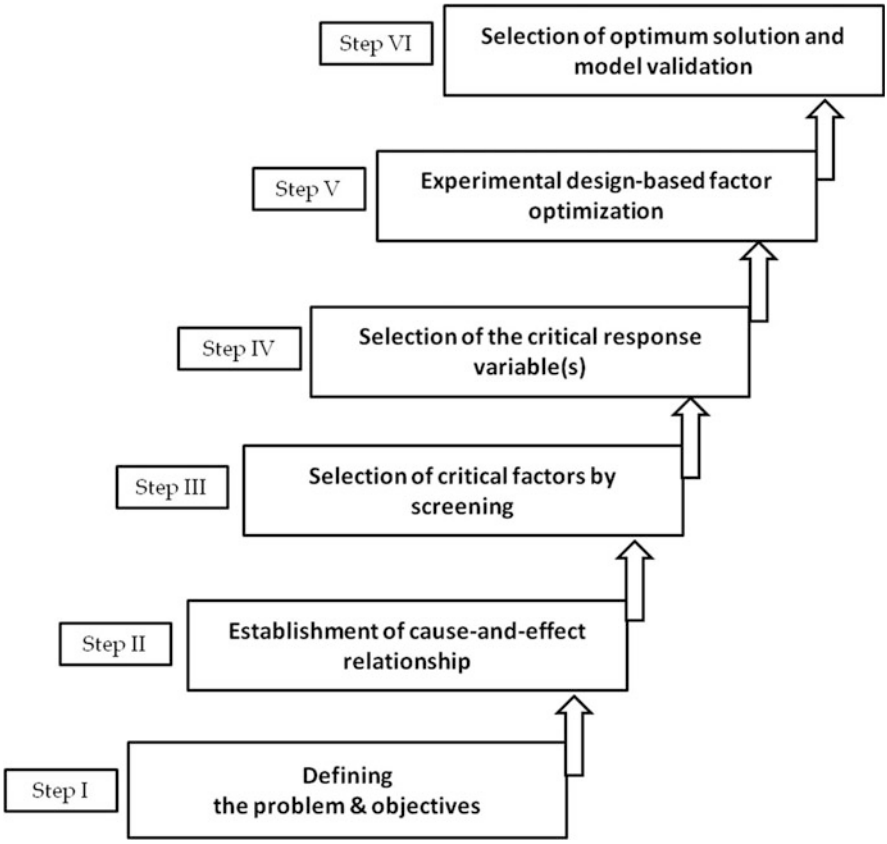

Fig. 1.1 Six-step approach used for implementation of the DoE in pharma product development

1.4.3 Selection of Critical Factors by Screening

When a system or process is new, it is usually important to identify the vital factors which have the most influence on the response(s) of interest. Often there are a lot of factors involved in any pharmaceutical product or process, and the experimenters do not know much about the system. Hence, it is better to perform screening in the beginning of the experiment in order to eliminate any unwanted factors. Usually, prioritization exercise is carried out on the basis of prior knowledge and experience. Also, screening experimental designs are used for identifying suitable factors. Figure 1.2 pictorially depicts a list of commonly used screening experimental designs.

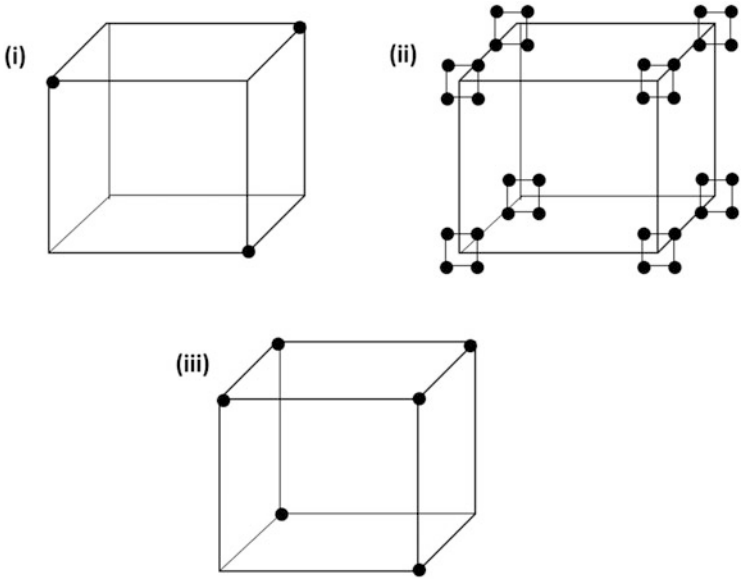

Fig. 1.2 Examples of various experimental designs used for factor screening study, (**a**) fractional factorial design, (**b**) Taguchi design, (**c**) Plackett-Burman design

1.4.4 Selection of the Critical Response Variable(s)

The selection of critical response variables is carried out on the basis of product-specific and patient-centric attributes. While selecting the response variable(s), the experimenter must consider useful information related to the impact of input factors on the safety and efficacy of the product. It is ideal to access the likely impact of the factors on the response and measures to mitigate the same.

1.4.5 Experimental Design-Based Factor Optimization

Use of response surface mapping (RSM) designs is very helpful for optimizing the factor effect on the related responses. Figure 1.3 pictorially depicts a list of commonly used screening experimental designs. With the help of a RSM design, a set of experiments are performed containing rational combination of the factors. The obtained data is subjected to analysis by fitting with various mathematical models such as linear, quadratic, cubic, and quartic models. A suitable model was chosen based on the validity of the model for statistical parameters like correlation coefficient, predicted error, and lack of fit analysis.

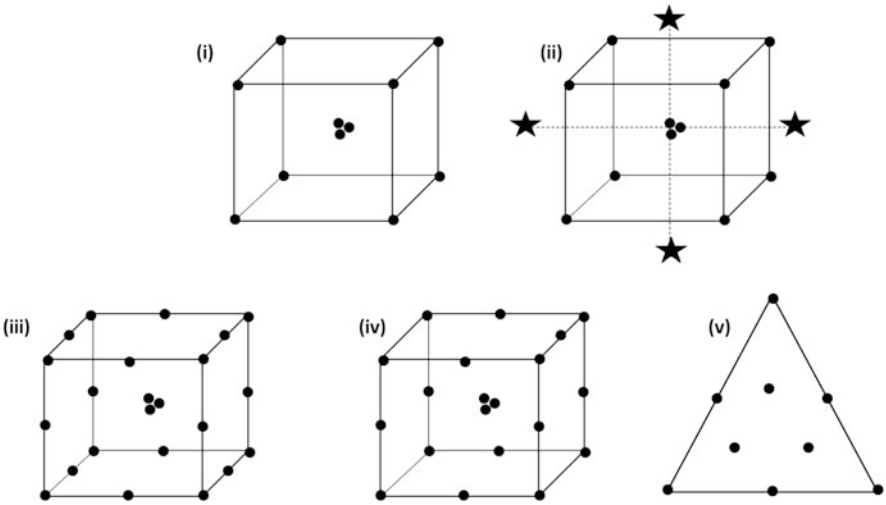

Fig. 1.3 Examples of various experimental designs used for factor screening study, (**a**) full factorial design, (**b**) central composite design, (**c**) Box-Behnken design, (**d**) optimal design, (**e**) mixture design

1.4.6 Selection of Optimum Solution and Model Validation

The selection of optimum solution is carried out on the basis of varied tools like graphical optimization (by brute-force method, overlay plots) and numerical optimization (by desirability and objective function) and artificial neural networks (ANN) (Fig. 1.4). The optimum solution is usually demarcated in the design space which is the narrower region within the knowledge space, as shown in Fig. 1.5.

1.5 Experimental Design Selection and Types

The approach of experimental design selection depends on the nature of experiment, number of factors, and flexibility of conducting the number of experiments. Design selection also depends on the selection of empirical model to describe statistical cause-and-effect relationship. Experimental designs are basically classified into two types such as the screening designs and the response surface designs [1, 8, 9]. The details regarding various experimental designs have been discussed below.

1.5.1 Screening Designs

Screening designs are an efficient way to identify the significant main effects of the factors. The term "screening" refers to an experimental plan that is intended to find the few significant factors from a list of many potential ones. Alternatively, a

Fig. 1.4 Various optimum search techniques used for experimental design-based optimization

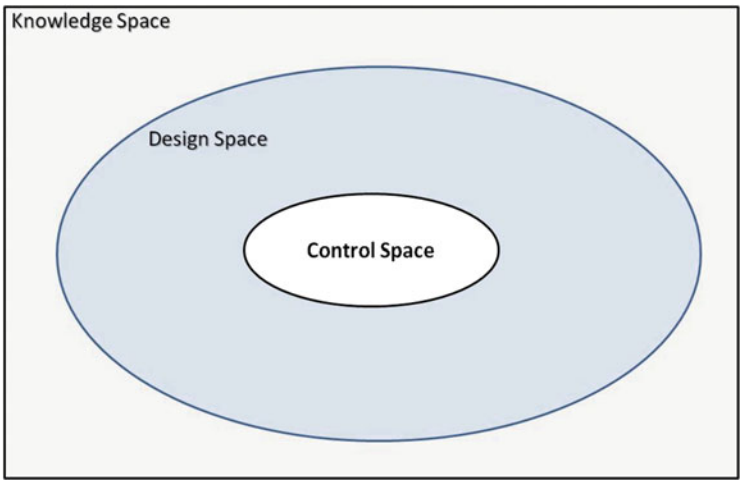

Fig. 1.5 Various types of spaces encountered during experimental design optimization

screening design is primarily used to identify significant main effects only, while the interaction effects are biased due to confounding nature of the factors. Fractional factorial design, Taguchi design, and Plackett–Burman design are primarily used for the purpose of factor screening.

1.5.1.1 Fractional Factorial Design

FFDs are recommended when the number of factors in an experiment ranges between 3 and 7. Such designs require two levels (−1 and +1) for preparing a matrix with combination of the factors. FFDs are expressed by X^{k-p}, where "X" indicates the number of levels and "k" indicates the number of factors and p describes the fractionation used.

1.5.1.2 Taguchi Design

Such design relies on the use of orthogonal arrays which provide a set of well balanced (minimum) experiments and serve as objective functions for optimization. Taguchi design starts with minimum of three factors and produces four experimental runs at two levels without requisite of any center point runs.

1.5.1.3 Plackett–Burman Design

Plackett–Burman design (PBD) is used for screening study for a minimum of 11 factors and produces 12 experimental runs. The number of runs (n) produced by the design is produced in the multiple of 4, 8, 12, 24. Hence, PBD is useful in screening factors in an experiment with large number of factors.

1.5.2 Response Surface Designs

Response surface designs or optimization design are used for optimizing the critical factors identified by factor screening. Such design employs nonlinear models like quadratic, cubic, and quartic models. Select instances of the response surface designs include full factorial design, central composite design, Box–Behnken design, optimal design, and mixture designs, which have been discussed below.

1.5.2.1 Full Factorial Design

These designs are represented by X^k, where X indicates number of factors and k indicates number of levels. A full factorial design may also be called a fully crossed design. A full factorial design generates experimental runs based on the factorial points and generates a linear polynomial model. Moreover, center points can also be placed in a full factorial design to increasing its power for better predictability. Such design is also capable of identifying the main effect as well as interaction effects.

1.5.2.2 Central Composite Design

Central composite design (CCD) is an effective statistical design which provides information exclusively on the effect of experiment variables. It is widely used for response surface optimization of the experiments, which employs second-order (quadratic) model for the response variable without using a complete three-level factorial experiment. It is employed when factorial designs detect the presence of curvature in the data, thus requires augmentation from an erstwhile linear design to the quadratic response surface design.

1.5.2.3 Box–Behnken Design

Box–Behnken design (BBD) is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require three levels of each factor. The designs have limited capability for orthogonal blocking compared to the central composite designs.

1.5.2.4 Optimal Designs

The optimality of a design depends on the statistical model and is assessed with respect to a statistical criterion, which is related to the variance-matrix of the estimator. Specifying an appropriate model and specifying a suitable criterion function both require understanding of statistical theory and practical knowledge with designing experiments. Further, optimal designs are of different types such as D-optimal, I-optimal, and A-optimal. These designs utilize three levels for each of the selected factors and are most commonly used for factor optimization study.

1.5.2.5 Mixture Designs

In a mixture experiment, the independent factors are proportions of different components of a blend. In another way, the fact that the proportions of the different factors must sum to 100% complicates the design as well as the analysis of mixture experiments. Mixture designs can be of different types such as simplex lattice designs, simplex centroid designs, and optimal designs. Among these variants of mixture designs, optimal designs are most commonly used for optimization of factors. Further, optimal designs are of different types such as D-optimal, I-optimal, and A-optimal. These designs utilize three levels for each of the selected factors and are most commonly used for factor optimization study.

1.6 Applications of DoE in Drug Product Development

For the development of pharmaceutical drug products, the use of systematic approaches for product development has now become a routine practice. Federal regulatory authorities are bringing new regulations for efficient, effective, and cost-

effective product development techniques for ultimately improving the cost of drug products to make them affordable without compromising their quality, safety, and efficacy [10, 11]. DoE, a multivariate approach to product development, has invariably proved its worth for producing robust drug products. Hence, applicability of DoE is possible at all stages of product development, which also helps in ultimately saving time, efforts, and resources. A variety of pharmaceutical products containing multitude of excipients for diverse functional applications for oral and non-oral drug delivery have been developed.

1.6.1 Oral Drug Delivery Systems

A variety of oral drug delivery systems have been developed using the systematic DoE approach. Such delivery systems include tablets, capsules, powders, granules, pellets, microspheres, etc., while liquid dosage forms include dry syrups, emulsions, suspensions, etc. Select instances of the literature reports available on DoE optimization of oral drug delivery systems have been described in Table 1.1.

1.6.2 Non-oral Drug Delivery Systems

A variety of non-oral drug delivery systems have been developed using the systematic DoE approach, which include dosage forms such as parenteral, topical, transdermal, ocular, otic, and inhalational preparations. Select instances of the literature reports on DoE optimization of non-oral drug delivery systems have been provided in Table 1.2.

1.7 Conclusion and Future Perspectives

In a holistic development practice, product and process understanding are the twin keystones of DoE approach. The more the formulator knows about the system, the better he can define it, and the higher precision he can monitor it with. As with any other coherent scientific methodology, DoE also requires holistic envisioning of the formulation development characteristics. Notwithstanding the enormous utility of this DoE-based philosophy in developing optimal solutions, it leads research mindsets to evolve the "out-of-box" strategies too. Besides, the current regulations laid by ICH through Q8 guidance highlight the pharmaceutical development using Quality by Design (QbD) principles. QbD particularly emphasizes on the application of DoE tools for systematic product development practice. Hence, the regulatory importance of DoE has augmented manifold in attaining efficient pharmaceutical development.

Table 1.1 Examples of the DoE-based optimization of oral drug delivery systems

Drug	Experimental design	Key findings	References
Immediate/modified release tablets			
Pioglitazone	Box–Behnken design	Enhancement in the oral bioavailability of the drug	[12]
Cefuroxime axetil	Central composite design	Improved gastroretentive ability and bioavailability	[13]
Amoxicillin trihydrate	Box–Behnken design	Pulsatile drug release behavior and improved oral bioavailability	[14]
Zidovudine and lamivudine	Central composite design	Improved gastroretentive ability and bioavailability	[15]
Cinnarizine	Central composite design	Enhancement in the bioavailability due to the prolonged gastric residence time	[16]
Meloxicam	Response surface design	Resulted in positive effects on disintegration time, wetting time, and mechanical strength	[17]
Deacetyl mycoepoxydience	Central composite design	Got improved dissolution and oral bioavailability of the DM	[18]
Microspheres/microparticulate systems			
Lacidipine	Central composite design	Improved stability, extending the core's shelf life, and providing a sustained and controlled release	[19]
Rabeprazole sodium	Box–Behnken design	Controlled drug release profile and acid resistance	[20]
Itopride hydrochloride	Box–Behnken design	Improved gastroretentive ability and bioavailability	[21]
Riboflavin sodium phosphate	Response surface design	It provides optimum encapsulation and better gastric stability	[22]
Etoposide	Factorial design	Received better stability and enhanced intracellular drug delivery	[23]
Paclitaxel	Taguchi design	Improved drug loading capacity and reversal from cancer drug resistance	[24]
Nanoemulsifying drug delivery systems			
Rosuvastatin calcium	D-optimal mixture design	Enhancement in the bioavailability and lymphatic targeting ability of the drug	[25]
Valsartan	Central composite design	Improvement in the dissolution rate and drug absorption	[26]
Lovastatin	Face-centered cubic design	Improvement in the dissolution rate and drug absorption	[27]
Olmesartan medoxomil	D-optimal mixture design	Enhancement in the bioavailability and lymphatic targeting ability of the drug	[28]
Irbesartan	Box–Behnken design	Improvement in the dissolution rate and drug absorption	[29]

(continued)

Table 1.1 (continued)

Drug	Experimental design	Key findings	References
Paclitaxel	D-optimal mixture design	Enhancement in the bioavailability and lymphatic targeting ability of the drug	[30]
Ondansetron hydrochloride	Box–Behnken design	Improvement in the dissolution rate and drug absorption	[31]
Lopinavir and darunavir	D-optimal mixture design	Enhancement in the bioavailability and lymphatic targeting ability of the drug	[32]
Mangiferin	D-optimal mixture design	Enhancement in the bioavailability and lymphatic targeting ability of the drug	[33]
Tamoxifen and naringenin	D-optimal mixture design	Improvement in the dissolution rate and drug absorption	[34]
Candesartan cilexetil	D-optimal mixture design	Improvement in the drug absorption performance	[35]
Lovastatin	Box–Behnken design	Improvement in the dissolution rate and drug absorption	[36]
Nanoparticulate delivery systems			
Olmesartan medoxomil	Box–Behnken design	Achievement in the intestinal lectin receptor targeting of the drug	[37, 38]
Ganoderic acid	Box–Behnken design	Improvement in the biopharmaceutical attributes of the drug	[39]
Rosuvastatin	Box–Behnken design	Improvement in the LDL-receptor targeting of the drug	[40]
Lopinavir	Central composite design	Enhancement in the biopharmaceutical performance of the drug	[41]
Resveratrol	Box–Behnken design	Improvement in the biopharmaceutical attributes of the drug	[42, 43]
Smilax alba Smilax china	Box–Behnken design	Improvement in the biopharmaceutical performance of the drug	[44]
Moxifloxacin hydrochloride	Central composite design	Reduction in the periodontal infection with the application of the drug	[45]

Table 1.2 Examples of the DoE-based optimization of non-oral drug delivery systems

Drug	Experimental design	Dosage form	Key findings	Reference
Topical drug delivery systems				
Isotretinoin	Response surface design	Topical gel	The nanocarrier showed good entrapment, while the size was found to be in the nanosized range	[46]
Aceclofenac	Response surface design	Topical gel	The resulted gel formulation got improved permeation profile and exhibited excellent anti-inflammatory action	[47]
Methoxsalen	D-optimal mixture design	Topical gel	The results indicated improvement in the skin permeability and retention of the drug	[48]
Lidocaine and prilocaine	D-optimal mixture design	Topical gel	The results indicated improvement in the skin permeability and retention of the drug	[49]
Transdermal drug delivery systems				
Dexibuprofen	Response surface design	Transdermal patches	The result of optimized formulation showed uniform thickness, low moisture uptake, and highly acceptable drug loading	[50]
Diflunisal	Central composite design	Nanolipidic carriers	The optimized formulation showed smaller particle size, high entrapment, and better skin retention ability	[51]
Glimepiride	Plackett–Burman design	Transdermal glimepiride liposomal films.	The optimized formulation obtained maximum entrapment capacity and optimum drug release	[52]
Injectable drug delivery systems				
Valrubicin	Response surface design	Injectable nanoparticle	Particle size observed in nano range with high formulation stability	[53]
Small interfering RNA	Response surface design	PLGA nanoparticles	Encapsulation efficiency was increased with sustained drug release profile	[54]
Ocular drug delivery systems				
Moxifloxacin hydrochloride	Response surface design	Solid lipid nanoparticles	Improved efficiency and sustained drug release behavior	[55]
Curcumin	Central composite design	Nanostructured lipid carriers	Enhanced stability and permeation of curcumin	[56]

(continued)

Table 1.2 (continued)

Drug	Experimental design	Dosage form	Key findings	Reference
Intranasal drug delivery systems				
Levodopa	Response surface design	Microparticles inhaler	Smaller particle size, better dissolution, and permeability of the drug	[57]
Lorazepam	Response surface design	In-situ gelling microemulsion via intranasal route	High formulation stability, drug loading, and controlled drug release behavior	[58]

References

1. Singh B, Raza K, Beg S (2013) Developing "optimized" drug products employing "designed" experiments. Chem Ind Dig 23:70–76
2. Singh B, Saini S, Lohan S, Beg B (2017) Systematic development of nanocarriers employing quality by design paradigms. In: Mishra, Kesherwani PK, Amin MIM (eds) Nanotechnology-based approaches for targeting and delivery of drugs and genes. Academic Press, New York, pp 110–148
3. Beg S, Swain S, Rahman M et al (2019) Application of design of experiments (DoE) in pharmaceutical product and process optimization. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic Press, New York, pp 43–64
4. Beg S, Rahman M, Panda SS (2017) Pharmaceutical QbD: omnipresence in the product development lifecycle. Eur Pharm Rev 22:58–64
5. Beg S, Hasnain MS (2019) Pharmaceutical quality by design: principles and applications. Academic Press, New York
6. Beg S, Rahman M, Robaian MA et al (2020) Pharmaceutical drug product development and process optimization: effective use of quality by design. CRC Press, New York
7. Singh B, Beg S (2013) Quality by design in product development life cycle. Chron Pharmabiz 22:72–79
8. Beg S, Hasnain MS, Rahman M et al (2019) Chapter 1 - introduction to quality by design (QbD): fundamentals, principles, and applications. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic Press, New York, pp 1–17
9. Beg S, Rahman M, Swain S (2020) Quality by design applications in pharmaceutical product development. Pharma Focus Asia, pp. 1–5
10. Beg S, Al Robaian M, Rahman M, Imam SS, Alruwaili N, Panda SK (2020) Pharmaceutical drug product development and process optimization: effective use of quality by design. CRC Press, Boca Raton, FL
11. Taleuzzaman M, Chuhan S, Gilani SJ, Imam SS, Beg S (2020) Systematic product and process development tools in life cycle management. In: Pharmaceutical drug product and process optimization. CRC Press, Boca Raton, FL, pp 33–52
12. Bonthagarala B, Dasari V, Kotra V, Swain S, Beg S (2019) Quality-by-design based development and characterization of pioglitazone loaded liquisolid compact tablets with improved biopharmaceutical attributes. J Drug Deliv Sci Technol 51:345–355
13. Bansal S, Beg S, Garg B, Asthana A, Asthana GS, Singh B (2016) QbD-oriented development and characterization of effervescent floating-bioadhesive tablets of cefuroxime axetil. AAPS PharmSciTech 17(5):1086–1099

14. Beg S, Swain S, Gahoi S, Kohli K (2012) Design, development and evaluation of chronomodulated drug delivery systems of amoxicillin trihydrate with enhanced antimicrobial activity. Curr Drug Deliv 10(2):174–187
15. Singh B, Garg B, Bhatowa R, Kapil R, Saini S, Beg S (2017) Systematic development of a gastroretentive fixed dose combination of lamivudine and zidovudine for increased patient compliance. J Drug Deliv Sci Technol 37:204–215
16. Sethi S, Mangla B, Kamboj S et al (2018) A QbD approach for the fabrication of immediate and prolong buoyant cinnarizine tablet using polyacrylamide-g-corn fibre gum. Int J Biol Macromol 117:350–361
17. Iurian S, Tomuta I, Bogdan C et al (2016) Defining the design space for freeze-dried orodispersible tablets with meloxicam. Drug Dev Ind Pharm 42:1977–1989
18. Du J, Zhou Y, Wang L et al (2018) Deacetyl mycoepoxydience nanocrystals dispersible tablets formulation and in vitro study. J Nanosci Nanotechnol 18:3850–3855
19. Sultana S, Bhavna, Iqbal Z et al (2009) Lacidipine encapsulated gastroretentive microspheres prepared by chemical denaturation for Pylorospasm. J Microencapsul 26:385–393
20. Swain S, Behera UA, Beg S, Sruti J, Patro CN, Dinda SC, Rao MEB (2012) Design and characterization of enteric-coated controlled release mucoadhesive microcapsules of Rabeprazole sodium. Drug Dev Ind Pharm 39(3):468–480
21. Bansal S, Beg S, Asthana A, Garg B, Asthana GS, Kapil R, Singh B (2016) QbD-enabled systematic development of gastroretentive multiple-unit microballoons of itopride hydrochloride. Drug Deliv 23(2):437–451
22. Zhang AY, Fan TY (2009) Optimization of riboflavin sodium phosphate loading to calcium alginate floating microspheres by response surface methodology. Beijing Da XueXue Bao 41:682–686
23. Yadav KS, Sawant KK (2010) Formulation optimization of etoposide loaded PLGA nanoparticles by double factorial design and their evaluation. Curr Drug Deliv 7:51–64
24. Dong X, Mattingly CA, Tseng M et al (2009) Development of new lipid-based paclitaxel nanoparticles using sequential simplex optimization. Eur J Pharm Biopharm 72:9–17
25. Beg S, Katare OP, Singh B (2017) Formulation by design approach for development of ultrafine self-nanoemulsifying systems of rosuvastatin calcium containing long-chain lipophiles for hyperlipidemia management. Colloids Surf B Biointerfaces 159:869–879
26. Bandyopadhyay S, Beg S, Katare OP et al (2015) QbD-oriented development of self-nanoemulsifying drug delivery systems (SNEDDS) of valsartan with improved biopharmaceutical performance. Curr Drug Deliv 12:544–563
27. Beg S, Sandhu PS, Batra RS et al (2015) QbD-based systematic development of novel optimized solid self-nanoemulsifying drug delivery systems (SNEDDS) of lovastatin with enhanced biopharmaceutical performance. Drug Deliv 22:765–784
28. Beg S, Sharma G, Thanki K, Jain S, Katare OP, Singh B (2015) Positively charged self-nanoemulsifying oily formulations of olmesartan medoxomil: systematic development, in vitro, ex vivo and in vivo evaluation. Int J Pharm 493(1–2):466–482
29. Swain S, Beg S, Sahu PK, Jena BR, Babu SM (2019) Formulation development, statistical optimization and characterization of the self-microemulsifying drug delivery system (SMEDDS) of irbesartan. Nanosci Nanotechnol Asia 9(2):210–228
30. Beg S, Kaur R, Khurana RK, Rana V, Sharma T, Singh B (2019) QbD-based development of cationic self-nanoemulsifying drug delivery systems of paclitaxel with improved biopharmaceutical attributes. AAPS PharmSciTech 20(3):118
31. Beg S, Jena SS, Patra CN, Rizwan M, Swain S, Sruti J, Rao ME, Singh B (2012) Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. Colloids Surf B: Biointerfaces 101:414–423
32. Garg B, Beg S, Kaur R, Kumar R, Katare OP, Singh B (2018) Long-chain triglycerides-based self-nanoemulsifying oily formulations (SNEOFs) of darunavir with improved lymphatic targeting potential. J Drug Target 26(3):252–266

33. Khurana RK, Beg S, Burrow AJ, Vashishta RK, Katare OP, Kaur S, Kesherwani P, Singh KK, Singh B (2017) Enhancing biopharmaceutical performance of an anticancer drug by long chain PUFA based self-nanoemulsifying lipidic nanomicellar systems. Eur J Pharm Biopharm 121:42–60
34. Sandhu PS, Kumar R, Beg S, Jain S, Kushwah V, Katare OP, Singh B (2017) Natural lipids enriched self-nano-emulsifying systems for effective co-delivery of tamoxifen and naringenin: systematic approach for improved breast cancer therapeutics. Nanomedicine 13(5):1703–1713
35. Sharma G, Beg S, Thanki K, Katare OP, Jain S, Kohli K, Singh B (2015) Systematic development of novel cationic self-nanoemulsifying drug delivery systems of candesartan cilexetil with enhanced biopharmaceutical performance. RSC Adv 5(87):71500–71513
36. Beg S, Sandhu PS, Batra RS, Singh B (2015) QbD-based systematic development of novel optimized solid self-nanoemulsifying drug delivery systems (SNEDDS) of lovastatin with enhanced biopharmaceutical performance. Drug Deliv 22(6):765–784
37. Beg S, Choudhry H, Zamzami MA, Alharbi KS, Rahman M, Singh B (2018) Nanocolloidal lipidic carriers of olmesartan medoxomil surface-tailored with Concanavalin-A for lectin receptor targeting. Nanomedicine 13(24):3107–3128
38. Beg S, Saini S, Bandopadhyay S, Katare OP, Singh B (2018) QbD-driven development and evaluation of nanostructured lipid carriers (NLCs) of olmesartan medoxomil employing multivariate statistical techniques. Drug Dev Ind Pharm 44(3):407–420
39. Rahman M, Beg S, Alharbi KS, Alruwaili NK, Alotaibi NH, Alzarea AI, Almalki WH, Alenezi SK, Altowayan WM, Alshammari MS, MAfzal S, Saleem VK (2020) Implications of solid lipid nanoparticles of ganoderic acid for the treatment and management of hepatocellular carcinoma. J Pharm Innov. https://doi.org/10.1007/s12247-020-09450-4
40. Beg S, Jain S, Kushwah V, Bhatti GK, Sandhu PS, Katare OP, Singh B (2017) Novel surface-engineered solid lipid nanoparticles of rosuvastatin calcium for low-density lipoprotein-receptor targeting: a quality by design-driven perspective. Nanomedicine 12(4):333–356
41. Garg B, Beg S, Kumar R, Katare OP, Singh B (2019) Nanostructured lipidic carriers of lopinavir for effective management of HIV-associated neurocognitive disorder. J Drug Deliv Sci Technol 53:101220
42. Poonia N, JK Narang V, Lather S, Beg T, Sharma B, Singh DP (2019) Resveratrol loaded functionalized nanostructured lipid carriers for breast cancer targeting: systematic development, characterization and pharmacokinetic evaluation. Colloids Surf B: Biointerfaces 181:756–766
43. Poonia N, Lather V, Narang JK, Beg S, Pandita D (2020) Resveratrol-loaded folate targeted lipoprotein-mimetic nanoparticles with improved cytotoxicity, antioxidant activity and pharmacokinetic profile. Mater Sci Eng C 114:111016
44. Qadir A, Aqil M, Ali A, Warsi MH, Mujeeb M, Ahmad FJ, Ahmad S, Beg S (2020) Nanostructured lipidic carriers for dual drug delivery in the management of psoriasis: systematic optimization, dermatokinetic and preclinical evaluation. J Drug Deliv Sci Technol 57:101775
45. Beg S, Dhiman S, Sharma T, Jain A, RK Sharma A, Jain BS (2020) Stimuli responsive in situ gelling systems loaded with PLGA nanoparticles of moxifloxacin hydrochloride for effective treatment of periodontitis. AAPS PharmSciTech 21(3):76
46. Raza K, Singh B, Singal P et al (2013) Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. Colloids Surf B Biointerfaces 105:67–74
47. Jana S, Ali SA, Nayak AK et al (2014) Development of topical gel containing aceclofenac-crospovidone solid dispersion by "quality by design (QbD)" approach. Chem Eng Res Des 92:2095–2105
48. Garg BJ, Garg NK, Beg S, Singh B, Katare OP (2016) Nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: formulation optimization, in vitro evaluation and preclinical assessment. J Drug Target 24(3):233–246

49. Negi P, Singh B, Sharma G, Beg S, Raza K, Katare OP (2016) Phospholipid microemulsion-based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD-based development and evaluation. Drug Deliv 23(3):941–957
50. Akhlaq M, Arshad MS, Mudassir AM et al (2016) Formulation and evaluation of anti-rheumatic dexibuprofen transdermal patches: a quality-by-design approach. J Drug Target 24:603–612
51. Kaur A, Bhoop BS, Chhibber S et al (2017) Supramolecular nano-engineered lipidic carriers based on diflunisal-phospholipid complex for transdermal delivery: QbD based optimization, characterization and preclinical investigations for management of rheumatoid arthritis. Int J Pharm 533:206–224
52. Ahmed OAA, Kurakula M, Banjar ZM et al (2015) Quality by design coupled with near infrared in formulation of transdermal glimepiride liposomal films. Int J Pharm Sci 104:2062–2075
53. De T, Miller T, Yim Z et al (2018) Abstract 3718: an injectable nanoparticle formulation of valrubicin. Cancer Chem 78(13):3718. https://doi.org/10.1158/1538-7445.AM2018-3718
54. Cun D, Jensen DK, Maltesen MJ (2011) High loading efficiency and sustained release of siRNA encapsulated in PLGA nanoparticles: quality by design optimization and characterization. Eur J Pharm Biopharm 77:26–35
55. Khurana LK, Singh R, Singh H et al (2018) Systematic development and optimization of an in-situ gelling system for moxifloxacin ocular nanosuspension using high-pressure homogenization with an improved encapsulation efficiency. Curr Pharm Des 24:1434–1445
56. Lakhani P, Patil A, Taskar P (2018) Curcumin-loaded nanostructured lipid carriers for ocular drug delivery: design optimization and characterization. J Drug Deliv Sci Technol 47:159–166
57. Bartos C, Pallagi E, Szabó-Révész P et al (2018) Formulation of levodopa containing dry powder for nasal delivery applying the quality-by-design approach. Eur J Pharm Sci 123:475–483
58. Shah V, Sharma M, Pandya R et al (2017) Quality by design approach for an in situ gelling microemulsion of Lorazepam via intranasal route. Mater Sci Eng C Mater Biol Appl 75:1231–1241

Design of Experiments for the Development of Solid Oral Dosage Forms

2

Sarwar Beg, Suryakanta Swain, and Bikash Ranjan Jena

Abstract

Design of Experiments (DoE) has now been considered as an indispensible tool in the pharmaceutical product development. Among a variety of dosage forms, the solid dosage forms are one of the most versatile delivery systems for diverse therapeutic applications. Such dosage forms include tablets, capsules, granules, pellets, and many more, where manufacturing of such dosage forms include multifunctional excipients and a number of unit operations are also involved. Hence, the use of DoE has definite merits for efficient development of the solid dosage forms with ultimate advantage of saving the resources and attaining the robust product quality and regulatory flexibility. In this regard, the present chapter provides an overview account on the manufacturing of solid dosage forms, and particularly highlights the current challenges and opportunity associated with them.

Keywords

Experimental designs · Solid dosage forms · Product quality · Quality attributes · Robustness · Regulatory flexibility

S. Beg (✉)
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India

S. Swain
School of Health Sciences, Department of Pharmaceutical Science, Kaziranga University, Jorhat, Assam, India

B. R. Jena
Southern Institute of Medical Sciences, College of Pharmacy, SIMS Group of Institutions, Guntur, Andhra Pradesh, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_2

2.1 Introduction

The modern era based pharmaceutical drug innovation and its incessant development is an extremely hard-hitting task, costly, and challenging judgment to the research scientist during manufacturing. It requires nonstop efforts, advanced systematic industrial technologies to develop the novel molecules to the society and common people. Current approach of designing the drug therapeutic systems aim for dispensing the *right* medication at *right* time at *right* target site. Such systems, therefore, provide enhanced efficacy through instant drug delivery in a continual and site-specific manner. After remaining shrouded for several years, the designing of drug delivery systems is fast evolving to yield enormous benefits to the patients as well as society [1]. However, the solid dosage manufacturing has established their incredible approaches for transforming the effectiveness in theranostics. The therapeutic or clinical applications entail the extensive patients as well as the consent from regulatory agencies. Therefore the researcher scientists need to develop such dosage forms that are conspicuously better than the existing ones. This in turn causes the co-ordinate effort among scientific researchers and taking up the methodical approaches for raising such dosage forms [2–4].

A plethora of variable excipients and selected drug substances with proper understating of their critical quality attributes and critical process parameters are required to develop the solid oral dosages. A control strategy may be implemented to optimize the formulation composition manufacturing process, desired product quality performance. The traditional approach of optimizing a formulation or process essentially entails studying the influence of one variable at a time (OVAT), while keeping others as constant. Using the OVAT ideology, only a single aspect is investigated, while the system as a whole remains unexplored. With this novel approach, the clarification of a number of hard assets can be accomplished, but the accomplishment of correct optimum composition is never acceptable. This may be attributed to the incidence of interactions, i.e., the impact of one or more factors upon others. The concluding product may be satisfactory but frequently suboptimal range, since an improved formulation might still subsist for the deliberate conditions. The applied OVAT methodology come to blows in "just satisfactory" solutions, as a detailed study of all variables is prohibitive. As it is hard to establish "cause and effect" relationships using traditional techniques, this becomes highly challenging to simultaneously consider all the variables [5, 6].

Several challenges occur due to the inconsistent product quality and robustness in batch-to-batch performance due to inadequate knowledge of causal relationships among the factors and responses during the OVAT approach. However, the systematic optimization approaches applying the multivariate techniques are widely practiced to alleviate such tribulations. These coherent approaches comprise of the application of Design of Experiments (DoE) coupled with multivariate statistical analysis as a function of the factor(s). These chemometric tools are very useful over the traditional approaches with several shortcomings, in order to provide number of

benefits. DoE helps in performing both the factor screening to identify the "influential" factors and "optimization" of them. One can replicate product and process performance with huge advantages of saving resources [7–10].

2.2 DoE Application for Developing the Solid Dosage Forms

The principles of DoE can be applied in diverse facets of the product development and for enhancing robustness of the dosage forms. DoE includes systematic understanding of the product performance by optimizing the corresponding product attributes and process parameters. By comprehending the critical parameters in the formulation, especially the solid oral drug products, DoE helps in applying systematic, scientific, and technological principles to match the product and process performance for innovation and growth. A complete study assessment of a conventional advancement approach with DoE enhanced principles is also crucial for designing the solid dosage forms. The physiological, chemical, and pharmacological attributes of the drug substance and excipients are ultimately responsible for governing pharmaceutical products' quality. The pharmaceutical product advancement plan's critical objectives using DoE need to be accomplished for the patient. Understanding of constituent is an add-on advantage of a variety of dosage forms for harnessing holistic essence of the concept. A more challenging perceptive of pharmaceutical solid dosage form manufacturing is high degree of variability encountered due to variation in the critical quality attributes, where DoE provides customized solutions and ultimate benefits for developing the optimized products [10].

2.2.1 In the Development of Tablet Dosage Forms

Execution of DoE approach during the formulation development can remove defects in tablets and can improve the robustness of the tablet formulations. Hence, for the formulation scientists, faster advancement to conquer time-taking process in developed phase can be might influential to resolve the formulation tribulations at a pilot scale level. In order to exterminate these time-taking issues during production of tablet dosage form, a DoE paradigm draws closer within finest protection during scale-up to produce the product with enhanced stability, quality, and efficacy. Hence it is most vital for OTC prescription, (generic-drug formulators) to bring on the solid dosage products in to the market. As confirmed in the ICH guidelines that on series of pharmaceuticals, ICH Q8 [R2], "the endeavor of pharmaceutical growth is to plan a quality product and its industrialized feasibility for large scale manufacturing by maintaining the same quality and efficacy." It was previously documented that all the noteworthy philosophy completely performed as per ICH recommended stability (Q8, Q9, and Q10) and as per patient compliance [10].

Table 2.1 Examples of the important input variables (process parameters) and their impact on the used for the three widely used tablet manufacturing

Tablet manufacturing techniques	Critical process parameters	Critical quality attributes
Direct compression	• Pre-compression force • Tablet weight and height • Punch design and geometry • Machine speed and forced compression	• Weight uniformity • Hardness • Appearance • Friability • Tablet weight • Disintegration • Dissolution
Wet granulation	• Configuration of impeller, chopper speed of mixer granulator • Binder addition time	• Uniformity of mixture • Moisture content, uniformity, impurity study
Roller compaction	• Speed of roller • Pressure of roller & types • Gap arrangements	• Ribbon density, thickness, and hardness • Particle size of the granules

USFDA is concerned on "Quality systems originated to build up the cGMP compliance" which encloses DoE principles for robust and effective product optimization and developments. This philosophy laid the framework for viewpoint of regulatory appraisal in customer credentials. The wide recompense of DoE model in turn forecast the suitable execution of new and pioneering strategies for expansion of new pharmaceutical products like tablet dosage forms which are accepted be secure, cost-effective, and even extra competent for use and their disposal to the market place. The solid oral dosages (e.g., tablets) ought to duly designed, intended to eradicate destructive syndromes; with its ground-breaking implications or significance on the patient centric approach. Undoubtedly, the admired and most convenient dosage forms can be an oral solid dosage form [OSD], which are reasonably less price to the manufacturer and even more profitable for drug making, merchandise during its mass productivity to the society. Table 2.1 enlists the list of significant variables input process parameters and critical quality attributes (CQAs) associated with the industrialized process of tablet dosage forms.

2.2.2 In the Development of Capsule Dosage Form

The hard gelatin capsules cells are known as the premier conventional dosage forms, whereas the empty capsules are supplied, since DoE targets to ascertain a solid manufactured goods and process understanding in order to boost the safety, efficacy, uniqueness as well as appropriate quality of overall product, e.g., Capsule dosage forms. The hard capsule is known to be an absolute excipient for DoE drug design, development, and acceptable variability. The critical quality attributes (CQAs) of capsule dosage forms include the faster filling of capsules in the machineries without any dimensional distinctiveness and mass changeability. Absolute disintegration of

Table 2.2 Examples of the important input variables (process parameters) and their impact on the used for the capsule manufacturing

Manufacturing processes	Critical process parameters	Critical quality attributes
Capsule filling operation	• Speed of rotating tray • Plate of vibration • Design of cones and height • Size of capsule shells and fill volume	• Disintegration & dissolution • Content uniformity • Weight uniformity

capsule cells is one of the significant necessities for targeting of total drug release and enhancement of dissolution profiles so that the disintegration time can be measured as input quality attributes essential for assurance of quality principally during *in vivo* manifestation for empty hard gelatin capsule cells. Based upon the final product's fineness and performance, it is quite important to pursue DoE paradigm due to its interaction on the product variability reported in critical quality parameters such as moisture content, weight which in turn necessitate to be embedded. Table 2.2 provides the list of input material attributes and process parameters, and critical quality attributes associated with the manufacturing of capsule dosage forms.

2.2.3 In the Tablet Coating Process

Tablets coating is conceivably one of the antique procedures of pharmaceutical industries that put into practice yet in extension. The premium sugar-coating process was an adherent scheming process. Mostly the drugs are dissimilar based upon their diverse extraordinary individualities such as its taste, obnoxious odor, modest are little sensitive to light or oxides and few of them are hygroscopic in nature. Because of this tablet coating is the alternative to resolve such troubles in conformist pharmaceutical dosage form. Earlier sugar coating was typically adopted from the confectionary industries. At present, it is replaced with superior film coating with distinct rationale applications, as the sugar-coating procedure was prolong to some extents 3–4 days. Film coating has several advantages like protection against moisture, pH, and taste masking. The overall potential of coating therapeutic dosage (tablets) can be categorized into three prime components: action, capability, and promotion. Film coating intensifies an applications of thin polymer layer to solid dosages so that majority of dosage forms seen are film coated as of accurate coating of immediate discharge of film dosage form, which conclude the discovery of color recognition, enhancement of stability, color, flavor masking, etc. Film coating can increase the method capacity and handling all through mass production. Hence, it has been recognized that coating process in pharma industries is highly challenge. In case of ideal solvents typically quite a lot of excipients are notified like pigments, plasticizer, opacifier, etc. The film coating subjected to the formulation carries a unified polymer that acts as a layer forming agent. Film coating intends deposition of

Table 2.3 Examples of instrumental and process parameters useful during DoE optimization of tablet coating process

Type of tablet coating process	Critical instrumental parameters	Critical process parameters
Conventional pan coating	• Pan design and geometry • Presence of baffles • Pan speed and spray nozzle size	• Humidity of air inlet and outlet • Temperature of air inlet and outlet • Product bed temperature • Air flow rate • Coating solution type
Fluidized bed coating	• Top spray of bottom spray • Blower drive speed • Pan design and geometry	• Humidity of air inlet and outlet • Temperature of air inlet and outlet • Atomization pressure • Product bed temperature • Atomization air pressure • Fluidization pattern • Coating solution type

a layer of coating material and drying a uniform coating formulation on the surface of substrate to form a uniform film. In order to obtain a unified tablets quality and polished coating, proper directive of process-based parameters, design space are enormously significant. An improperly or inadequate developed film coating practice could elevate out multiple defects in tablet productions like color difference twining, chipping, erosion, from particular tablet to tablet, reduced solubility, enhancement, and steadiness of developed product [11]. Table 2.3 specifies a list of important variables input process parameters and critical quality attributes associated with the manufacturing of tablet coating process.

2.2.4 In the Pharmaceutical Pellets Manufacturing

The solid dosage manufacturing occupies a remarkable position in drug research and innovation. Among all the selective dosages, the pellets is intensifying reflection as frequent units operations for possessing unanticipated qualities, including fewer outcome by gastric vacant time, reliable distribution in GI tract leads to increasing rate of drug absorption, with decreasing the irritation chances; which in generalized GI tract area. In contrast to the single-unit dosage forms, the multiple-unit sustained-release dosages like pellets are alleged to encompass numerous favorable paybacks. They can distribute in the GIT, consistently then maximizing drug absorption and reducing peak plasma concentrations, minimizing the risk of local GI tract irritation and dose dumping. The DoE-based enteric coating process development specified capable output which used in scale-up activity. The exact scale-up of pellets strictly relied on absolute process variables optimization, accepting the risk associated with variables and execution of scale-up factor calculation provided by the vendor. During the spray portion, the pellets coating is preferred as an extremely strongest

Table 2.4 Examples of instrumental and process parameters useful during DoE optimization of pellets

Pellet manufacturing process	Critical instrumental parameters	Critical process parameters
Extrusion-spheronization process	• Extruder plate dimension • Extruder sieve geometry • Spheronizer plate dimension and grooves geometry	• Extrusion time and extruder speed • Spheronization time and speed

procedure than that of extra methods of pelletization; since it is concerned with numerous process variables, literal quality characteristics of product. As per literature reviews, a lot of realistic method parameters are involved like humidity, temperature, inlet air flow, atomization air pressure, spray rate, etc., for drug product quality; whereas diameter of nozzle tip, types of filter bags; timings of drying are even significant that specifically intended upon practical skills [11]. Table 2.4 enlists the list of important variables input process parameters and critical quality attributes associated with the manufacturing process of pellet dosage forms.

2.3 Conclusion and Future Directions

In the era of systematic pharmaceutical development, the use of DoE tools has shown tremendous importance for its wider ability by industry and regulatory authorities like FDA, EMA, MHRA, and many more for the science and risk-based holistic manufacturing practice to achieve pharmaceutical innovation. Since solid dosage form development constitutes the majority of the healthcare market, hence the use of systematic tools for producing such dosage forms in an efficient manner with high robustness and negligible variability is quite important. Although there is no specific federal regulatory regulation available for practical implementation of experiential designs, yet the existing ICH Q8, Q9, and Q10 are guiding tools for explaining the current state of regulatory requirements. As per the ICHQ8 and Q9 guidances specifically emphasizes on the critical analytical factors for effectual method control and enhancement, it is extremely crucial to implement DoE into practice in order to achieve rationale understanding of the product and process behavior.

References

1. Singh B, Raza K, Beg S (2013) Developing “optimized” drug products employing “designed” experiments. Chem Ind Dig 23:70–76
2. Singh B, Saini S, Lohan S, Beg B (2017) Systematic development of nanocarriers employing quality by design paradigms. In: Mishra KPK, Amin MIM (eds) Nanotechnology-based approaches for targeting and delivery of drugs and genes. Elsevier, Diego, pp 110–148

3. Beg S, Swain S, Rahman M et al (2019) Chapter 3 - application of design of experiments (DoE) in pharmaceutical product and process optimization. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic, New York, pp 43–64
4. Beg S, Rahman M, Panda SS (2017) Pharmaceutical QbD: omnipresence in the product development lifecycle. Eur Pharm Rev 22:58–64
5. Beg S, Hasnain MS (2019) Pharmaceutical quality by design: principles and applications. Academic, New York
6. Swain S, Jena BR, Madugula D, Beg S (2019) Chapter 6 - application of quality by design paradigms for development of solid dosage forms. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic, New York, pp 72–79
7. Singh B, Beg S (2013) Quality by design in product development life cycle. Chron Pharmabiz 22:72–79
8. Beg S, Hasnain MS, Rahman M et al (2019) Chapter 1 - Introduction to quality by design (QbD): fundamentals, principles, and applications. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic, New York, pp 1–17
9. Beg S, Rahman M, Swain S (2020) Quality by design applications in pharmaceutical product development. Pharm Focus Asia 2020:1–5
10. Beg S, Al Robaian M, Rahman M, SS Imam NA, Panda SK (2020) Pharmaceutical drug product development and process optimization: effective use of quality by design. CRC Press, Boca Raton
11. Taleuzzaman M, Chuhan S, Gilani SJ, Imam SS, Beg S (2020) Systematic product and process development tools in life cycle management. In: Pharmaceutical drug product and process optimization. CRC Press, Boca Raton, pp 33–52

Design of Experiments for the Development of Topical Drug Products

3

Nishtha Chaurawal and Kaisar Raza

Abstract

The systematic development of pharmaceutical products using quality tools is highly essential for attaining robust performance by minimizing variability. Design of experiment (DoE) is now considered as an indispensable tool in pharmaceutical development to obtain quality products. Among a variety of dosage forms, the topical dosage forms are useful for providing a localized therapeutic effect of the drugs. Such dosage forms include liposomes, ethosomes, niosomes, transfersomes, and many more, where the manufacturing of such dosage forms includes multifunctional excipients and a number of unit operations. Hence, the use of DoE provides greater flexibility and is highly effective for the efficient development of topical products with ultimate savings of the resources. The present chapter, therefore, provides a holistic account on the implementation of DoE approach for the manufacturing of topical drug products and also highlights the current challenges and opportunities associated with them.

Keywords

Quality by design · Design of experiment · Topical delivery · Liposomes · SLNs · D-optimal design · Box–Behnken design

N. Chaurawal · K. Raza (✉)
Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Ajmer, Rajasthan, India
e-mail: drkaisar@curaj.ac.in

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_3

3.1 Introduction

Over the years, drug delivery systems are the epitome of the medicines arena. A drug delivery system is defined as a formulation that, after administration, enables the therapeutic agent to reach its site of action and shows therapeutic response without affecting the non-target tissues, cells, and organs. The area of drug delivery systems is broad, which also covers the targeted systems and controlled release systems of drug delivery [1]. Since ancient times, the drug is delivered by the most common traditional route of administration, i.e., oral and parenteral. For more than two decades, the research was preceding to overcome the problems (such as poor absorption, difficulty in dose intake, first pass metabolism) related to the traditional route of drug administration, which leads to the researchers to use the skin as a carrier for the drug delivery. The researchers found that the delivery of drugs via skin can successfully reduce the side effects related to other routes of administrations [2]. The delivery of therapeutic agents via skin was very challenging for the researchers. Since the late 1970s, intensive research has been carried out to understand the mechanism of drug penetration through the skin to enable the design of delivery systems for topical absorption [3]. Skin delivery can be of different types, such as transdermal delivery, dermal delivery, and topical delivery. In recent times, the demand for topical administration of drugs is increasing because of the high patient compliance and topical or systemic delivery of drugs whenever possible [4]. The topical delivery system is defined as the method of application of the formulation to the uppermost layer of the skin for the treatment of local skin diseases. In topical drug delivery, the skin penetration of drugs into the deeper tissues or systemic circulation is unnecessary for the therapeutic response of the drug [3]. There are several conventional topical formulations available in the market, as ascribed in Table 3.1. The topical administration of the active therapeutic agent mostly depends upon its physicochemical properties, its ability to permeate the skin, the structure of carrier, and skin conditions [5]. The formulation's role is also very

Table 3.1 Marketed conventional topical formulations

Drugs	Commercial products	Dosage forms	Indications	Manufacturer
Betamethasone propionate	Diprolene	Cream, ointment	Psoriasis	Merck Sharp & Dohme
Dithranol	Micanol	Cream	Psoriasis	Riemser
Diclofenac sodium	Voltinax	Gel	Anti-inflammatory, analgesic	Naxpar Ltd.
Tacrolimus	Aptac	Ointment	Fungal infections	Naxpar Ltd.
Capsaicin	Zostrix	Cream	Analgesic	Bradley Marketing Sol.
Menthol	Eucerin	Lotion	Dermatitis	Eucerin Ltd.
Neomycin sulfate	Neosporin	Ointment	antibacterial	Johnson & Johnson
Miconazole	Mycoban	Cream	Fungal infection	ICM Pharma

relevant in the topical delivery as the therapeutic efficacy of the drug can be altered by the interaction between the vehicle and the skin. The formulation assists the drug substance to reach the target site and also helps in the management of drug transport, active duration, and dosage integrity. To prepare and design an optimized formulation with the above characteristics, understanding the skin's structural features is also necessary along with the vehicle, which has a crucial role in the drug delivery mechanism. The vehicle super-saturation can improve the thermodynamic activity of the formulation for drug penetration through the skin. Therefore, topical delivery came out as a ray of hope in the treatment of various skin conditions like fungal infections, inflammation, bacterial infections, and skin cancers [6].

However, the conventional topical formulations were unable to provide the desired therapeutic efficacy and showed high degradation of the drug at the skin surface, making scientists research on nanocarriers based topical drug delivery. The nano-drug delivery systems have various applications over conventional drug delivery, such as allowing the drug to cross the skin membrane easily at a shallow dose and enhancing therapeutic efficacy. The nanocarriers encapsulate the drug, which can also retrain its degradation on the skin. Table 3.2 shows various types of nanocarriers designed by the researchers for the topical delivery of active molecules and their specific treatment [7]. Nanodelivery systems are suitable strategies for enhancing the rate and extent of drug penetration and improving drug absorption [8]. These all properties of nanocarriers such as potency, targeting ability, and permeability are depended upon its characterization studies, i.e., size, polydispersity index, percentage of components, and surface charge. Therefore, newer analytical and characterization techniques are necessary to determine these properties related to nanoformulations and control them during production and synthesis. The new drug delivery systems which can be utilized for the skin penetration of therapeutic agent were largely unexplored even after the numerous scientific research in this field. However, to overcome these hurdles, the scientific and systematic approaches were

Table 3.2 Topical drugs encapsulated in nanocarriers

Drugs	Nanocarriers	Indications
Benzyl peroxide	Liposomal gel	Acne
Prednisolone	Magnetic liposomes	Allergic dermatitis
Hydroxyzine	Liposomes	Skin allergy
Idoxuridine	Liposomal gel	Herpes simplex
Methotrexate	Ethosomes	Psoriasis
Dithranol	Liposomes and niosomes	Psoriasis
NB-002	Nanoemulsion	Fungal infection
Tacrolimus	Nanoparticles	Psoriasis
Cyclosporine A	Solid lipid nanoparticles	Atopic dermatitis
Capsaicin	Flexible membrane vesicles	Musculoskeletal pain
Coal tar	Lipid-coated microparticles	Psoriasis
Corticosteroid	Skin-lipid liposomes	UV induced erythema
Finasteride	Liposomes	Acne, androgenetic alopecia

employed by the researchers to optimize the process design and nanoformulations [9, 10]. In topical delivery, the incomplete understanding of process production is the main reason for the researchers to implement Quality by Design (QbD) approach to optimize nano-drug delivery systems, which can lead to the development of optimized nano-based formulations. The main advantage of the QbD concept is the development of high-quality drug products without extensive regulatory oversight [11]. QbD has implemented one of its components called the design of experiment (DoE) to develop an optimized pharmaceutical product. Besides product development, QbD has applications in the process design, robust manufacturing process, quality attributes, regulatory flexibility, and also improves the regulatory communication between industry and regulators. In the topical drug delivery, DoE provides real-time quality control with real-time release and decreases post-approval changes in the formulation. The various topical delivery formulations developed with the implementation of DoE have been discussed further in this chapter [12].

3.2 Advantages of Topical Drug Delivery

The approach related to drug delivery's topical route provides various advantages over other drug administration routes. Figure 3.1 represents the advantages of topical delivery. These include no systemic absorption of drugs, high concentration of drug at the target site, avoidance of the first pass metabolism sustained delivery of the drug, ease of administration, enhancement in bioavailability, a continuation of dosage regimen with short biological half-lives, non-invasive application of formulation, and patient compliance. Topical drug delivery also decreased the

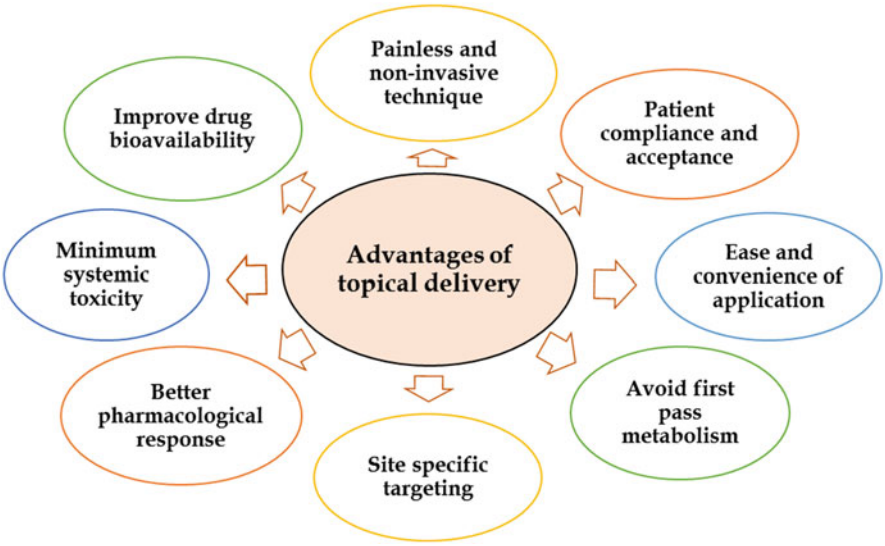

Fig. 3.1 Advantages of topical delivery

peak-associated side effects and ensured a steady-state profile, which helps in the increment of drug level above minimum therapeutic concentration [13]. The topical administration of drugs provides relief in a broad range of therapeutic indications. According to the researchers, various studies showed that the topical delivery of drugs exhibited sustained pharmacological action due to the high rate and extent of drug delivery in a reasonable time to achieve local therapeutic concentration [14]. This can enhance the absorption of drugs compared to other routes of administration [15]. The delivery of nanocarriers via topical administration also possesses several advantages: sustained release of the drug, skin targeting, increased uptake, a base to the bioactive compounds, avoiding direct contact of harmful drugs with the skin, and reduces adverse effects related to the drug. The most crucial application of topical delivery is to treat contagious skin diseases such as atopic dermatitis, skin cancer, and psoriasis. The encapsulation of bioactive agents into nanocarriers also reduces its degradation when coming in contact with the skin at the time of topical delivery [16].

3.3 Challenges in Topical Delivery

The skin is the largest organ of the body and consists of three main layers called the outermost epidermis layer (50–150 μm thick), inner dermis layer (about 250 μm thick), subcutaneous fat tissues. The nutrients maintain their vitality by crossing through dermal–epidermal junction because of the lack of blood vessels in the epidermis. The epidermis is further divided into five different layers named as stratum corneum (SC), stratum lucidum, stratum granulosum (granular layer), stratum spinosum (spinous layer), and stratum germinativum (basal layer). The epidermis with the absence of SC is called the viable epidermis, which is responsible for the barrier nature of skin for topical drug delivery, and most of the cutaneous disorders are also found in it [17]. The transportation of substances from the skin occurs mainly via three important routes by the passive diffusion process. The transportation routes are transcellular, intercellular, and trans-appendageal route [18]. As suggested by various researchers, the most effective penetration route for topical drug delivery is the trans-appendageal route, and some drugs also used the intercellular pathway between the corneocytes to cross the skin membrane [19].

The penetration of substances from hair follicles is also possible. It was demonstrated that skin penetration through the hair follicle pathway could enhance the penetration and absorption of bioactive agents for drug delivery. Hair follicles also work as a center for the depot formation, which can multiply the SC storage capabilities [20]. Figure 3.2 represents all the major routes of topical delivery of drugs. Instead of these routes of drug transportation via the skin, the protein transporters present in the skin also play a vital role in the transportation of substances. Therefore, the localization and expression of various tight junction proteins present at the epithelial layers and hair follicles have been altered during the disease stage of the skin, such as psoriasis. The main barrier in topical delivery is

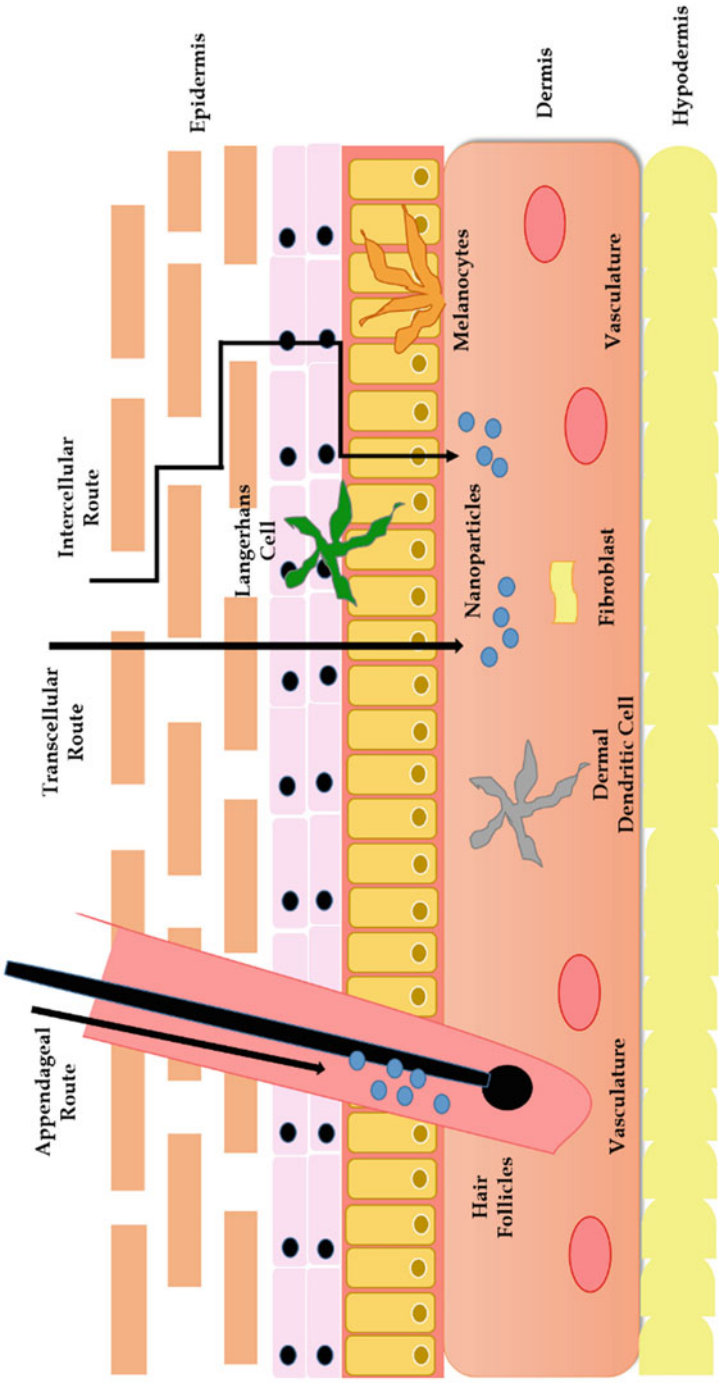

Fig. 3.2 Pictorial representation of topical drug delivery

the SC, which causes the challenge that the drug has to cross the SC and reach viable epidermis or systemic circulation to show the therapeutic efficacy.

Then another challenge is the balance between the drug penetration through SC and its buildup in the skin to ensure the therapeutic concentration of the drug [2]. Though molecules are unable to fully and readily pass through the SC membrane, they can only cross the membrane. The rate of percutaneous absorption of molecules is determined by the state of SC hydration [7]. Andrea and co-workers suggested that the skin permeability was increased by the removal of SC, whereas after removing the full epidermis, the magnitude of skin permeation enhanced by 1–2 orders of magnitude. The results manifested that skin permeability can be altered with different magnitudes by the influence of different skin layers [21]. The researchers also investigated that the permeation of active molecules through the SC is more in the diseased skin (e.g., psoriasis, dermatitis, and fungal infection) compared to the intact skin. The challenges in the penetration of the drugs across the skin are not only derived of the skin structure, but also depend on the nature of the topical delivery vehicle. One such factor in the resistance of permeability is the exogenous substances [22]. The characteristics of drugs/substances play an essential role in topical delivery. Although most of the substances have different physicochemical properties such as poor aqueous solubility, high permeability, and different molecular structures, which make them rigid to cross skin membrane, to penetrate the skin layer effectively, the drug should possess log partition coefficient value (log P) between 1 and 4 and a molecular weight less than 500 Da [23]. Despite all the above challenges, the permeation of drugs across the skin can be enhanced by various chemical and physical techniques such as radiofrequency, iontophoresis, electroporation, microneedles, magnetophoresis, and ultrasound. These techniques are very advantageous in topical delivery, but their utilization is limited due to their toxicity and lack of feasibility. However, the delivery of peptides, proteins, nucleotide fragments, vaccines, or cancer therapeutics does not still fit in the criteria of the attractive prospects of topical delivery via different techniques and strategies [24]. These challenges or drawbacks are the significant factors for the implementation of nanodelivery in the topical application, which could also expand the range of drugs available for the topical administration [25].

3.4 Role of Nanocarriers in Topical Delivery

As discussed above, conventional topical formulations have numerous side effects and limitations related to efficacy, safety, and patient compliance. The skin is a comprehensive and easy route for drug application, but it is not evident that drug is reaching its site of action. To overcome these side effects, the researchers' designed novel drug delivery systems without reducing the efficacy of therapeutic agents. The therapeutic strategies for the management of skin disease have also been available due to the new delivery of a drug [26, 27]. The nanocarriers provide satisfactory pharmacokinetic data such as low absorption of the drug as compared to conventional formulations. In general, the use of nanocarriers offers several advantages over

other drug delivery systems. These are used to enhance the stability of drugs by chemical or physical means, increase the solubility of hydrophobic drugs, deliver higher concentrations of drugs to the target site due to and enhanced permeation and retention (EPR) effect, and offers sustained and controlled release of the drug. The nanocarriers are modified with cell-specific ligands and anticipate the targeted treatment of drugs. Nanocarriers enhance the permeation of therapeutic agents through the SC by accumulating in the hair follicles and provide drug release into the deeper skin layers. There are various nanoparticles for topical delivery, such as lipid-based nanocarriers, polymeric nanoparticles, emulsified nanocarriers, dendrimers, and organic nanoparticles [28]. When the nanocarriers are formulated with the implementation of DoE, the above applications become more dominant, which have been discussed further in this chapter.

3.5 Applications of DoE in the Development of Topical Drug Delivery Systems

3.5.1 Lipid-Based Systems

3.5.1.1 Liposomes

Liposomes are the lipid-based vesicular drug delivery systems made up of one or more lipid bilayers. They are biocompatible and biodegradable and prepared by natural phospholipids with mixed lipid chains. They can entrap hydrophilic, lipophilic, and amphiphilic drugs. The drug can be either intercalated into a lipid bilayer or encapsulated in the aqueous phase. The physicochemical properties of the drug and lipid composition decide the location of drugs in liposomes [29]. The topical administration of drugs using liposomal formulation is a promising area for the concern of skin diseases. At the International Pharmaceutical Federation Congress (1979), the first report enlightened the evidence of altered drug deposition during the topical delivery of drugs via liposomal formulation was presented [30]. This report provided the base for further research articles indicating that entrapment of drugs into liposomes enhanced the skin deposition of drugs and reduced its absorption into the blood. The liposomes might reduce the percutaneous absorption of the drug by increased local action. These studies claim that liposomes can be useful for drug delivery by topical route [31]. Therefore, it is necessary to prepare the liposomes with optimized liposomal composition, high-throughput production, and better in vivo kinetics. QbD implementation in liposomal design and production can be suitable for optimizing elements, identifying suitable composition, and high screening methodologies. The various type of designs is utilized for the same [32]. All lipid-based nanocarriers with their DoE are enlisted in Table 3.3. The first optimization studies for liposomes were reported in 1988 by the well-known researcher Gregoriadis [58].

Singh et al. in 2005 reported that the optimization of formulation with traditional approaches is unpredictable, uneconomical, invariable, and time-consuming. Therefore, the use of systemic optimization techniques with response surface methodology

Table 3.3 List of experimental designs used in lipid-based nanocarriers

Drug	Nanocarriers	Lipids/surfactant	Experimental design	References
Nimesulide	Liposomes	Phospholipon 90G	Factorial design	Singh et al. [33]
Benzocaine	Liposomes	Phosphatidylcholine	D-optimal design	Mura et al. [34]
5-fluorouracil	Liposomes	phosphatidylcholine	Full factorial design	Hussain et al. [35]
Tretinoin	Liposomes	Phospholipon 85G	Full factorial design	Bavarsad et al. [36]
CoQ10 and PTA	Liposomes	Soy phosphatidylcholine	Central composite design	Çelik et al. [37]
Papain	Liposomes	Soy phosphatidylcholine	Box–Behnken design	Chen et al. [38]
Lornoxicam	Liposomes	Soy lecithin	Central composite design	Joseph et al. [39]
Acetazolamide	Liposomes	Soy phosphatidylcholine	D-optimal design	Naguib et al. [40]
Diacerein	Elastosomes	Span 60	Full factorial design	Aziz et al. [41]
Sodium stibogluconate	Liposomes	Phospholipon 90G	Box–Behnken design	Dar et al.
Insulin	Liposomes	Phosphatidylcholine	Fractional factorial design	Dawoud et al. (2018)
Clotrimazole	Ethosomes	Soy lecithin	Factorial design	Akhtar and Pathak [42]
Psoralen	Ethosomes	Soy lecithin	Factorial design	Pathak and Kumari [43]
Tramadol	Ethosomes	Phospholipon 90G	Factorial design	Ahmed et al. [44]
Triamcinolone	Ethosomes	Soy lecithin	Box–Behnken design	Akhtar et al. [45]
Piroxicam	Proniosomes	Span 60	Box–Behnken design	Solanki et al. [46]
Methotrexate	Niosomes	Span 60	Box–Behnken design	Abdelbary and Aboughaly [47]
Diacerein	Niosomes	Tween 40 and Tween 60	Box–Behnken design	El-Say et al. [48]
Coenzyme Q10	Proniosomes	Span 85	*I*-optimal mixture design	Yadav et al. [49]
Acyclovir	Niosomes	Tween 60 and Span 60	Full factorial design	Jacob et al. [50]
Diacerein	Niosomes	Span 60	Box–Behnken design	Moghddam et al. [51]
Methotrexate	Niosomes	Span 20 and Tween 40	Box–Behnken design	Zidan et al. [52]

(continued)

Table 3.3 (continued)

Drug	Nanocarriers	Lipids/surfactant	Experimental design	References
Halobetasol propionate	SLNs	Tween 80	Full factorial design	Bikkad et al. [53]
Terbinafine Hcl	SLNs	Compritol 888 ATO	Full factorial design	Vaghasiya et al. [54]
Isotretinoin	SLNs	Phospholipon 90G	Face-centered cubic design	Raza et al. [55]
Auraptene	SLNs	Glyceryl palmitostearate	Central composite design	Daneshmand et al. [56]
Fluocinolone acetonide	NLCs	Compritol 888 ATO	Box–Behnken design	Pradhan et al. [57]

(RSM) can overcome these side effects and help choose the best formulation. The researchers employed full factorial design with two independent variables at three levels, and the response variables selected were percent drug diffused, percent drug entrapment (EE%), and percent drug leakage for the optimization of the liposomal formulation. The other process variables were kept constant throughout the study. The Design-Expert software was selected, and a response surface plot was formed to fit the second-order polynomial equation. The results concluded that the full factorial design was successful in optimizing liposomal formulation, and liposomes showed high potential carriers for the topical delivery of nimesulide. The linearity with negligible deviation was shown between the observed and predicted values. The prophecy of the best liposomal formulation can be achieved using optimization methods [33]. Mura et al. [34] implemented a D-optimal design for the development of benzocaine liposomes. The primary purpose of the design implementation was to identify the essential factors influencing the response. Afterward, Doehlert design was applied for the response surface plot of the preliminary phase factors. Then, ANOVA was used for the validation of the regression model. Then, the formulation factors and their best level for response optimization were identified by the graphical analysis of the effects. The results suggested that benzocaine's permeability to the skin layer was increased because of the formulation optimization. The in vivo studies of optimized formulation indicated the enhancement of the benzocaine therapeutic efficacy compared to the previous best benzocaine liposomal formulation. This confirmed the relationship between the intensity of anesthetic effect and the in vitro drug permeation rate of benzocaine and demonstrating the actual effectiveness of the proposed approach for the liposomal formulation optimization [34].

Li et al. [59] prepared daptomycin loaded flexible nanoliposomes for topical skin therapy. They applied the Design-Expert version 8.1 to design the experimental groups and analyze the results. The results depicted that the optimized nanoliposomes exhibited narrow particle size distribution, high stability, and increase drug loading capacity. The in vitro skin permeability data showed a high diffusion capacity of the daptomycin into the skin compared with the naive drug.

Further, the optimized nanoliposomes indicated that the drug could rapidly achieve high-level concentration in the skin and underlying tissues. Hence, they may serve the potential clinical applications of daptomycin using a topical delivery approach to current intravenous delivery [59]. Hussain et al., in 2015, designed 5-fluorouracil loaded elastic liposomes for the management of skin cancer. The elastic liposomes were optimized by employing full factorial experimental design using Design-Expert software. A total of nine liposomal formulations were prepared using dependable variables against independent variables. The study results indicated that the optimized 5-fluorouracil loaded elastic liposomes had desired quality and pharmacokinetics compared to the plain drug. The liposomes manifested optimum permeation ability and overcame therapeutic limitations for the drug's topical delivery, which was absent in the conventional dosage form [35].

Bavarsad et al. in 2016 reported penetration-enhancer vesicles (PEV) for topical delivery of tretinoin using Transcutol, and a full factorial design was employed for the formulation optimization. The three-dimensional response surface plot stated that by increasing the phospholipid concentration up to 20%, the drug release was decreased in 15 min. The optimized PEV exhibited high incorporation efficiency and enhanced skin drug penetration compared to the conventional cream formulation due to the solubilizing properties of the Transcutol. The optimum PEV showed less hyperkeratosis without hyperplasia, high patient compliance, and caused mild adverse effects such as peeling, erythema, and burning in comparison to the conventional cream formulation [36]. Çelik et al. [37] developed coenzyme Q10 (CoQ10) and D-panthenyl triacetate (PTA)-loaded liposomes for topical delivery. For this purpose, a central composite design was used with three factors studied at five different levels. The experimental design aided in the preparation of CoQ10 and PTA liposomes successfully for the first time, and the results showed enhancement in EE% and drug loading capacity. Hence, the report concluded that the encapsulation of these two compounds could be a promising way for the effective delivery of both compounds simultaneously [37]. Chen et al. [38] prepared novel elastic liposomes for skin delivery of papain by implementing three-level three-variable Box–Behnken Design. The Design-Expert software was used for data prediction. The results showed an increment in EE%, high drug deposition in skin, and higher cumulative amounts and fluxes of optimized papain loaded liposomes compared to the papain solution. The topical delivery of liposomes could inhibit the hypertrophic scar in rabbit ears, including decreased collagen fibers, decreased microvessels, and signaling pathway regulation. The optimized elastic liposomes were found to be effective in topical scar treatment [38].

Naguib et al. [40] prepared acetazolamide loaded penetration-enhancing hybridized vesicles for the topical delivery of glaucoma. The formulation was optimized by applying a highly accurate and robust D-optimal mixture statistical design. The prepared and optimized liposomes are evident in high physicochemical and biological properties, large particle size, high drug release, increased EE%, excellent stability, and high drug bioavailability compared to conventional liposomes. The optimized acetazolamide loaded liposomes also showed lesser side effects as compared to the conventional acetazolamide loaded liposomes [40].

Joseph et al. [39] developed lornoxicam loaded liposomes for topical delivery. The central composite design was implemented for formulation optimization. The two variables (phospholipid and cholesterol content) and two responses (EE% and in vitro drug release) and the Design-Expert software were selected for the optimization. The response surface plot was formed to evaluate the independent variables' response to the dependent variables (Fig. 3.3). The plot showed that the EE% was the same at the medium content of phospholipid and cholesterol, but as their concentration decreases, the EE% was also decreased and vice versa. The highest and lowest levels increased the drug release, while at other levels, the drug release was decreased. Thus, the study concluded that there was an enhancement in the half-life of the optimized formulation. The optimized liposomes had stability limitation for 3–4 weeks and large particle size compared to the naïve drug. The ex vivo permeation studies and in vitro skin toxicity studies showed that the low doses could be efficient for the topical delivery of the optimized formulation, which can enhance the efficacy of the drug with fewer side effects [39].

Aziz et al., in 2018, developed diacerein loaded elastosomes for topical delivery to treat osteoarthritis. The full factorial design was implemented for the optimization of elastosomes, and the results showed high skin permeation and retention capacity of diacerein compared to the plain drug. Thus, it is concluded that the topical delivery of the drug can reduce its adverse effects on oral administration [41].

Dar et al., in 2018, developed sodium stibogluconate loaded nano-deformable liposomes for topical treatment of leishmaniasis. A Box–Behnken design was selected with three levels and three factors to optimize the formulation using RSM. The study's result stated that the surface area of the optimized formulation decreased with an increase in the vesicle size of the liposomes. The optimized topical liposomes reduced the side effects, which were seen in the intralesional or intravenous delivery of the sodium stibogluconate [60]. Dawoud et al. [61] prepared insulin chitosan mucoadhesive liposomal gel for wound healing. The Box–Behnken design was implemented with three critical process parameters for liposomes optimization. The optimization of liposomes availed high stability and sustained release profile to the insulin for the wound healing. Chen et al. in 2020 prepared zedoary turmeric oil and tretinoin encapsulated liposomal gel for topical delivery of psoriasis using orthogonal design. The results suggested that the optimized formulation allowed high EE%, solubility, and stability. The optimized liposomal gel increased the tretinoin content in the skin and reduced administration frequency compared to the naïve drug [61].

3.5.1.2 Ethosomes

Touitou and his co-workers have discovered a new type of vesicular system named ethosomes. Ethosomes differ from the liposomes in their structure, mechanism of action, and mode of application [62]. Ethosomes are defined as the non-invasive vesicular drug delivery systems and carry drugs deep into the skin layers or systemic circulation. These are malleable, soft lipid vesicles containing phospholipids, alcohol (in high concentration), and water. Ethosomes may vary in the size range from 10 nm to few microns. Ethosomes are well-established nanocarriers for local

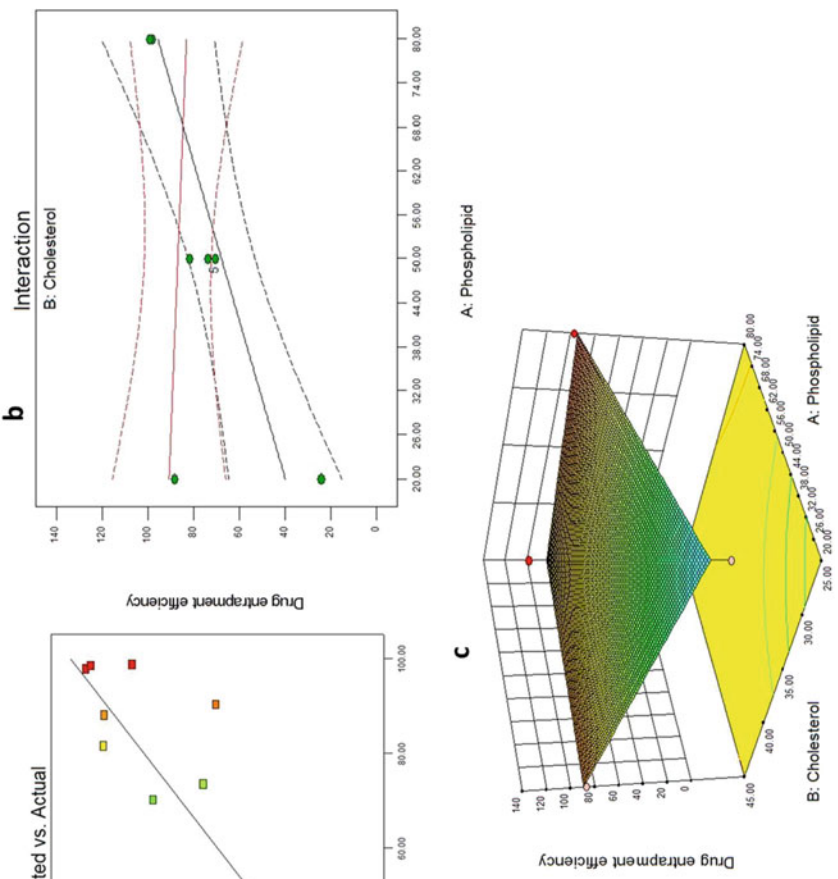

Fig. 3.3 (a) Relationship between the predicted and actual values, (b) interaction between the variables and responses, (c) 3D surface plots for variables of (**a**) entrapment values (**b**) drug release. Figure source: Joseph et al. [39]

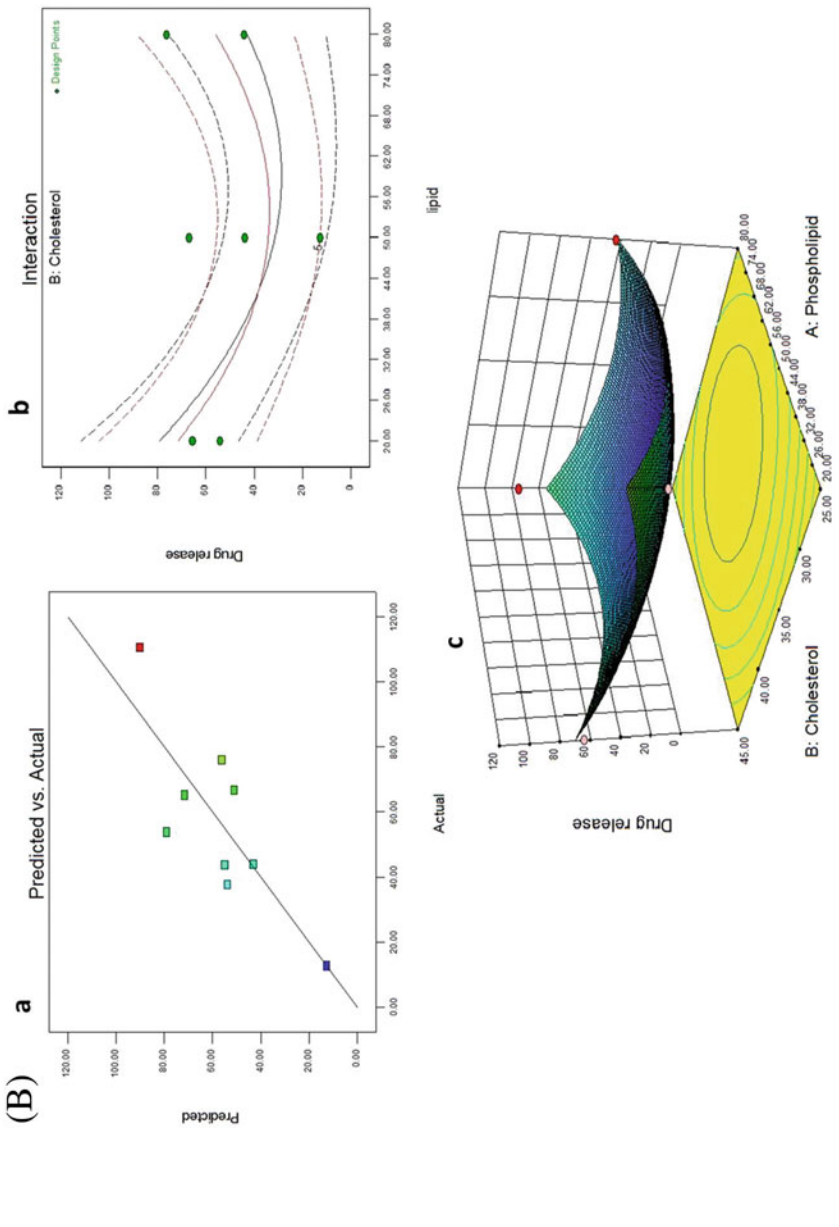

Fig. 3.3 (continued)

delivery of drugs and can penetrate the skin layers with high transdermal flux. The high concentration of ethanol (20–50%) in the ethosomes is the reason for its high permeation ability because the ethanol can disturb the organization of skin lipid bilayer. The ethosomes also possess the same stability as conventional vesicles [63]. Akhtar et al., in 2012, designed Cavamax W7 composite ethosomal gel for topical delivery of clotrimazole using factorial design. The design was validated by the Design-Expert software version 8.0.5. The response surface plot showed that EE % was increased with an increase in the concentration of soya lecithin and Cavamax W7. The optimized ethosomes were found to be stable, offered better efficiency, and higher steady-state flux than the reference ethosomes. The in vivo study of optimized ethosomes indicated the high permeability of the vesicles into the skin layers. Thus, the Cavamax W7 ethosomes were discovered as the superior nanocarrier systems for the clotrimazole topical delivery [42].

Pathak and Kumari [43] prepared Cavamax W7 composite ethosomal gel of psoralen by implementing the factorial design, and the design was validated by the Design-Expert software version 8.0.5. The response surface plot found that EE% was increased at the highest level of soy lecithin and Cavamax W7. The results concluded that the permeation of optimized formulation was more than that of the reference ethosomes, even without using a permeation enhancer. The optimized psoralen ethosomes had the potential to minimize the adverse effects associated with photosensitivity of psoralen and were the efficient carrier for the topical delivery of the drug [43]. Ahmed et al. developed tramadol loaded nanoethosomal transgel for the topical delivery. The three-factor three-level Box–Behnken design was used for formulation optimization. It was observed that the vesicle size had a direct positive relationship with the phospholipid concentration, and the permeability of the system was increased with a decrease in particle size. Therefore, the optimized ethosomal gel possessed high analgesic efficacy, enhanced skin permeability and bioavailability, improved patient compliance, and avoided frequent dosing compared to the oral marketed formulation [44].

Garg et al. [64] prepared methoxsalen loaded ethosomes-based hydrogel for the treatment of vitiligo. The two-factor, three-level face-centered design was implemented for the optimization of ethosomes. The response surface plot showed that at a higher level of ethanol and lower phospholipid levels, the vesicles' size decreased. Hence, the QbD approach in the optimization of formulation provided detailed knowledge about the ethosomal gel quality and the formulation attributes. Overall, the optimized formulation imparted better therapeutic efficiency and higher flux in comparison to the commercial product and hydroethanolic formulation [64]. Akhtar et al. [45] designed nanoethosomal glycolic vesicles of triamcinolone acetonide to manage atopic dermatitis using a three-level Box–Behnken experimental design. The design was applied to investigate the sensitivity of the responses against the variations in the experimental variables. The research group concluded that the optimized formulation not only provided an enhancement in the drug entrapment, but also offered better stability, higher drug release flux, and uniform, and more profound permeability compared to the reference ethosomes. The carbomer gel of the optimized ethosomal glycolic vesicles was found to be clear

and transparent at a pH range of skin. Overall, the nanoethosomal vesicles were suitable for the topical delivery of triamcinolone acetonide [45].

3.5.1.3 Niosomes

Niosomes are the vesicular systems composed of non-ionic surfactants and are multilamellar. They do not contain phospholipids, making them different from the liposomes [65]. Niosomes can be used as the drug delivery vehicles to deliver the drugs to the lung, ocular system, skin, brain, liver, tumor organ, etc. They are very successful in the topical delivery of drugs as they increase the SC properties by enhancing their smoothness or decreasing transepidermal water loss [66]. They have various topical delivery applications such as drug stability, sustained drug release, high skin penetration, and the ability to encapsulate both hydrophilic and lipophilic drugs [67]. DoE implementation in the niosomes can avoid excessive effort, time, and materials for the formulation development and provide significance of different variables.

Solanki et al. formulated piroxicam loaded proniosomes for the topical delivery employing the three-factor three-level Box–Behnken statistical design. The response surface plots depicted that on decreasing the concentration of cholesterol, EE% of the niosomes was also decreased. The optimized niosomes were found to be less leaky and small particle size. The study concluded that the optimized proniosomes exhibited all the desired properties for topical delivery [46]. Abdelbery et al., in 2015, prepared methotrexate loaded niosomes for the treatment of psoriasis. A three-level three-factor Box–Behnken design was applied for the formulation optimization, and the independent variables selected were the methotrexate concentration in hydration medium, the total weight of niosomal components, and surfactant. The dependent variables selected were EE% and particle size. The EE% was reported to decrease along with an increase in hydration volume. The reason for increment in particle size was the excessive amount of drugs inside the niosomes. The in vivo study displayed that the niosomes were non-irritant after applied topically. Thus, it is concluded that the methotrexate loaded niosomes had the therapeutic efficacy to treat psoriasis, and preclinical applications on animal models were necessary to establish the safety of the niosomes [47].

El-say et al. developed niosomal gel for the topical delivery of diacerein using the Box–Behnken design model. The three independent variables selected were the percentage of stearylamine, hydrophilic–lipophilic balance, and concentration of surfactant and sonication time. The dependent variables selected were the EE%, particle size, and percentage of diacerein release. The stat graphics model with two ANOVA was used for the statistical analysis. It was found that the increase in sonication time can reduce the vesicle size, and a decrease in both percentages of stearylamine and surfactant might decrease the EE%. Also, the optimized formulation resulted in a suitable topical application and exhibited high pharmacodynamic and anti-inflammatory activity compared to the conventional gel of diacerein [48]. Jacob et al. developed acyclovir loaded niosomal gel for the topical application, and 3^2 full factorial design was implemented for the niosomes optimization. From the contour response plot, it was observed that there is no significant difference in the

particle size between various formulations. The vesicle size and EE% were changed while changing the concentration of surfactant and cholesterol. The study's result stated that the formulation manifested high bioavailability and sustained release of drugs compared to conventional dosage form [50].

Moghddam et al. prepared diacerein loaded niosomes for the treatment of psoriasis by applying 3^3 Box–Behnken design. The optimized niosomal formulation exhibited high EE%, reasonable particle size, and homogenous surface morphology. The developed formulation was found to be the better carrier for diacerein delivery [51]. Arafa et al. in 2017 employed Minitab software for the optimization of pregabalin loaded niosomes. The data exhibited that EE% of the formulation had a direct relationship with the cholesterol ratio. However, the in vitro release study showed that the drug delivery followed Higuchi and Fickian release model. The ex vivo permeation of niosomes was increased as compared to the hydrogels. Therefore, it is investigated that the hydrogels loaded niosomes are the only capable carrier for the controlled release of pregabalin [68].

Al-Mahallawi et al. [69] designed methotrexate loaded ultra-permeable niosomes by implementing a 3^3 Box–Behnken experimental design with Design-Expert software to evaluate the design. The independent variables selected were the Cremophor RH 40 percentage, stabilizer percentage, and sonication time, and the dependent variables evaluated were the EE%, vesicle size, and polydispersity index. The response surface plot depicted that the sonication time and stabilizer percentage had a negative impact on the EE%, and increasing stabilizer percentage might enhance the vesicle size of the niosomes. Thus, the results concluded that the optimized formulation showed better stability profile and in vivo data evaluated the high amount of drug deposition in the rat skin compared to the methotrexate solution [69].

3.5.1.4 Solid Lipid Nanocarriers (SLNs)

SLNs were first discovered in 1991; they are the lipid nanocarriers containing the properties of both lipid-based systems and the polymeric nanoparticle systems. SLNs encapsulate drugs at the lipid matrix composed of a solid lipid or mixture of solid lipids. They have various advantages such as drug protection against enzymes or degradation, controlled drug release, biocompatibility. SLNs are better nanocarriers for skin delivery as they enhanced the permeation of encapsulated drug [70].

Bikkad et al. developed halobetasol propionate loaded SLNs for the topical delivery using 3^2 full factorial design. Amount of Tween 80 and the content of glycerol monostearate were selected as the independent factors, and particle size and EE% were the dependent factors. The Design-Expert software was used for data analysis, and the response surface plot showed that the particle size was increased with decrease in the ratio of Tween 80: glyceryl monostearate and EE% increased with increase in the ratio of Tween 80 and glyceryl monostearate. The optimized formulation exhibited controlled drug release, less skin irritation, better stability, lower side effects, and high therapeutic efficacy compared with the conventional gel [53]. Raza et al. in 2013 developed isotretinoin loaded SLNs for the treatment of skin acne. They employed a face-centered cubic design for the optimization of response

variables. From the response surface plot, it resulted that the phospholipid had a positive relationship with the particle size and zeta potential. The optimized SLNs were highly stable, with a low aggregation tendency of the dispersed phase. The SLNs could be the better option for the isotretinoin topical delivery [55].

Daneshmand et al. [56] prepared SLNs for the auraptene delivery as an anti-inflammatory agent. A central composite design with a five-factor three responses was used for the analysis of variables. The optimized formulation exhibited high EE % and low skin irritancy. The in vitro data provided controlled release of the drug as compared to the conventional cream. The optimized SLNs revealed high anti-inflammatory efficacy and maximum local effect of the drug at the skin with no sensitization [56].

3.5.2 Emulsion Based Systems

3.5.2.1 Microemulsion

Hoar and Schulman, in 1943, prepared a single-phase, uniform, non-conducting, transparent solution called microemulsions. Microemulsions are thermodynamically stable, transparent mixtures of oil, water, and surfactant. These have various advantages such as high stability, better bioavailability, deliver both hydrophilic and lipophilic drugs. Microemulsions are different from other nanocarriers in such a way that they are amphiphilic and have multi-drug delivery [71]. Table 3.4 shows the list of designs used in these systems. Microemulsion can enhance drug penetration from the skin, making it a suitable option for the topical delivery of drugs. In

Table 3.4 List of experimental designs used in emulsified systems and polymeric systems

Drug	Nanocarriers	Experimental design	References
Aceclofenac	Microemulsion	D-optimal design	Todosijević et al. [72]
Lidocaine and prilocaine	Microemulsion	D-optimal design	Negi et al. [73]
Clobetasol propionate	Microemulsion	D-optimal design	Patel et al. [74]
Sertaconazole	Microemulsion	Full factorial design	Radwan et al. [75]
Rotigotine	Microemulsion	Central composite design	Wang et al. [76]
Synthetic chalcone	Nanoemulsion	Full factorial design	de Mattos et al. [77]
Kojic monooleate	Nanoemulsion	D-optimal design	Afifah et al. [78]
Metronidazole	Nanoemulsion	D-optimal design	Yu et al. [79]
Chlorpromazine	PNPs	Box–Behnken design	Alvarez-Trabado et al. [80]
Cetrimide	PNPs	Central composite design	Marto et al. [81]
Terconazole	Micelle	Full factorial design	Abd-Elsalam et al. [82]
Simvastatin	Micelle	Full factorial design	Varshosaz et al. [83]
Vinpocetine	Micelle	Orthogonal design	Zhou et al. [84]

recent times, researchers take an interest in the in silico techniques such as DoE for the prediction of microemulsion phase behavior.

Patel et al. designed clobetasol propionate loaded microemulsion gel for the treatment of vitiligo. The D-optimal design was implemented for the optimization of formulation, and the independent variables selected were the amount of mixture of surfactant and co-surfactant (S_{mix}) and oil concentration. The dependent variables selected were the globule size and solubility of the drug. The response surface plot evaluated that the decrease in globule size is directly proportional to the amount of oil proportion. The results concluded that the optimized microemulsion exhibited better skin permeation, high skin retention, and less skin irritation than the marketed formulation. Therefore, the optimized topical microemulsion gel could be a useful option for the treatment of vitiligo [74]. Negi et al. in 2014 developed lidocaine and prilocaine loaded microemulsion hydrogel for topical delivery using D-optimal mixture design. The formulation variables selected were the Smix, water, and oil, and the response variables chosen were skin retention, globule size, percent cumulative permeation, and skin permeation flux. The response analysis was carried out, and the response surface plot (Fig. 3.4) evaluated that with an increase in the water level, the globule size was decreased, and percent skin retention of both the drugs increased. The thermodynamic activity of the drug decreased with an increase in the surfactant concentration and the drug's affinity.

The response surface plot proved that the maximum water content and minimum S_{mix} could enhance the globule size, % skin retention, and permeation flux and percutaneous absorption. The results concluded that the optimized microemulsion hydrogel showed high skin absorption and skin permeation compared to the commercial cream [73]. Radwan et al. prepared sertaconazole loaded microemulsion gel as an antifungal agent. The formulation was optimized, employing factorial design and Design-Expert software. The results indicated that the optimized microemulsions impart a high skin retention activity and better antimycotic activity as compared to the marketed formulation. Moreover, optimized microemulsion did not show any histopathological changes on rat skin as proved to be non-irritant as compared to marketed cream. Thus, the dermal delivery could be achieved by the sertaconazole loaded optimized microemulsion [75].

3.5.2.2 Nanoemulsions

Nanoemulsions are the emulsified carriers composed of oil, surfactant, and water. Their droplet size is smaller than the microemulsion. They are usually opaque to blue-white [71]. Nanoemulsions have various advantages compared to a conventional emulsion, such as increased interfacial area, better stability, high solubility, and improved bioavailability.

Yu et al. applied a D-optimal design for the optimization of metronidazole loaded nanoemulsions with anti-rosacea properties. The droplet size, % skin retention, and metronidazole content were selected as the independent variables, and the oil content, water, and S_{mix} were used as the dependent variables. The response surface plot evaluated that at the moderate levels, optimized nanoemulsion showed less droplet size and better skin retention properties and high therapeutic efficacy

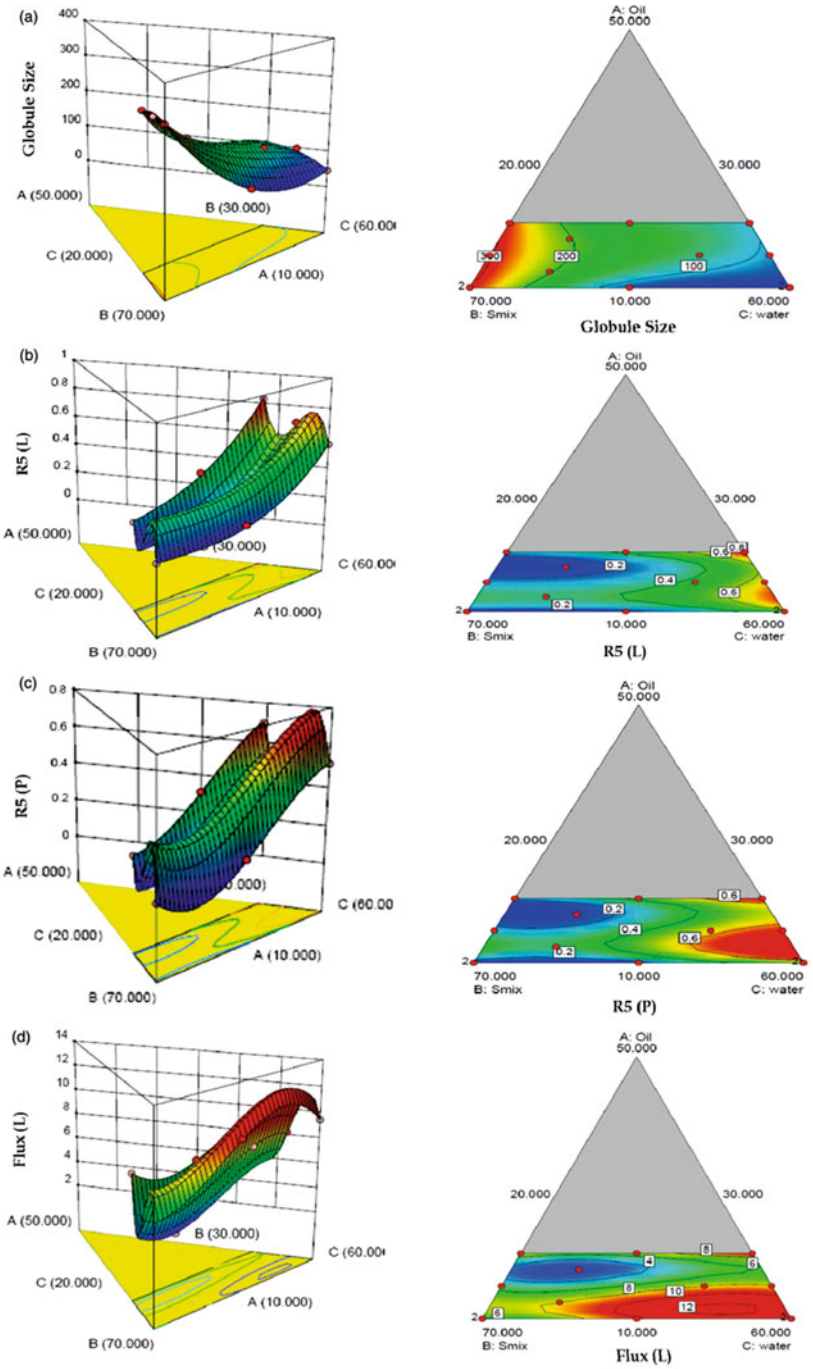

Fig. 3.4 3D response surface plots and 2D contour plots of (**a**) globule size of microemulsion, (**b**) skin retention of lidocaine, (**c**) skin retention of prilocaine, (**d**) permeation flux of lidocaine, (**e**) permeation flux of prilocaine, (**f**) skin permeation of lidocaine, (**g**) skin permeation of prilocaine (adapted from [73])

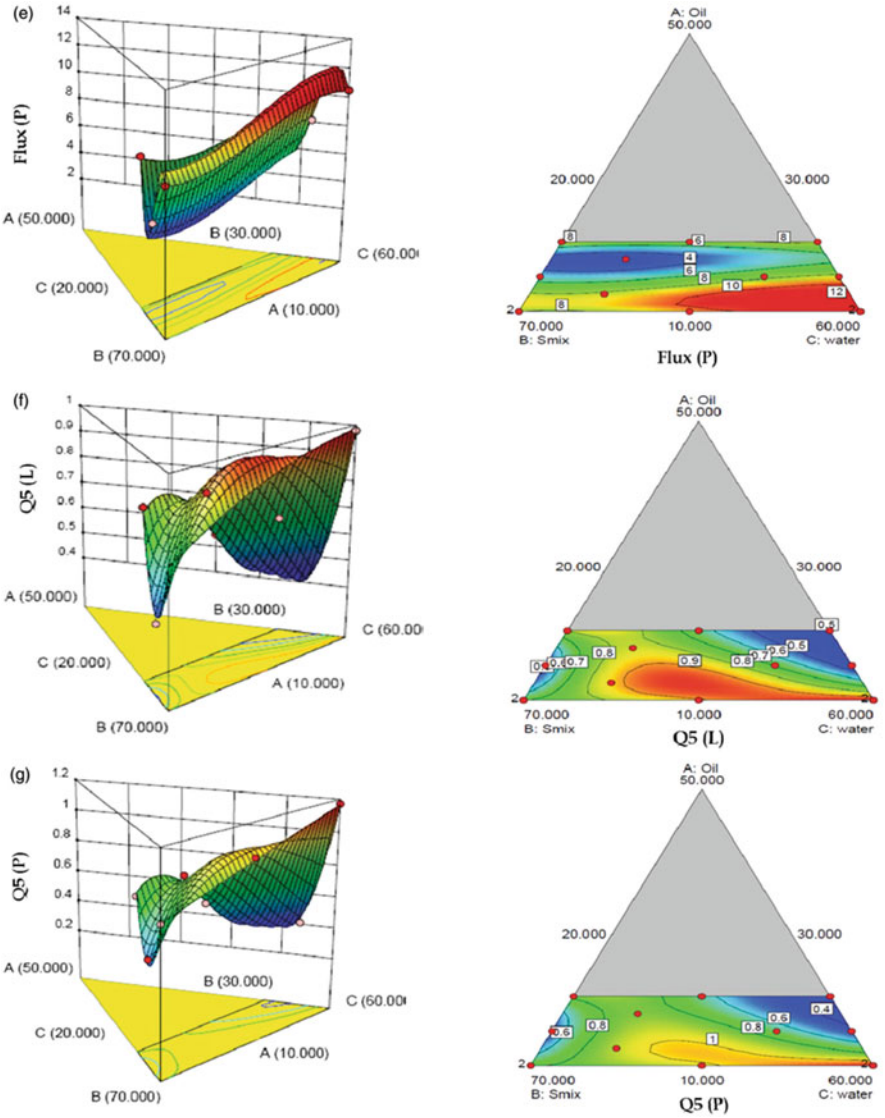

Fig. 3.4 (continued)

[79]. Afifah et al., in 2018, prepared kojic monooleate loaded nanoemulsions for the treatment of hyperpigmentation in the skin. A five-factor one-response D-optimal design was used for the evaluation of nanoemulsions. The three-dimensional surface plot stated that the droplet size was decreased with increasing xanthan gum and

surfactant content. The in vitro data of the optimized formulation suggested that the microemulsion was less toxic and safe for the cosmeceutical applications [78].

3.5.3 Polymer-Based Systems

3.5.3.1 Polymeric Nanoparticles (PNPs)

PNPs are biomaterials with immense potential, simple design, higher biocompatibility and biomimetic properties. In the drug delivery systems, the main characteristics of PNPs are controlled particle size and excellent solubility and elasticity. PNPs are of two types, i.e., nanocapsules and nanospheres. They can reach its target site at the desired time and level [85]. Topical delivery of PNPs can provide protection to drugs from degradation, reduce side effects of toxic drugs via controlled disease, and increase skin penetration [86].

Marto et al., in 2018, developed novel human neutrophil elastase loaded starch nanocapsule implementing the central composite design. The design was used to evaluate the response of independent variables on the dependent variables. The independent variables selected were the amount of drug, Tween 80 content, lipid content, and percentage of surfactant and drug loading, particle size, zeta potential, and EE% are the dependent variables. The MODDE software was used for the data evaluation, and it was found that the enhancement in the content of lipid and Tween 80 led to a decrease in the particle size and increase in the EE%. The result of the study concluded that the optimized nanocapsules exhibited high efficacy and skin permeation activity and showed a promising carrier for topical delivery [81]. Pizarro et al. prepared fluorometholone loaded nanoparticles for inflammatory disorders. The formulation was optimized using three factors (concentration of drug, surfactant, and polymer) and three levels (particle size, zeta potential, polydispersity index, and EE%) central composite design matrix. The response surface plot showed that the particle size increased with an increase in polymer concentration and decreased with an increase in the surfactant concentration. The zeta potential and polydispersity index exhibited that the formulation is monodispersed in nature with very high EE%. The skin permeation and anti-inflammatory studies showed that the optimized formulation had better skin permeability than commercial formulation. However, the optimized fluorometholone NPs were found to be successful for the topical delivery to reach the posterior segment of the eye [87].

3.5.3.2 Micelles

Micelles are polymeric nanocarriers composed of mainly surfactants and polymers. The micelles are formed only, when the surfactant concentration reaches to or above the critical micellar concentration and these nanostructures consist of a solvophobic core and solvophilic shell. The hydrophobic drugs can encapsulate in the core of polymeric micelles, and shell provides stability to the micelles. The micelles shell has the ability to prevent protein adsorption and opsonization. However, micelles have various advantages in topical drug delivery, such as high biocompatibility,

better drug deposition, and high skin permeation [88]. The polymeric micelles have been reported to increase the drug's solubility at the skin site, sustained drug release.

Varshosaz et al. developed chitosan gel containing nanomicelles for the effective topical delivery of simvastatin for wound healing using irregular full factorial design. The independent variables chosen for the optimization were the hydration temperature, polymer/drug ratio, organic solvent and hydration time, and the responses were the EE%, particle size, zeta potential, drug release, and drug loading efficiency. From the response surface plot (Figs. 3.5 and 3.6), the drug release was found to be rapid at first and then followed by sustained release. The hydration temperature affected the particle size. The EE% was increased with an increase in the drug content. Thus, the study concluded that the optimized formulation showed high wound healing ability and could be a promising carrier for topical delivery [83]. Zhou et al. prepared vinpocetine loaded micelles with the implementation of orthogonal design to optimize micelles. They found that the optimized micelles

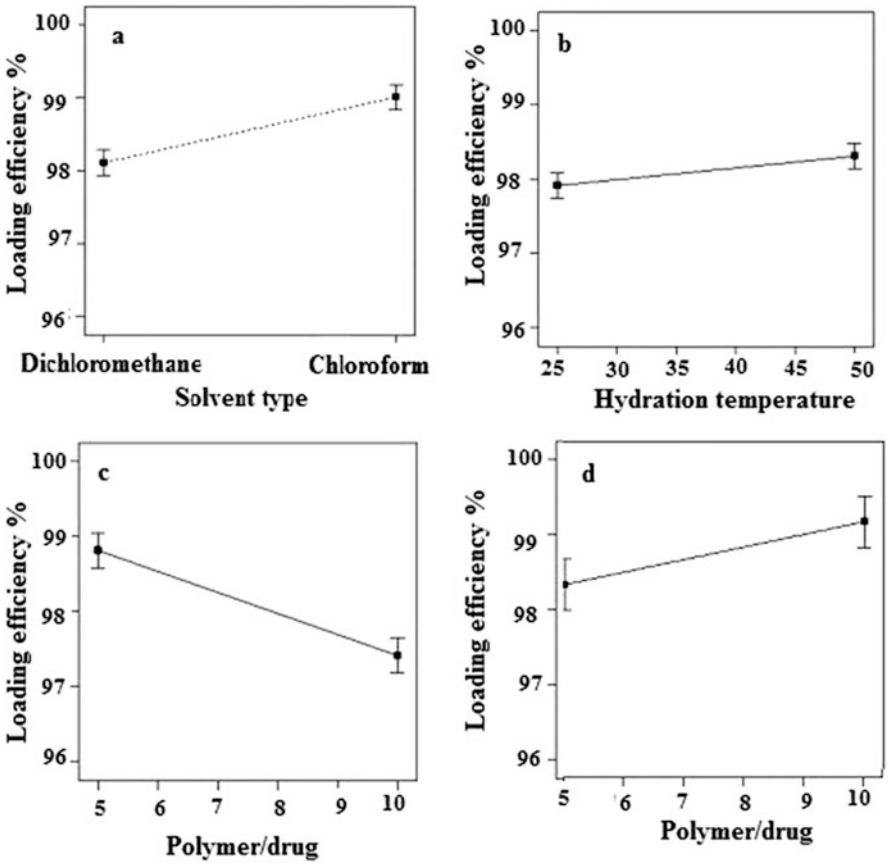

Fig. 3.5 The effects of different studied parameters on loading efficiency of nanomicelles

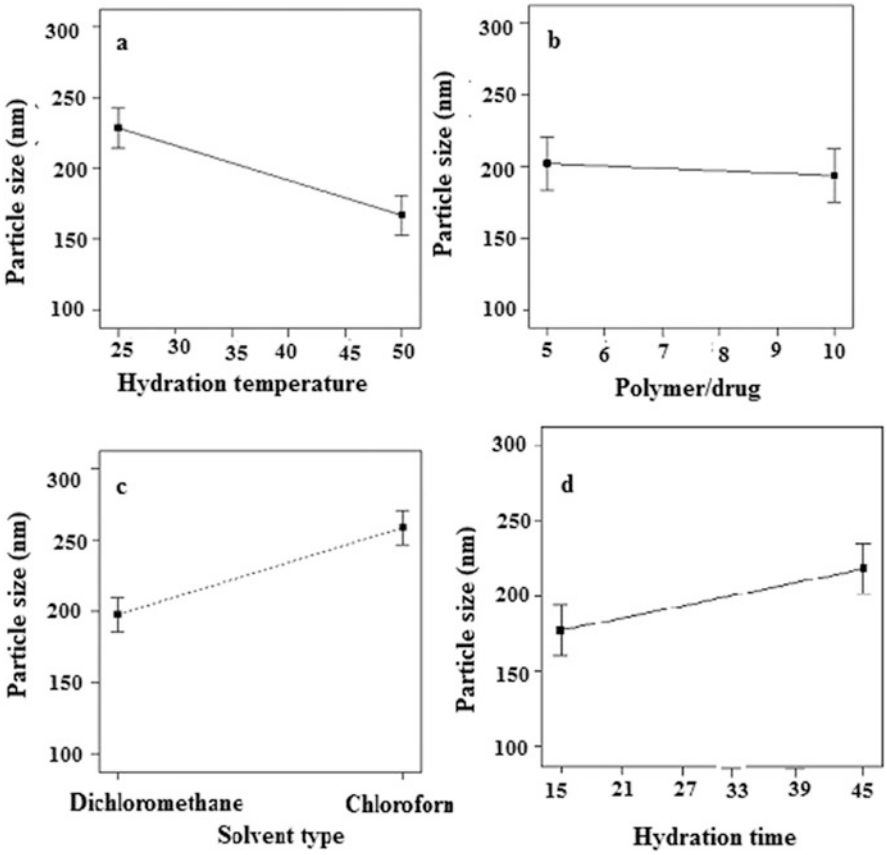

Fig. 3.6 The effects of different studied parameters on the particle size of nanomicelles. Figures source: Varshosaz et al. [83]

exhibited a sustained release profile and prolonged plasma concentration compared to the vinpocetine injection [84].

3.6 Conclusion

The skin is the easiest, non-invasive, and an appealing route for the drug administration, which initiates the research towards the topical drug delivery system. The main drawback of the topical delivery is the SC barrier which resists the entry of bioactive agents. Henceforth, nanosystems such as liposomes, niosomes, SLNs, microemulsions, PNPs become the widely formulated technologies to overcome these side effects and improve the efficacy of drugs at the skin. It requires many efforts to prepare a nanosystem, either raw materials, preparation methods, or characterization techniques. At the duration of the formulation of nanosystems,

various process and formulation parameters are required, and their optimization is crucial for the desired quality of the product for topical delivery. Thus, the DoE is a vital optimization technique for the optimization of nanosystems and ensures desirable product design with a highly potential final topical product.

References

1. Lavik EB, Kuppermann BD, Humayun MS (2012) Drug delivery, 5th edn. Elsevier, London. https://doi.org/10.1016/B978-1-4557-0737-9.00038-2
2. Petrilli R, Lopez RFV (2018) Physical methods for topical skin drug delivery: concepts and applications. Braz J Pharm Sci 54:1–19. https://doi.org/10.1590/s2175-97902018000001008
3. Touitou E (2002) Drug delivery across the skin. Expert Opin Biol Ther 2:723–733. https://doi.org/10.1517/14712598.2.7.723
4. Lee H, Song C, Baik S, Kim D, Hyeon T, Kim DH (2018) Device-assisted transdermal drug delivery. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2017.08.009
5. Simões A, Veiga F, Figueiras A, Vitorino C (2018) A practical framework for implementing quality by design to the development of topical drug products: Nanosystem-based dosage forms. Int J Pharm 548:385–399. https://doi.org/10.1016/j.ijpharm.2018.06.052
6. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, Wurm EMT, Yoong C, Robertson TA, Soyer HP, Roberts MS (2011) Nanoparticles and microparticles for skin drug delivery Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev 63:470–491. https://doi.org/10.1016/j.addr.2011.01.012
7. Katare O, Raza K, Singh B, Dogra S (2010) Novel drug delivery systems in topical treatment of psoriasis: rigors and vigors. Indian J Dermatol Venereol Leprol 76:612–621. https://doi.org/10.4103/0378-6323.72451
8. Marto J, Gouveia LF, Gonçalves LM, Gaspar DP, Pinto P, Carvalho FA, Oliveira E, Ribeiro HM, Almeida AJ (2016) A quality by design (QbD) approach on starch-based nanocapsules: a promising platform for topical drug delivery. Colloids Surf B Biointerfaces 143:177–185. https://doi.org/10.1016/j.colsurfb.2016.03.039
9. Kraft JC, Freeling JP, Wang Z, Ho RJY (2014) Emerging research and clinical development trends of liposome and lipid nanoparticle drug delivery systems. J Pharm Sci. https://doi.org/10.1002/jps.23773
10. Li J, Qiao Y, Wu Z (2017) Nanosystem trends in drug delivery using quality-by-design concept. J Control Release. https://doi.org/10.1016/j.jconrel.2017.04.019
11. Rathore AS (2009) Roadmap for implementation of quality by design (QbD) for biotechnology products. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2009.06.006
12. Pramod K, Tahir M, Charoo N, Ansari S, Ali J (2016) Pharmaceutical product development: a quality by design approach. Int J Pharm Investig 6:129. https://doi.org/10.4103/2230-973x.187350
13. Sharma G, Thakur K, Raza K, Singh B, Katare OP (2017) Nanostructured lipid carriers: a new paradigm in topical delivery for dermal and transdermal applications. Crit Rev Ther Drug Carrier Syst 34:355–386. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2017019047
14. Robert MEM, Kalia YN (2006) New developments in topical antifungal therapy. Am J Drug Deliv. https://doi.org/10.2165/00137696-200604040-00006
15. Maibach HI (2000) Introduction. In: Dermatopharmacology of topical preparations. Springer, Berlin, pp 1–2. https://doi.org/10.1007/978-3-642-57145-9_1
16. Gupta M, Agrawal U, Vyas SP (2012) Nanocarrier-based topical drug delivery for the treatment of skin diseases. Expert Opin Drug Deliv 9:783–804. https://doi.org/10.1517/17425247.2012.686490

17. Boer M, Duchnik E, Maleszka R, Marchlewicz M (2016) Structural and biophysical characteristics of human skin in maintaining proper epidermal barrier function. Postep Dermatol Alergol 33(1):1–5. https://doi.org/10.5114/pdia.2015.48037
18. Ruela ALM, Perissinato AG, de Lino ME, Mudrik PS, Pereira GR (2016) Evaluation of skin absorption of drugs from topical and transdermal formulations. Braz J Pharm Sci 52:527–544. https://doi.org/10.1590/s1984-82502016000300018
19. Fluhr J, Lademann J (2009) Editorial: Emphasis on skin physiology. Skin Pharmacol Physiol. https://doi.org/10.1159/000235823
20. Lauterbach A, Müller-Goymann CC (2015) Applications and limitations of lipid nanoparticles in dermal and transdermal drug delivery via the follicular route. Eur J Pharm Biopharm 97:152–163. https://doi.org/10.1016/j.ejpb.2015.06.020
21. Luís A, Ruela M, Perissinato AG, Esselin M, Lino DS (2016) Evaluation of skin absorption of drugs from topical and transdermal formulations. Braz J Pharm Sci 52:527–544
22. Singh Malik D, Mital N, Kaur G (2016) Topical drug delivery systems: a patent review. Expert Opin Ther Pat 26:213–228. https://doi.org/10.1517/13543776.2016.1131267
23. Ng KW (2018) Penetration enhancement of topical formulations. Pharmaceutics 10:10–12. https://doi.org/10.3390/pharmaceutics10020051
24. Cevc G, Blume G (1992) Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. BBA-Biomembranes 1104:226–232. https://doi.org/10.1016/0005-2736(92)90154-E
25. Roberts MS, Mohammed Y, Pastore MN, Namjoshi S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE (2017) Topical and cutaneous delivery using nanosystems. J Control Release 247:86–105. https://doi.org/10.1016/j.jconrel.2016.12.022
26. Castro GA, Coelho ALLR, Oliveira CA, Mahecha GAB, Oréfice RL, Ferreira LAM (2009) Formation of ion pairing as an alternative to improve encapsulation and stability and to reduce skin irritation of retinoic acid loaded in solid lipid nanoparticles. Int J Pharm 381:77–83. https://doi.org/10.1016/j.ijpharm.2009.07.025
27. Gupta M, Vyas SP (2012) Development, characterization and in vivo assessment of effective lipidic nanoparticles for dermal delivery of fluconazole against cutaneous candidiasis. Chem Phys Lipids 2012:454–461. https://doi.org/10.1016/j.chemphyslip.2012.01.006
28. Goyal R, Macri LK, Kaplan HM, Kohn J (2016) HHS Publ Access 2016:77–92. https://doi.org/10.1016/j.jconrel.2015.10.049.Nanoparticles
29. Bangham AD, Standish MM, Weissmann G (1965) The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. J Mol Biol 13:253–259. https://doi.org/10.1016/S0022-2836(65)80094-8
30. Mezei M, Gulasekharam V (1980) Liposomes - a selective drug delivery system for the topical route of administration I. Lotion dosage form. Life Sci 26:1473–1477. https://doi.org/10.1016/0024-3205(80)90268-4
31. Egbaria K, Weiner N (1990) Liposomes as a topical drug delivery system. Polym Mater Sci Eng Proc ACS Div Polym Mater Sci Eng 63:59–63
32. Wong A (2010) Quantitative modeling of the high-throughput production and in vivo kinetics of (drug-encapsulating) liposomes. PLoS One 5:e0010280. https://doi.org/10.1371/journal.pone.0010280
33. Singh B, Mehta G, Kumar R, Bhatia A, Ahuja N, Katare O (2005) Design, development and optimization of nimesulide-loaded liposomal systems for topical application. Curr Drug Deliv 2:143–153. https://doi.org/10.2174/1567201053585985
34. Mura P, Capasso G, Maestrelli F, Furlanetto S (2008) Optimization of formulation variables of benzocaine liposomes using experimental design. J Liposome Res 18:113–125. https://doi.org/10.1080/08982100802118540
35. Hussain A, Haque MW, Singh SK, Ahmed FJ (2016) Optimized permeation enhancer for topical delivery of 5-fluorouracil-loaded elastic liposome using design expert: part II. Drug Deliv 23:1242–1253. https://doi.org/10.3109/10717544.2015.1124473

36. Bavarsad N, Akhgari A, Seifmanesh S, Salimi A, Rezaie A (2016) Statistical optimization of tretinoin-loaded penetration-enhancer vesicles (PEV) for topical delivery. DARU J Pharm Sci 24:1–12. https://doi.org/10.1186/s40199-016-0142-0
37. Çelik B, Sağıroğlu AA, Özdemir S (2017) Design, optimization and characterization of coenzyme Q10- and D-panthenyl triacetate-loaded liposomes. Int J Nanomedicine 12:4869–4878. https://doi.org/10.2147/IJN.S140835
38. Chen YY, Lu YH, Ma CH, Tao WW, Zhu JJ, Zhang X (2017) A novel elastic liposome for skin delivery of papain and its application on hypertrophic scar. Biomed Pharmacother 87:82–91. https://doi.org/10.1016/j.biopha.2016.12.076
39. Joseph J, Vedha Hari BN, Ramya Devi D (2018) Experimental optimization of Lornoxicam liposomes for sustained topical delivery. Eur J Pharm Sci 112:38–51. https://doi.org/10.1016/j.ejps.2017.10.032
40. Naguib SS, Hathout RM, Mansour S (2017) Optimizing novel penetration enhancing hybridized vesicles for augmenting the in-vivo effect of an anti-glaucoma drug. Drug Deliv 24:99–108. https://doi.org/10.1080/10717544.2016.1233588
41. Aziz DE, Abdelbary AA, Elassasy AI (2018) Fabrication of novel elastosomes for boosting the transdermal delivery of diacerein: statistical optimization, ex-vivo permeation, in-vivo skin deposition and pharmacokinetic assessment compared to oral formulation. Drug Deliv 25:815–826. https://doi.org/10.1080/10717544.2018.1451572
42. Akhtar N, Pathak K (2012) Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. AAPS PharmSciTech 13:344–355. https://doi.org/10.1208/s12249-012-9754-y
43. Pathak K, Kumari S (2013) Cavamax W7 composite psoralen ethosomal gel versus cavamax W7 psoralen solid complex gel for topical delivery: a comparative evaluation. Int J Pharm Investig 3:171. https://doi.org/10.4103/2230-973x.121284
44. Ahmed S, Sarim Imam S, Zafar A, Ali A, Aqil M, Gull A (2016) In vitro and preclinical assessment of factorial design based nanoethosomes transgel formulation of an opioid analgesic. Artif Cells Nanomed Biotechnol 44:1793–1802. https://doi.org/10.3109/21691401.2015.1102742
45. Akhtar N, Verma A, Pathak K (2017) Feasibility of binary composition in development of nanoethosomal glycolic vesicles of triamcinolone acetonide using Box-behnken design: in vitro and ex vivo characterization. Artif Cells Nanomed Biotechnol 45:1123–1131. https://doi.org/10.1080/21691401.2016.1202261
46. Solanki AB, Parikh JR, Parikh RH (2007) Formulation and optimization of piroxicam proniosomes by 3-factor, 3-level Box-Behnken design. AAPS PharmSciTech 8. https://doi.org/10.1208/pt0804086
47. Abdelbary AA, Aboughaly MHH (2015) Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: application of Box-Behnken design, in-vitro evaluation and in-vivo skin deposition study. Int J Pharm 485:235–243. https://doi.org/10.1016/j.ijpharm.2015.03.020
48. El-Say KM, Abd-Allah FI, Lila AE, Hassan AESA, Kassem AEA (2016) Diacerein niosomal gel for topical delivery: development, in vitro and in vivo assessment. J Liposome Res 26:57–68. https://doi.org/10.3109/08982104.2015.1029495
49. Yadav NK, Nanda S, Sharma G, Katare OP (2016) Systematically optimized coenzyme q10-loaded novel proniosomal formulation for treatment of photo-induced aging in mice: characterization, biocompatibility studies, biochemical estimations and anti-aging evaluation. J Drug Target 24:257–271. https://doi.org/10.3109/1061186X.2015.1077845
50. Jacob S, Nair AB, Al-Dhubiab BE (2017) Preparation and evaluation of niosome gel containing acyclovir for enhanced dermal deposition. J Liposome Res 27:283–292. https://doi.org/10.1080/08982104.2016.1224897
51. Moghddam SRM, Ahad A, Aqil M, Imam SS, Sultana Y (2016) Formulation and optimization of niosomes for topical diacerein delivery using 3-factor, 3-level Box-Behnken design for the

management of psoriasis. Mater Sci Eng C 69:789–797. https://doi.org/10.1016/j.msec.2016.07.043
52. Zidan AS, Mokhtar Ibrahim M, Megrab NAE (2017) Optimization of methotrexate loaded niosomes by Box–Behnken design: an understanding of solvent effect and formulation variability. Drug Dev Ind Pharm 43:1450–1459. https://doi.org/10.1080/03639045.2017.1318907
53. Bikkad ML, Nathani AH, Mandlik SK, Shrotriya SN, Ranpise NS (2014) Halobetasol propionate-loaded solid lipid nanoparticles (SLN) for skin targeting by topical delivery. J Liposome Res 24:113–123. https://doi.org/10.3109/08982104.2013.843192
54. Vaghasiya H, Kumar A, Sawant K (2013) Development of solid lipid nanoparticles based controlled release system for topical delivery of terbinafine hydrochloride. Eur J Pharm Sci 49:311–322. https://doi.org/10.1016/j.ejps.2013.03.013
55. Raza K, Singh B, Singal P, Wadhwa S, Katare OP (2013) Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. Colloids Surf B Biointerfaces 105:67–74. https://doi.org/10.1016/j.colsurfb.2012.12.043
56. Daneshmand S, Jaafari MR, Movaffagh J, Malaekeh-Nikouei B, Iranshahi M, Seyedian Moghaddam A, Tayarani Najaran Z, Golmohammadzadeh S (2018) Preparation, characterization, and optimization of auraptene-loaded solid lipid nanoparticles as a natural anti-inflammatory agent: in vivo and in vitro evaluations. Colloids Surf B Biointerfaces 164:332–339. https://doi.org/10.1016/j.colsurfb.2018.01.054
57. Pradhan M, Singh D, Murthy SN, Singh MR (2015) Design, characterization and skin permeating potential of fluocinolone acetonide loaded nanostructured lipid carriers for topical treatment of psoriasis. Steroids 101:56–63. https://doi.org/10.1016/j.steroids.2015.05.012
58. Gregoriadis G (1988) Liposomes as a drug delivery system: optimization studies. Adv Exp Med Biol 1988:151–159. https://doi.org/10.1007/978-1-4684-7908-9_12
59. Li C, Zhang X, Huang X, Wang X, Liao G, Chen Z (2013) Preparation and characterization of flexible nanoliposomes loaded with daptomycin, a novel antibiotic, for topical skin therapy. Int J Nanomedicine 8:1285–1292. https://doi.org/10.2147/IJN.S41695
60. Dar MJ, Din FU, Khan GM (2018) Sodium stibogluconate loaded nano-deformable liposomes for topical treatment of leishmaniasis: macrophage as a target cell. Drug Deliv 25:1595–1606. https://doi.org/10.1080/10717544.2018.1494222
61. Dawoud MHS, Yassin GE, Ghorab DM, Morsi NM (2019) Insulin mucoadhesive liposomal gel for wound healing: a formulation with sustained release and extended stability using quality by design approach. AAPS PharmSciTech 20:1–15. https://doi.org/10.1208/s12249-019-1363-6
62. Touitou E, Godin B (2007) Ethosomes for skin delivery. J Drug Deliv Sci Technol 17:303–308. https://doi.org/10.1016/S1773-2247(07)50046-8
63. Aggarwal D, Nautiyal U (2016) Ethosomes: a review. Int J Pharm Med Res 4:354–363
64. Garg BJ, Garg NK, Beg S, Singh B, Katare OP (2016) Nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: Formulation optimization, in vitro evaluation and preclinical assessment. J Drug Target 24:233–246. https://doi.org/10.3109/1061186X.2015.1070855
65. Rajera R, Nagpal K, Singh SK, Mishra DN (2011) Niosomes: a controlled and novel drug delivery system. Biol Pharm Bull 34:945–953. https://doi.org/10.1248/bpb.34.945
66. Hamishehkar H, Rahimpour Y, Kouhsoltani M (2013) Niosomes as a propitious carrier for topical drug delivery. Expert Opin Drug Deliv 10:261–272. https://doi.org/10.1517/17425247.2013.746310
67. Marianecci C, Di Marzio L, Rinaldi F, Celia C, Paolino D, Alhaique F, Esposito S, Carafa M (2014) Niosomes from 80s to present: the state of the art. Adv Colloid Interf Sci 205:187–206. https://doi.org/10.1016/j.cis.2013.11.018
68. Arafa MG, Ayoub BM (2017) DOE optimization of nano-based carrier of pregabalin as hydrogel: new therapeutic & chemometric approaches for controlled drug delivery systems. Sci Rep 7:1–15. https://doi.org/10.1038/srep41503

69. Al-Mahallawi AM, Fares AR, Abd-Elsalam WH (2019) Enhanced permeation of methotrexate via loading into ultra-permeable niosomal vesicles: fabrication, statistical optimization, ex vivo studies, and in vivo skin deposition and tolerability. AAPS PharmSciTech 20:1–10. https://doi.org/10.1208/s12249-019-1380-5
70. Sala M, Diab R, Elaissari A, Fessi H (2018) Lipid nanocarriers as skin drug delivery systems: properties, mechanisms of skin interactions and medical applications. Int J Pharm 535:1–17. https://doi.org/10.1016/j.ijpharm.2017.10.046
71. Callender SP, Mathews JA, Kobernyk K, Wettig SD (2017) Microemulsion utility in pharmaceuticals: implications for multi-drug delivery. Int J Pharm 526:425–442. https://doi.org/10.1016/j.ijpharm.2017.05.005
72. Todosijević MN, Cekić ND, Savić MM, Gašperlin M, Ranđelović DV, Savić SD (2014) Sucrose ester-based biocompatible microemulsions as vehicles for aceclofenac as a model drug: formulation approach using D-optimal mixture design. Colloid Polym Sci 292:3061–3076. https://doi.org/10.1007/s00396-014-3351-4
73. Negi P, Singh B, Sharma G, Beg S, Raza K, Katare OP (2016) Phospholipid microemulsion-based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD-based development and evaluation. Drug Deliv 23:951–967. https://doi.org/10.3109/10717544.2014.923067
74. Patel HK, Barot BS, Parejiya PB, Shelat PK, Shukla A (2013) Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: ex vivo permeation and skin irritation studies. Colloids Surf B Biointerfaces 102:86–94. https://doi.org/10.1016/j.colsurfb.2012.08.011
75. Radwan SAA, ElMeshad AN, Shoukri RA (2017) Microemulsion loaded hydrogel as a promising vehicle for dermal delivery of the antifungal sertaconazole: design, optimization and ex vivo evaluation. Drug Dev Ind Pharm 43:1351–1365. https://doi.org/10.1080/03639045.2017.1318899
76. Wang Z, Mu HJ, Zhang XM, Ma PK, Lian SN, Zhang FP, Chu SY, Zhang WW, Wang AP, Wang WY, Sun KX (2015) Lower irritation microemulsion-based rotigotine gel: Formulation optimization and in vitro and in vivo studies. Int J Nanomedicine 10:633–644. https://doi.org/10.2147/IJN.S74079
77. de Mattos CB, Argenta DF, de Melchiades GL, Cordeiro MNS, Tonini ML, Moraes MH, Weber TB, Roman SS, Nunes RJ, Teixeira HF, Steindel M, Koester LS (2015) Nanoemulsions containing a synthetic chalcone as an alternative for treating cutaneous leshmaniasis: optimization using a full factorial design. Int J Nanomedicine 10:5529–5542. https://doi.org/10.2147/IJN.S83929
78. Afifah SN, Azhar S, Ashari SE, Salim N (2018) Development of a kojic monooleate-enriched oil-in-water nanoemulsion as a potential carrier for hyperpigmentation treatment. Int J Nanomedicine 13:6465–6479. https://doi.org/10.2147/IJN.S171532
79. Yu M, Ma H, Lei M, Li N, Tan F (2014) In vitro/in vivo characterization of nanoemulsion formulation of metronidazole with improved skin targeting and anti-rosacea properties. Eur J Pharm Biopharm 88:92–103. https://doi.org/10.1016/j.ejpb.2014.03.019
80. Alvarez-Trabado J, López-García A, Martín-Pastor M, Diebold Y, Sanchez A (2018) Sorbitan ester nanoparticles (SENS) as a novel topical ocular drug delivery system: design, optimization, and in vitro/ex vivo evaluation. Int J Pharm 546:20–30. https://doi.org/10.1016/j.ijpharm.2018.05.015
81. Marto J, Ruivo E, Lucas SD, Gonçalves LM, Simões S, Gouveia LF, Felix R, Moreira R, Ribeiro HM, Almeida AJ (2018) Starch nanocapsules containing a novel neutrophil elastase inhibitor with improved pharmaceutical performance. Eur J Pharm Biopharm 127:1–11. https://doi.org/10.1016/j.ejpb.2018.01.011
82. Abd-Elsalam WH, El-Zahaby SA, Al-Mahallawi AM (2018) Formulation and in vivo assessment of terconazole-loaded polymeric mixed micelles enriched with Cremophor EL as dual functioning mediator for augmenting physical stability and skin delivery. Drug Deliv 25:484–492. https://doi.org/10.1080/10717544.2018.1436098

83. Varshosaz J, Taymouri S, Minaiyan M, Rastegarnasab F, Baradaran A (2018) Development and in vitro/in vivo evaluation of HPMC/chitosan gel containing simvastatin loaded self-assembled nanomicelles as a potent wound healing agent. Drug Dev Ind Pharm 44:276–288. https://doi.org/10.1080/03639045.2017.1391832
84. Zhou J, Zhang W, Pang J, Wang X, Zheng Z, Li Y, Yang F, Yang W (2020) Optimized preparation of vinpocetine micelles and in vivo evaluation of its pharmacokinetics in rats. Pharm Dev Technol 25:464–471. https://doi.org/10.1080/10837450.2019.1709501
85. El-Say KM, El-Sawy HS (2017) Polymeric nanoparticles: promising platform for drug delivery. Int J Pharm 528:675–691. https://doi.org/10.1016/j.ijpharm.2017.06.052
86. Zhang Z, Tsai PC, Ramezanli T, Michniak-Kohn BB (2013) Polymeric nanoparticles-based topical delivery systems for the treatment of dermatological diseases. Wiley Interdiscip Rev Nanomed Nanobiotechnol 5:205–218. https://doi.org/10.1002/wnan.1211
87. Gonzalez-Pizarro R, Silva-Abreu M, Calpena AC, Egea MA, Espina M, García ML (2018) Development of fluorometholone-loaded PLGA nanoparticles for treatment of inflammatory disorders of anterior and posterior segments of the eye. Int J Pharm 547:338–346. https://doi.org/10.1016/j.ijpharm.2018.05.050
88. Deshmukh AS, Chauhan PN, Noolvi MN, Chaturvedi K, Ganguly K, Shukla SS, Nadagouda MN, Aminabhavi TM (2017) Polymeric micelles: basic research to clinical practice. Int J Pharm 532:249–268. https://doi.org/10.1016/j.ijpharm.2017.09.005

Design of Experiments for the Development of Transdermal Drug Products

4

Mohini Mishra and Kaisar Raza

Abstract

Development of an impeccable pharmaceutical product is always challenging in terms of process development and the product quality. Formulations meant for transdermal applications are sophisticated systems employing a plethora of constituents and using the traditional hit and trial approach of formulation design will lead to a troublesome job. This chapter enables one to understand about the various critical quality attributes and critical material attributes that affect the quality target profile of the transdermal product. Transdermal drug delivery systems (TDDS) are specially designed systems that are meant to deliver therapeutic agents across the skin of the patient for systemic effects. These systems are a type of controlled release drug delivery systems that tend to deliver therapeutic agents at a fixed rate over a protracted period of time. Quality by Design (QbD) being a scientific method that take into account various risk factors (risk assessment), quality target product profile (QTPP), critical quality attributes (CQA), and a control strategy for the process and product development. QbD aims at development of a TDDS product to deliver the optimum amount of drug across the skin while minimizing the amount of drug load, thus resulting in the least possible amount of residual drug substance. QbD leads to better understanding of the product and manufacturing process that contributes to profound assessment of the effects of variations in raw materials and the manufacturing process on drug product quality.

M. Mishra · K. Raza (✉)
Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Ajmer, Rajasthan, India
e-mail: drkaisar@curaj.ac.in

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_4

Keywords

Transdermal drug delivery systems · Quality by design · Critical quality attributes · Critical material attributes · Quality target product profile

4.1 Introduction

Developing an impeccable pharmaceutical product is always challenging in terms of process development and the product quality. Traditionally, formulation scientists used to optimize the process of formulation development by changing one variable at a time (OVAT) while remaining others constant, this approach was unable to discover the interdependence of different variables and hence not a robust method for the optimization of formulation development process and also squandering of precious time and resources [1, 2]. The formulations developed by such a hit and trial method lead to suboptimal formulations and not the most optimal formulation, basically the success of any formulation in this situation depends on the prior knowledge, experimentation skills, acumen, and fortune of the pharmaceutical formulator. Formulations meant for transdermal applications are sophisticated systems employing a plethora of constituents and using the traditional hit and trial approach of formulation design will lead to a troublesome job [1, 3].

4.2 Transdermal Drug Delivery Systems

Transdermal drug delivery systems (TDDS) are specially designed systems that are meant to deliver therapeutic agents across the skin of the patient for systemic effects. These systems are a type of controlled release drug delivery systems that tend to deliver therapeutic agents at a fixed rate over a protracted period of time [4]. Besides providing a controlled and constant administration of the drug, these also provide a continuous input of drugs and abrogate pulsed entry of the therapeutic agents into systemic circulation, which often results in untoward side effects. Drug release and performance of TDDS is greatly influenced by their composition along with biophysical, morphological, and physicochemical properties of the skin [5]. In the year 1979, FDA approved the first transdermal system, i.e. Transderm-SCOP [5, 6], later on various TDDS were approved by FDA as are listed in Table 4.1. The validation of TDDS as evidenced from percutaneous absorption of the therapeutic agents can be warranted by the measurable drug levels in the systemic circulation, excretion of drug and its metabolites, and the therapeutic response of the patient to the therapy [5]. Matrix type TDDS contain one or more active ingredients dissolved or partially suspended in a mixture of various components, including adhesives, penetration enhancers, softeners, and preservatives, and are typically manufactured using solvent, hydrogel, or hot melt-based practices. Reservoir type TDDS similarly contain a variety of components in liquid or semi-solid form, however, reservoir type TDDS utilize a heat-sealed area to entrap the active gel between the backing membrane and

Table 4.1 Enlisting some of the TDDS with the year of their FDA approval and the conditions for which they are used

S. No.	TDDS	Condition	Year	References
1.	Transderm-SCOP	Nausea and vomiting due to motion sickness	1979	[7]
2.	ZECUITY™ (sumatriptan iontophoretic transdermal system)	Migraine	1992	[8]
3.	Nitro-Dur® (nitroglycerin transdermal system)	Angina	1995	[9]
4.	Gelnique (oxybutynin)	Overactive bladder	2009	[10]
5.	Duragesic® fentanyl transdermal system	Chronic pain	2009	[11]
6.	Neupro(rotigotine) patch	Parkinson disease	2013	[12]
7.	Microneedle based influenza vaccine patch	Influenza	2016	[13]
8.	Mirataz (mirtazapine transdermal ointment)	Weight loss in cats	2018	[14]
9.	Secuado® (asenapine) transdermal system	Schizophrenia	2019	[15]
10.	Twirla® (levonorgestrel and ethinylestradiol)	Hormonal contraceptive patch	2020	[16]

a microporous membrane. Because of the inherent failure modes and safety risks associated with the reservoir TDDS, FDA recommends TDDS manufacturers and applicants focus development efforts on matrix type TDDS [17, 18].

Advantages of TDDS over other drug delivery systems and dosage forms are as follows [4, 19]:

1. TDDS tend to avoid the first pass metabolism of drugs.
2. These systems provide a continuous input of therapeutic agents and hence reduced fluctuations in the plasma levels of drugs which in turn corresponds to better therapeutic effect.
3. As TDDS tend to provide a continuous input of therapeutic agents, these systems are a good choice to formulate therapeutic agents with short half-life and a low therapeutic index.
4. These systems provide ease of elimination of drug delivery systems in case of toxicity as they are externally applied and can be easily removed.
5. Patient compliance is of utmost importance for the success of any drug delivery system, because of lower dosing frequency the TDDS serve to be more patient compliant and hence a better choice of drug delivery systems.
6. These systems provide a continuous and controlled delivery of therapeutic agents for a protracted period of time and henceforth avoiding therapeutic failures.
7. TDDS lead to attenuated inter or intra-patient differences or variability.

4.3 Quality by Design Approach in the Designing of TDDS

Being a sophisticated drug delivery system TDDS comprise of a variety of constituents that in turn make it so unique, the various quality attributes (constituents) for a TDDS include:

The most important constituent of any drug delivery system is drug or therapeutic agent itself. In TDDS the drug tends to remain in direct contact with the release liners. Linings are used to provide a protection to the patch during the storage of the TDDS. Polyester film could be a good example of liner. The unique property of TDDS is that they can be adhered to the skin and hence adhesive is an important constituent of the TDDS that could have a remarkable effect on the product quality. Acrylates, Silicones, Polyisobutylene are some examples of adhesives that are widely used in TDDS. Chemicals like terpenoids, terpenes, pyrrolidones, alcohol, ethanol, methanol, sodium lauryl sulfate, pluronic F127, pluronic F68 are used as permeation enhancers that affect the release of the drug from the TDDS. In TDDS, there exists a layer that tends to protect the delivery system from the outer environment which is termed as backing layer. Cellulose derivatives, Polypropylene Silicon rubber, Poly vinyl alcohol are used in the fabrication of backing layer [5, 6]. Adhesive layer is an integral part of TDDS, this can have an effect on the quality of the product as well as patient compliance too. The removal of TDDS from the skin can result in different types of situations based on adhesive and cohesive forces interplaying between the TDDS and skin of the patient. The TDDS patch is peeled off leaving no visible residue of the adhesive on the skin of the patient and another situation can be when the adhesive is adhered to the skin of the patient and no residue of adhesive remained on the TDDS patch, these situations are often termed as adhesive failures. Cohesive failure is a situation when the adhesive tends to remain on the skin of the patient as well as TDDS patch. Situations other than when the TDDS patch is peeled off leaving no visible residue of the adhesive on the skin of the patient are considered as flaws in the TDDS and are subjected to be of quality failure [6]. Quality concerns such as improper adhering of the TDDS patch on to the skin of the patient, wrinkled patch, difficulty in removing the liner from the TDDS patch, and conglutination of the therapeutic agent inside the patch that affects the release of the therapeutic agent and hence therapeutic activity. In the year 2007, ADHD patch was reported for the concerns like improper adhesion to the skin and cold flow, likewise in the year 2008, problems of dry crystallization were reported for the Parkinson's disease patch [20]. Likewise, there exists several other quality attributes that affect product profile, hence a well-organized systematic approach of designing and development of TDDS should be implemented that saves time and increases efficiency not like the hit and trial one that consumes more time and resources. Quality by Design (QbD) being a scientific method that take into account various risk factors (risk assessment), quality target product profile (QTPP), critical quality attributes (CQA), and a control strategy for the process and product development. QbD aims at development of a TDDS product to deliver the optimum amount of drug across the skin while minimizing the amount of drug load, thus resulting in the least possible amount of residual drug substance. QbD leads to better understanding of the product and

manufacturing process that contributes to profound assessment of the effects of variations in raw materials and the manufacturing process on drug product quality (drug product quality attributes and product performance characteristics such as drug permeation/flux rate, adhesion, application site reaction, and safety/quality issues (e.g., residual drug substance, cold flow, and seal breakage of the liquid reservoir systems)) [21].

The development of TDDS via QbD approach requires a systematic methodology which is detailed as follows [3, 17, 20]:

- It starts with the establishment of the required or desired quality target product profile (QTPP).
- After establishment of the QTPP, the critical quality attributes (CQAs) are identified (based on prior art, experimentation, and risk assessment) that tend to have effect on the quality of the product.
- Based on CQAs of the product (TDDS) type and amount of excipients are selected for the product development of the desired quality.
- Next step is to select an optimized manufacturing process and defining a control strategy.

A more elaborated QbD approach of TDDS product development also requires identification of the material attributes and process parameters that eventually affect the quality of the product and establishment of a link between the material attributes and the process parameters. FDA has provided a complete guidance on the QbD approach towards designing of the TDDS, the various considerations and terms as described by FDA has been discussed herein:

4.4 Quality Target Product Profile

The QTPP corresponds to the quality characteristics that are required to be accomplished to guarantee the want to quality, safety, and efficacy of the TDDS product. QTPP are determined based on the therapeutic need, population of the patient, and other functional requirements. For an instance, based on application site of the product or the population (if pediatric) the size range (a QTPP) of the TDDS product is determined. The various QTPP element and their quality considerations are tabulated in Table 4.2 [17].

4.5 Critical Quality Attributes

Critical quality attributes (CQAs) are the characteristic properties (physical, chemical, or biological) of the finished products that require to be within the appropriate limits to assure the want to quality of the product. CQAs are determined based on the defined QTPP, prior art, experimentation, and the risk assessments. Each CQA either

Table 4.2 The various QTPP elements and their quality considerations for TDDS

S. No.	QTPP element	Quality considerations
1.	In vivo delivery of active ingredient to achieve therapeutic effect	Formulation design and manufacturing control
2.	Minimization of residual drug	Formulation design
3.	Adherence for duration of wear period	Excipient selection, component control, physical design (shape, dimensions, etc.), and manufacturing control
4.	Minimization of irritation	Formulation design
5.	Chemical and physical stability for shelf life	Formulation design, container closure attributes, storage conditions
6.	Non-drug substance-related impurities	Excipient selection and manufacturing control

alone or in relation to other CQAs must relate to the elements of the QTPP of the TDDS product [3, 17].

FDA has described CQAs for TDDS into four categories which are discussed as follows:

4.5.1 TDDS Product

For TDDS, CQAs generally include appearance (such as lack of visible crystals), uniformity of dosage units, dimensions, peel adhesion, permeation enhancer content, impurities and degradants, preservative/antioxidant content (if present), assay, in vitro drug release profile, release liner peel strength, tack, cold flow, shear strength, residual solvents, residual monomers, package integrity, and microbial limits.

4.5.2 Therapeutic Agent

TDDS belongs to a unique category of drug delivery systems which is specially designed for selected therapeutic agents and special diseased conditions. Physico-chemical and biological properties of the therapeutic agent can have an effect on the performance of the TDDS product and its manufacturing process, therefore selection of drug should be made wisely. Characteristics of drug like, molecular weight, pKa, pH, melting point, partition coefficient, and aqueous solubility have the potential to affect the rate of delivery of drug across the skin. Solid-state properties like crystal type, particle size, and polymorphism should be taken into consideration prior to the product development process as these have the tendency to affect the product quality and performance.

4.5.3 Excipients and Components

TDDS is a sophisticated drug delivery system category that includes a bundle of excipients and other components such as adhesives, tackifiers, rate controlling membranes, non-rate controlling membranes, permeation enhancers, solubilizers, agents such plasticizers or softeners, all these components have drastic potentials to have an impact on the quality and performance of TDDS. Selection of these excipients and components should be made wisely to assure quality attributes.

For an instance, as per the FDA guidance, an agent to qualify as an adhesive, the following attributes should be taken into consideration:

- For adhesive polymer(s) as raw material(s): molecular weight, polydispersity, spectroscopic analysis (e.g., infrared radiation (IR) absorption), thermal analysis, intrinsic or complex viscosity, and measurement of residual monomers, dimers, solvents, heavy metals, catalysts, and initiators.
- For adhesive as a laminate (in the absence of the active ingredient and other excipients): residual solvents, peel, tack, shear, and adhesion.
- For adhesive in the final product (along with drug substance and other excipients and components): identification, residual monomers, dimers, and solvents; impurities; loss on drying; and uniformity. Other properties to be considered include the viscoelastic properties (such as elastic modulus (G'), viscous modulus (G''), and creep compliance (J)), and functional properties including, but not limited to, peel, shear, adhesion, tack, in vitro drug release, and in vitro drug permeation.

The properties of an adhesive as raw material (e.g., rheology, including intrinsic viscosity and complex viscosity) can impact the final product quality attributes. Adhesive suppliers' specifications are often wide; thus, adhesive raw material received throughout the life cycle of the product may vary greatly within the adhesive suppliers' specifications. For example, the rheological properties of the adhesive lots used in the pivotal in vivo trial for TDDS (e.g., bioequivalence (BE), Pharmacokinetic (PK), adhesion studies) may not be consistent with the supplier's previously manufactured adhesive lots or their future adhesive lots. Therefore, applicants should request historical rheology values from the adhesive manufacturer to better understand their process capabilities and the potential influence of variability in the adhesive rheology on the final product. This can further assist applicants in assessing the need to establish or tighten internal controls for the raw material.

Identifying, evaluating, and properly controlling similar quality attributes of other key components of TDDS products will enhance product and process understanding of the TDDS throughout its life cycle.

4.5.4 Labelling

Inks used for labelling may have an interaction with the TDDS product; henceforth, proper assessment of inks during the product development process (extractable and leachable studies) is emboldened. Generally labelling is applied on the backing film. FDA recommends the use of printing ink color that has proper contrast and should stay behind on the product even after the removal of the patch from the skin or after disposal. TDDS that are colored to be in match with the human skin tone, clear or translucent invite errors during administration procedure (difficulty in finding TDDS on the skin of the patient, unable to remove TDDS properly, and applying new system on the existing one), that in turn may lead to product failure.

4.6 Case Studies

Garg et al., developed nanostructured lipid carriers of aceclofenac by QbD approach employing 3^3 factorial design for the optimization of the formulation. The research group evaluated the nanostructured lipid carriers of aceclofenac for the transdermal penetration potential and stability, the different surfactants and lipids were considered as critical material attributes (CMAs), while particle size, zeta potential, polydispersity index (PDI), entrapment efficiency, and in vitro drug release were selected as CQAs to attain QTPP. The cause and effect relationship between various CMAs and CQAs was analyzed by Ishikawa fishbone diagram with the help of Minitab 17.0.4 software. Further, they carried out risk assessment matrix (RAM) for prioritizing the high-risk factor related with each CMAs or critical process parameters (CPP) of the formulation. Later, they employed Taguchi orthogonal array design for the screening of critical formulation attributes (CFAs) (type of liquid lipid, type of solid lipid, type of surfactant, %surfactant, ratio of lipid mixture and liquid lipid, stirring speed, and temperature). Finally, eight formulations were formulated and evaluated for various CQAs and on the basis of these observations CFAs were finalized employing Design Expert® (v10.0) Software [22].

Dhingani et al., developed W/O type microemulsion based formulation of Atenolol for the transdermal application employing QbD approach. A three-factor D-optimal mixture design was used to study the correlation between the independent and critical dependent variables. Concentration of oil, concentration of surfactant mixture (surfactant and co-surfactant), and concentration of water were considered as independent variables. They carried out 16 runs in triplicates, and the critical responses were identified and evaluated using Unscrambler® 10.2 (CAMO AS, Norway, Switzerland) [23].

Akhlaq et al., formulated seventeen dexibuprofen transdermal patch employing the use of three-factor three-level Box–Behnken design for the optimization with the aid of Design Expert® (v 6.0) Software. Matrix polymer, penetration enhancer, and plasticizer were selected as independent variables by the research group [24].

Jain et al., employed full factorial design-based study for the optimization of diclofenac loaded ethosomes meant to be administered via transdermal route. They

reported that full factorial design enables simultaneous variation of all the formulation variables making quantification of the effects produced by them and any feasible interaction. The correlation between different formulation variables and the physicochemical properties was assayed by regression analysis with the help of using John Macintosh Program (JMP, SAS Institute, Cary, NC). Ethanol concentration and phosphatidylcholine: cholesterol ratios were selected as independent variables by the research group whereas vesicle size, zeta potential, elasticity, and entrapment efficiency as dependent variables [25].

Ahmed et al., formulated liposomal formulation of Glimepiride for the transdermal administration making use of QbD approach coupled with near infrared. For the assessment of cause and effect correlation, they employed Ishikawa fishbone diagram and selected liposomal vesicular size, entrapment efficiency and in vitro release from the liposomal films as CQAs for the determination of all the potential risks. Plackett–Burman design was naited as a screening design to identify the important factors that have an impact during the development process and further optimization. Seven independent variables were categorized into two categories: first category include factors that are involved in the formulation of liposomes such as phosphatidylinositol, drug concentrations, cholesterol, and pH of hydration medium, whereas the second category includes factors which are involved in the fabrication of transdermal film, namely, matrix-forming material (HPMC), permeation enhancer (DMSO), plasticizer (propylene glycol). Based on risk assessment studies the research group concluded that chemical (phospholipid, drug, and cholesterol concentrations), assessment method, physical (hydration medium, sonication parameters, freeze–thaw cycles), and instrumentation might have an impact in the entrapment efficiency of the Glimepiride liposomal formulation. The vesicular size was reported to be affected by the chemical, physical, suspension method, and the sizing method parameters. Further, they reported that the Glimepiride release from the transdermal film is affected by the liposomal related, dissolution method, transdermal matrix related, and instrumental variability [26].

Bakonyi et al., developed and evaluated the semi-solid drug carrier system for the transdermal administration of the drug lidocaine with the aid of QbD approach to be used as anesthetic. The QTPP identified by the research group are as follows with the justification being described in the brackets: route of administration (transdermal route of drug delivery by passes first pass metabolism and avoids systemic side effects and better patient compliance), therapeutic indication (local anesthesia is important for conditions like minor surface skin surgical, and at times when relief from irritation, itching, and pain is sought), site of activity (transdermal formulations are designed to allow penetration into the deeper areas of viable epidermis and dermis where nerve fibers are present), release profile (prolonged duration of local anesthetic action is required), stability (the product should be stable in terms of physical, biological, and chemical stability to avoid conditions like phase separation, crystal formation, change in pH, viscosity, and appearance of the product), dosage strength (5% lidocaine formulations are effective as local anesthetic), and dosage form (semi-solid systems are required). Further, the research group defined various CQAs in accordance with the QTPP are physical attributes, homogeneity of API in

drug product, solubility of API in drug product, moisturizing effect, viscosity, in vitro drug release, ex vivo drug release, TEWL (trans epidermal water loss), and dosage form type. After assessment of the risks involved in the QbD approach the various characteristics that found to influence the product quality are in vitro and ex vivo drug release (both 18%), dosage from type (15%), moisturizing effect and TEWL (both 13%). Based on the results concluded from RAM, the severity of different CQAs was identified as follows: in vitro and ex vivo drug release, dosage from type, moisturizing effect, and TEWL with the highest severity score (>300), which suggests that these factors are the most influential ones on the quality of product [27].

4.7 Conclusion

Quality based drug design approach is an excellent tool for the fabrication of transdermal formulations. The various critical quality attributes as well as critical material attributes that tends to affect the quality target product profile of the formulation can be determined at an early stage of development and hence will benefit the formulator in the selection of excipients. QbD leads to better understanding of the product and manufacturing process that contributes to profound assessment of the effects of variations in raw materials and the manufacturing process on drug product quality.

References

1. Raza K, Singh B, Singal P, Wadhwa S, Katare OP (2013) Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. Colloids Surf B Biointerfaces 105:67–74
2. Garg V, Singh H, Bhatia A, Raza K, Singh SK, Singh B et al (2017) Systematic development of transethosomal gel system of piroxicam: formulation optimization, in vitro evaluation, and ex vivo assessment. AAPS PharmSciTech 18(1):58–71
3. Singh B, Kumar R, Ahuja N (2005) Optimizing drug delivery systems using systematic "design of experiments." Part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst 2005:27–105
4. Sharma G, Thakur K, Raza K, Singh B, Katare OP (2017) Nanostructured lipid carriers: a new paradigm in topical delivery for dermal and transdermal applications. Crit Rev Ther Drug Carrier Syst 34(4):355–386
5. Shingade GM (2012) Review on: recent trend on transdermal drug delivery system. J Drug Deliv Ther 2:1
6. Wokovich AM, Prodduturi S, Doub WH, Hussain AS, Buhse LF (2006) Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute. Eur J Pharm Biopharm 64:1–8
7. Drugs@FDA: FDA-Approved Drugs [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=017874
8. Zecuity (Sumatriptan Iontophoretic Transdermal System): Uses, Dosage, Side Effects, Interactions, Warning [Internet]. [cited 2020 Jul 24]. Available from: https://www.rxlist.com/zecuity-drug.htm

9. (No Title) [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/anda/98/89884_Nitroglycerin Transdermal_Admindocs.pdf
10. (No Title) [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/202513s003lbl.pdf
11. (No Title) [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2005/19813s039lbl.pdf
12. (No Title) [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/021829lbl.pdf
13. Inactivated Influenza Vaccine Delivered by Microneedle Patch or by Hypodermic Needle - Full Text View - ClinicalTrials.gov [Internet]. [cited 2020 Jul 24]. Available from: https://clinicaltrials.gov/ct2/show/NCT02438423
14. Mirataz (mirtazapine transdermal ointment) - Veterinarians | FDA [Internet]. [cited 2020 Jul 24]. Available from: https://www.fda.gov/animal-veterinary/product-safety-information/mirataz-mirtazapine-transdermal-ointment-veterinarians
15. (No Title) [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/212268s000lbl.pdf
16. (No Title) [Internet]. [cited 2020 Jul 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/204017s000lbl.pdf
17. Fda, Cder, hillsi. Transdermal and topical delivery systems-product development and quality considerations guidance for industry DRAFT GUIDANCE [Internet]. [cited 2020 Jun 21]. Available from: https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
18. Prausnitz MR, Langer R, Author NB (2008) Transdermal drug delivery NIH public access author manuscript. Nat Biotechnol 26(11):1261–1268
19. Sharma G, Dhankar G, Thakur K, Raza K, Katare OP (2016) Benzyl benzoate-loaded microemulsion for topical applications: enhanced dermatokinetic profile and better delivery promises. AAPS PharmSciTech 17(5):1221–1231
20. Bandyopadhyay A. Transdermal drug delivery system-quality by design approach. 2017. Available from http://transdermalspecialties.com/home.html
21. Fda, Cder, ocheltreet. Guidance for Industry Residual Drug in Transdermal and Related Drug Delivery Systems [Internet]. 2011 [cited 2020 Jun 21]. Available from: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
22. Garg NK, Sharma G, Singh B, Nirbhavane P, Tyagi RK, Shukla R et al (2017) Quality by Design (QbD)-enabled development of aceclofenac loaded-nano structured lipid carriers (NLCs): an improved dermatokinetic profile for inflammatory disorder(s). Int J Pharm 517 (1–2):413–431
23. Dhingani A, Patel J, Garala K, Raval M, Dharamsi A (2014) Quality by design approach for development of W/O type microemulsion-based transdermal systems for atenolol. J Dispers Sci Technol 35(5):619–640
24. Akhlaq M, Arshad MS, Mudassir AM, Hussain A, Kucuk I, Haj-Ahmad R et al (2016) Formulation and evaluation of anti-rheumatic dexibuprofen transdermal patches: a quality-by-design approach. J Drug Target 24(7):603–612
25. Jain S, Patel N, Madan P, Lin S (2015) Quality by design approach for formulation, evaluation and statistical optimization of diclofenac-loaded ethosomes via transdermal route. Pharm Dev Technol 20(4):473–489
26. Ahmed OAA, Kurakula M, Banjar ZM, Afouna MI, Zidan AS (2015) Quality by design coupled with near infrared in formulation of transdermal glimepiride liposomal films. J Pharm Sci 104(6):2062–2075
27. Bakonyi M, Berkó S, Kovács A, Budai-Szűcs M, Kis N, Erős G et al (2018) Application of quality by design principles in the development and evaluation of semisolid drug carrier systems for the transdermal delivery of lidocaine. J Drug Deliv Sci Technol 44:136–145

Design of Experiments for the Development of Injectable Drug Products

5

Dhawal Chobisa

Abstract

Design of experiments (DoE) is a widely used statistical tool for planning experiments, collecting and analyzing data, and drawing valid conclusions. This chapter describes the basics of DoE, types of DoE designs, and rationale for the selection of a design. Applications of DoE in the development of pharmaceutical drug products are discussed with emphasis on injectable drug products. Also, a practical case study of the development of a nanoemulsion product is discussed in detail.

Keywords

Design of experiments (DoE) · Injectable drug products · Screening and optimization designs · DoE in the pharmaceutical drug products · Nanoemulsion · Design analysis · Residual and influence diagnostics

5.1 Introduction

The objective of the development of pharmaceutical drug products is to deliver safe and efficacious medicines to the patients reliably. It is crucial to ensure the desired drug product quality reproducibly. Failure to achieve the quality of drug products can lead to severe safety concerns and suboptimal therapeutic benefits for the patients. In early 2000, Quality by Design (QbD) based drug product development

D. Chobisa (✉)
Department of Industrial and Physical Pharmacy, College of Pharmacy, Purdue University, West Lafayette, IN, USA

Integrated Product Development Organization, Innovation Plaza, Dr. Reddy's Laboratories, Hyderabad, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_5

was adopted by the United States Food and Drug Administration (US-FDA) and other regulatory authorities to ensure the quality of the products [1–3].

Pharmaceutical drug products can be administered by various routes such as oral, topical, and injectable administration. The safety margin is very narrow with injectable (e.g. intravenous) dosage forms as they bypass the absorption step and quickly access the systemic circulation. QbD based development of injectable drug products involves the designing of drug delivery systems such as liposomes, polymeric nanoparticles, lyophilized powder, solutions, suspensions, and emulsions, selection of excipients, and product composition. Also, robust manufacturing processes and analytical methods are crucial to develop quality drug products. The understanding of unit operations such as sterilization is a unique challenge faced during the development of injectable dosage forms. Various six-sigma tools, including design of experiments (DoE), risk assessment, critical to quality (CTQ), affinity diagram, quality function deployment (QFD), failure mode and effects analysis (FMEA), statistical analysis, process capability analysis, control strategy, etc. are useful at different stages of product development. DoE is one of the most widely used tools for formulation and analytical method development, process optimization, and process validation [4, 5].

DoE is a systematic statistical approach that allows the evaluation of the impact of change in multiple input variables, known as factors, within the boundary of experimental design, on the output variables, known as responses. DoE is a robust data collection (by designing and conducting experiments) and analytical tool (by analysis and inferring data). The stochastic models, developed based on specific factors combination and obtained results, are used for identifying the effects of factors on responses and help understand the nature of interactions between two or more factors. DoE is useful for obtaining the "true optimum" (design space) with minimum possible experiments leading to faster and cost-effective product and process development. Also, an essential advantage of using DoE is that the experimenters can quantify the interactions between factors which cannot be determined with traditional one factor at a time (OFAT) approach [6–9].

5.2 Basics of DoE

This section describes basic concepts used frequently in DoE, which are essential to understand before discussing various types of experimental designs, the rationale of design selection, and case studies.

5.2.1 Randomization

R. A. Fisher introduced the concept of randomization in experimental designs in 1925. Randomized experiments are considered as "gold standards" for inferring unambiguous and valid conclusions from statistical data [10]. Systematic (but not randomized) experiments lead to judgment bias and inaccurate interpretation of the

data. Also, non-randomized experiments are vulnerable to confounding or hidden variables, also known as lurking variables, which vary with time. Examples of lurking variables include a change in temperature of different shelves in lyophilizer during series of experiments, the machine heats up, change in experimenter, temperature or humidity changes, etc. Randomization does not mean that experiments to be performed in any order occur to the experimenter; it must be a physical experimental process [11]. Methods for randomization include simple randomization (flipping a coin, throwing dice, and randomly select a card from a shuffled deck), block randomization (grouping in equal sample size), and stratified randomization (randomization in a way that controls and balances the effect of covariates) [12].

Randomization serves the following purposes [13].

- No selective bias to the results of experiments.
- Accurate and unbiased estimation of error effects.
- Ensures that the error effects are statistically independent.

5.2.2 Blocking

Blocking is a mathematical technique of removing variations associated with a known change during the experiment. For example, if two different batches (or lots) of a surfactant is needed to prepare an emulsion product; the change in manufacturer batch (or lot) of surfactant might affect the properties of the emulsion. Performing the experiments with two different blocks (each block of experiments with one batch of surfactant) normalizes the effect caused by batches of surfactant. Blocking helps to reduce variability due to known reasons for experiments that may take several days, may involve different experimenters, and may subject to known changes in experimental conditions. However, the experimenter should be careful during block selection. Blocking should not be applied to a factor if the experimenter is interested in studying the effect of that factor on the response. For example, in the above emulsion experiments, blocking should not be used on surfactant if the experimenter is interested in evaluating the effect of different batches of surfactant on emulsion properties. Blocking can be applied to more than one factor during the experiments [6, 14].

Blocking serves the following purposes [14].

- Ensures that the blocked variable does not spoil the evaluation of other variables.
- Precise estimation of an experimental error (by removing the effect of a blocked variable from error calculation).
- In a few cases, it is possible to measure the effect of the blocked variable on the response.

5.2.3 Orthogonality

Independent variables (factors) affecting the dependent variable (response) are orthogonal if they are not correlated. For example, the concentration of the oil phase and concentration of surfactant are two orthogonal independent variables affecting the stability of the globule size distribution of an emulsion.

Orthogonality is an indicator of the independence of factors. In the DoE layout, each column is representative of a factor. It is important to estimate the effect of a factor (and interactions) independently without the interference of other factors. Orthogonality ensures independent estimation of the effect of a factor [15, 16].

5.2.4 Replication

Replication can be defined as the repetition of the same set of experimental conditions more than once. All similar experiments are known as replicates. The variability in the response for a similar set of experimental conditions indicates that the source must be something other than the factors controlled during the experiments. The objectives of replicate experiments are to determine the experimental error and reduce the bias due to uncontrolled variables. It increases the signal to noise (S/N) ratio if the noise is due to uncontrolled variables. The experimental error can also be determined if the process is in statistical control for a time. The standard error of mean (SEM) (standard deviation of the theoretical distribution of the sample means) can be expressed by $\sqrt{(s/n)}$, where s stands for the standard deviation (measure of dispersion of individual values) and n stands for the number of samples. It is desirable to have a higher number of samples and a lower standard deviation to achieve lower SEM. Replicates can increase the number of samples, whereas blocking helps decrease the standard deviation [17].

5.2.5 Confounding/Aliasing

Confounding or aliasing refers to the inability of clean estimation of effects and interactions. Effects that cannot be estimated independently of each other are considered confounded or aliased. It is the price experimenter pays with the fractional factorial designs because experiments for all combinations of factor levels are not possible with a reduced number of experiments, i.e., not enough degree of freedom. For example, if the estimate of effect X_4 in four-factor experiment is $(X_4 + X_1X_2X_3)$, then the main effect X_4 is aliased with 3-way interaction $X_1X_2X_3$. It cannot be concluded whether the significant effect, if any, is due to X_4 alone, interaction $X_1X_2X_3$, or both [18, 19].

Confounding is undesirable. However, it is not practically possible to perform experiments for all combinations of factor levels at industrial settings due to time and cost constraints. Confounding is a decision for an experimenter to make for knowingly confound (higher order) interactions with main effects while generating the

experimental design. The good news for pharmaceutical scientists is that the higher-order interactions (3 factor interactions (3FI) and above), generally, have been observed to be insignificant in most cases [18, 19].

5.2.6 Resolution

The resolution of an experimental design refers to the degree of confounding, i.e., the degree to which the main effects are confounded with 2 or 3 or higher factor interactions. The number of resolution of design indicates interactions confounded with main effects; for example, resolution III means the main effects are confounded with 2-factor interactions (2FI). Similarly, in resolution IV designs, main effects are confounded with 3-factor interactions (3FI), and 2FI are confounded with 2FI. Generally, 2FI has a significant effect on responses. It is advisable to choose designs with a higher resolution. Resolution V or higher designs are good for characterization, and resolution IV designs are adequate for screening purposes. The resolution III designs should only be used for ruggedness testing and comparisons. The resolution term is not applicable to full factorial designs as they do not have a confounding effect [7, 20].

5.2.7 Model

A model is a mathematical relationship such as equations and formula constructed using statistical methods that relate changes in one or more factors to the changes in responses. Based on the nature of collected data, different models might be helpful such as linear models, interaction models, quadratic models with curvature in one or more variables, cubic models, etc.

Caution: Before we move forward to the types and selection of experimental designs, it is essential to understand what DoE can give us. *The design of experiments is not the panacea*. Statistical modeling works best with a sound scientific approach. Understanding the scope of experiments, selection of appropriate factors and levels, and suitable DoE design is the key to successful DoE. Factors and levels (operating ranges within the experimental boundary) should be selected based on scientific rationale. A few pre-DoE experiments might help to decide the factors and ranges to use in DoE. Also, tools such as a cause-and-effect relationship (fishbone diagram) and risk analysis are useful for the selection of factors.

5.3 Types and Criteria for Selection of an Experimental Design

5.3.1 Types of Experimental Designs

DoE designs include factorial and fractional designs, Placket–Burman, Taguchi design, response surface methods, etc. Following are the types of DoE designs categorized according to the objective of the experiments [21].

5.3.1.1 Comparative Objective

If the primary objective is to identify whether a factor, out of several studied factors, is significant. Randomized block designs are useful for comparative purposes.

5.3.1.2 Screening Objective

If the primary objective is to screen out a few vital factors out of several investigated factors that affect the responses. The selected important factors can be studied further for optimization. Fractional factorial designs, Placket–Burman, Taguchi designs, etc. are useful for screening purposes.

5.3.1.3 Optimization Objective

The optimization objective includes identification and quantification of the main effects and higher-order interactions, resulting in a design space. Several product and process development experiments have the goal of optimization to ensure reproducible quality. Various designs are used for optimization purposes, such as response surface methods (RSM) designs, including central composite, Box–Behnken, and optimal designs. Mixture designs such as simplex lattice, simplex centroid, and optimal designs are useful if the factors are proportions of a mixture. Mixture designs are used to find out the optimum composition/fraction of factors to achieve desired responses.

5.3.2 Rationale for Selection of an Experimental Design

The selection of an experimental design depends on parameters such as the objective of the experiments, number of factors to be investigated, available resources such as feasibility of maximum number experiments, time, material, cost, etc. A higher number of experiments provide more information, but in most practical cases, it is not feasible to invest more resources and a long time. Faster development of quality drug products is the key.

Full factorial designs are useful if the number of factors to be studied is less than 5. In case of factorial designs, the number of experiments is determined by $2^K$where K = number of factors. Full factorial design for 3, 4, and 5 factors suggest 8, 16, and 32 experiments, respectively. The number of experiments increases exponentially as the number of factors increases, for example, 64 (6 factors), 128 (7 factors), 256 (8 factors), and so on, which is cumbersome and time-consuming. Screening designs are useful to select a few important factors out of many. Fractional factorial,

minimum run screening, Plackett–Burman, and Taguchi designs are useful for screening (number of factors 5 or more). Response surface designs are used for characterization and optimization. These designs can be applied using the selected important factors, generally but not limited to 2–4, from the screening studies [14].

5.4 Case Study—Screening DoE for a Sterile Nanoemulsion Product

5.4.1 Introduction

5.4.1.1 Product

ABC (1% w/v) nanoemulsion.

5.4.1.2 Objective

The objective of the screening design was to find out the most important factors affecting the responses, i.e., selection of a vital few factors from the trivial many. Selected factors were studied for optimization purposes.

5.4.1.3 Factors

Factors were selected based on domain knowledge and a risk assessment (Table 5.1).

5.4.1.4 Responses

Responses are the critical quality attributes of the drug product.

- Globule size distribution (Z-average and PDI).
- In vitro drug release (IVR) at 1 h, 6 h, and 12 h.

Table 5.1 Factors for screening DoE

Factors	Factor names	Units	Type	Subtype	Minimum	Maximum
A	Particle size of drug (D50)	μ	Numeric	Continuous	10	30
B	Viscosity of oil phase[a]	N s/m^2	Numeric	Continuous	0.550	0.750
C	Homogenization temperature	^{0}C	Numeric	Continuous	30	60
D	Homogenization pressure	Bar	Numeric	Continuous	200	1000
E	Homogenization time	Minutes	Numeric	Continuous	5	25
F	Preservative	Wt. %	Numeric	Continuous	0.1	2

[a]Oil phase refers to the mixture of mineral oil, drug, and surfactant

Table 5.2 Estimated and confounded terms of screening DoE design

Estimated term	Confounded terms
A	A + BCE + DEF
B	B + ACE + CDF
C	C + ABE + BDF
D	D + AEF + BCF
E	E + ABC + ADF
F	F + ADE + BCD
AB	AB + CE
AC	AC + BE
AD	AD + EF
AE	AE + BC + DF
AF	AF + DE
BD	BD + CF
BF	BF + CD
ABD	ABD + ACF + BEF + CDE
ABF	ABF + ACD + BDE + CEF

5.4.2 Experimental Design

5.4.2.1 Selection of Design

A fractional factorial design (2^{6-2}, resolution IV; software—Design-Expert®Version 12.0.9.0, Stat-Ease Inc.) was selected. Resolution IV design allows clean estimation of main effects. The 2FI confound with other 2FI, which might not be a concern for screening purpose. Table 5.2 shows the confounded terms of the designs, which show that the main effects are confounded with 3FI (insignificant in most cases). Minimum run screening design (a set of two-level designs) can be used if it is necessary to reduce the experimental runs. However, minimum run designs are extremely sensitive to missing data. Even one missing data reduce the resolution of design to III, which means the main effects will be confounded with 2FI [22].

The power of the design (the ability of the design to detect the significant effects) should be more than 80% for practical purposes. It is advisable to decide the S/N ratio based on desirable difference to detect (signal) and the variability in the measurements of responses (noise).

5.4.2.2 Design Layout

Table 5.3 shows the design layout with factors combination and responses obtained after the experiments. Experiments were performed in a randomized manner to avoid bias.

5.4.2.3 Design Summary

Two-level factorial design with reduced 3FI without center points or blocks was built. Tables 5.4 and 5.5 show the summary and descriptive statistics of factors and responses, respectively.

Table 5.3 Design layout with responses

		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Response 1	Response 2	Response 3	Response 4	Response 5
Std	Run	A:Particle size of drug (D50)	B: Viscosity of oil phase	C: Homogenization temperature	D: Homogenization pressure	E: Homogenization time	F: Preservative	Z-average	PDI	IVR 1 h	IVR 6 h	IVR 12 h
		Micron	N s/m^2	Degree C	Bar	Minutes	Wt. %	Nm		%	%	%
2	1	30	0.55	30	200	25	0.1	610	0.17	22.1	57.8	82.3
1	2	10	0.55	30	200	5	0.1	829	0.27	14.4	50.1	78.8
8	3	30	0.75	60	200	25	0.1	550	0.12	24.2	58.8	87.7
10	4	30	0.55	30	1000	25	2	715	0.2	18.6	54.6	79.7
7	5	10	0.75	60	200	5	0.1	598	0.13	23.1	59.9	86.5
13	6	10	0.55	60	1000	25	0.1	239	0.07	29.5	65.4	93.7
12	7	30	0.75	30	1000	5	0.1	777	0.25	17.2	53.1	79
6	8	30	0.55	60	200	5	2	576	0.16	24.1	51.8	76.2
15	9	10	0.75	60	1000	5	2	269	0.09	27	62.8	90
4	10	30	0.75	30	200	5	2	832	0.26	15.5	48.8	72.8
5	11	10	0.55	60	200	25	2	544	0.15	25.9	57.7	82.2
9	12	10	0.55	30	1000	5	2	781	0.22	16.2	46.6	68.5
11	13	10	0.75	30	1000	25	0.1	722	0.23	17.9	45.9	70.1
16	14	30	0.75	60	1000	25	2	245	0.1	29.2	66.2	92.4
14	15	30	0.55	60	1000	5	0.1	275	0.12	30.1	63.4	94.5
3	16	10	0.75	30	200	25	2	617	0.18	22.8	44.8	70.9

Table 5.4 Summary of factors with associated descriptive statistics

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Particle size of drug (D50)	Micron	Numeric	10.00	30.00	−1 ↔ 10.00	+1 ↔ 30.00	20.00	10.33
B	Viscosity of oil phase	N s/m2	Numeric	0.5500	0.7500	−1 ↔ 0.55	+1 ↔ 0.75	0.6500	0.1033
C	Homogenization temperature	Degree C	Numeric	30.00	60.00	−1 ↔ 30.00	+1 ↔ 60.00	45.00	15.49
D	Homogenization pressure	Bar	Numeric	200.00	1000.00	−1 ↔ 200.00	+1 ↔ 1000.00	600.00	413.12
E	Homogenization time	Minutes	Numeric	5.00	25.00	−1 ↔ 5.00	+1 ↔ 25.00	15.00	10.33
F	Preservative	Wt. %	Numeric	0.1000	2.00	−1 ↔ 0.10	+1 ↔ 2.00	1.05	0.9812

Table 5.5 Summary of responses with associated descriptive statistics

Response	Name	Units	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Transform	Model
R1	Z-average	Nm	16.00	Factorial	239	832	573.69	210.64	3.48	None	Mean
R2	PDI		16.00	Factorial	0.07	0.27	0.1700	0.0632	3.86	None	Mean
R3	IVR 1 h	%	16.00	Factorial	14.4	30.1	22.36	5.22	2.09	None	Mean
R4	IVR 6 h	%	16.00	Factorial	44.8	66.2	55.48	7.07	1.48	None	Mean
R5	IVR 12 h	%	16.00	Factorial	68.5	94.5	81.58	8.58	1.38	None	Mean

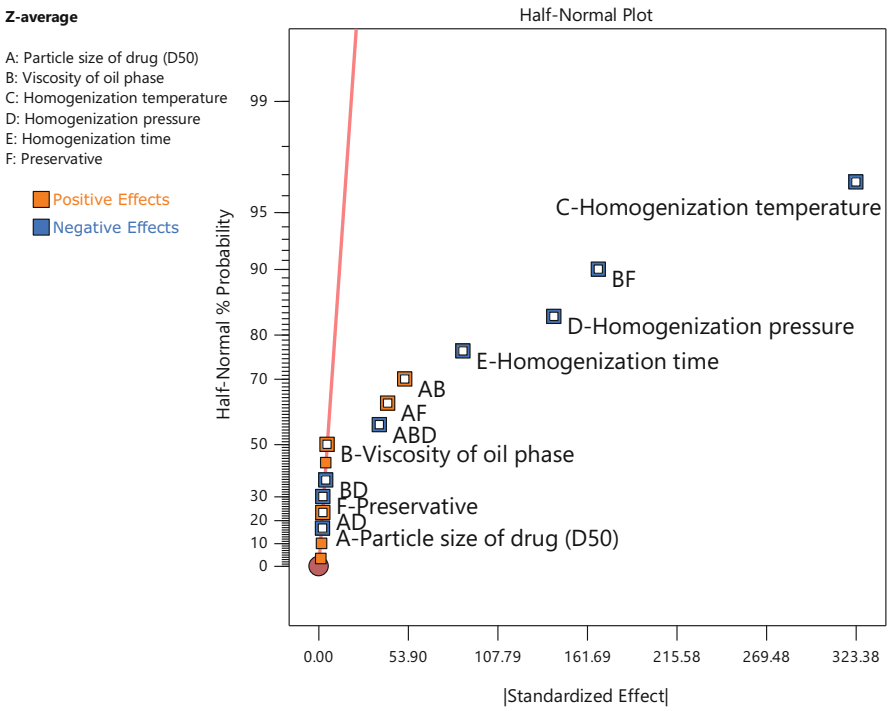

Fig. 5.1 Half-normal plot for respone 1(Z-average)

5.4.3 Effects Analysis

5.4.3.1 Response 1: Z-average

Half-normal plot: It assesses the relative significance of factors or interaction terms. It is a scale to determine the impact of factors or interaction terms on response. The terms that have more significant estimated effects appear away from the line in the right corner [23, 24]. Half-normal plot for a response (Fig. 5.1) shows that the main effects homogenization temperature (factor C), homogenization pressure (factor D), and homogenization time (factor E) have the larger effects. Also, interactions terms BF, AB, AF, and ABD are significant as they are away from the line. We can identify and focus on three main effects (C, D, and E) for further evaluation.

Normal plot: Normal probability plot of estimated effects is another tool to assess the relative impact/significance of factors or terms on response. The terms on the line have minimal effect, whereas terms on either side of the line represent the higher impact (greater the distance from the line, higher the impact) [25]. Figure 5.2 shows similar results as of the half-normal plot. Half-normal plot is another way of representing the normal plot with only positive values (conversion of estimated effects in absolute numbers).

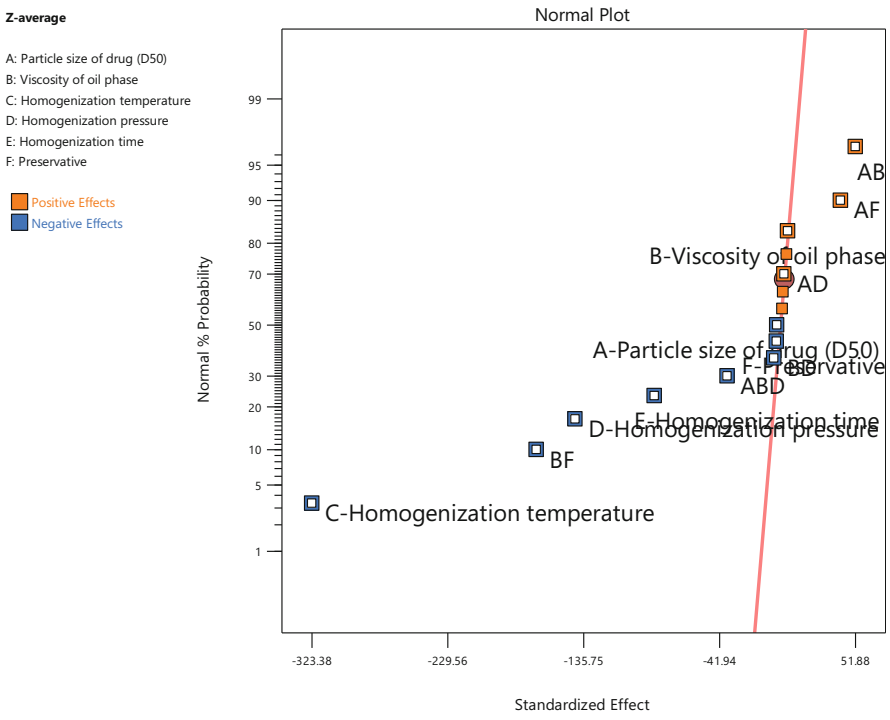

Fig. 5.2 Normal plot for response 1(Z-average)

Pareto chart: Pareto chart is a graphical way to present the selected model terms and their significance [26]. It contains two different t-limits (Bonferroni and standard t-limit). The values for both limits change based on the selected model terms (Fig. 5.3).

5.4.3.2 Response 2: PDI

Half-normal and normal plots: The half-normal and normal plots for the response PDI show that homogenization temperature (factor C) has a significant effect on PDI as compared to any other factor (Figs. 5.4 and 5.5).

Pareto chart: Fig. 5.6 shows that only homogenization temperature (factor C) exceeds the Bonferroni limit, which is more conservative than the standard t-critical. No other factor was found significant.

5.4.3.3 Response 3: IVR 1 h

Based on the effect's analysis by half-normal plot, normal plot, and pareto chart (data not shown), main effects homogenization temperature (factor C) and homogenization time (factor E) and the interaction term BF observed to be significant. Homogenization temperature (factor C) crossed the Bonferroni limit, whereas homogenization time (factor E) and BF crossed t-limit.

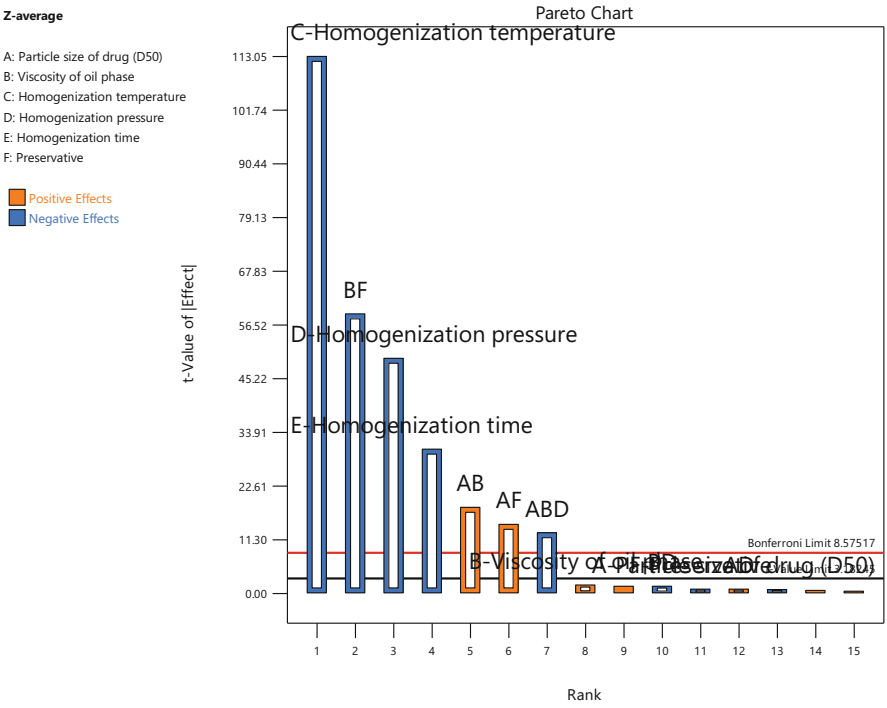

Fig. 5.3 Pareto chart for response 1 (Z-average)

5.4.3.4 Response 4: IVR 6 h

Based on the effect's analysis by half-normal plot, normal plot, and pareto chart (data not shown), only homogenization temperature (factor C) significantly affected (crossed the Bonferroni limit) the IVR at 6 h.

5.4.3.5 Response 5: IVR 12 h

Only homogenization temperature (factor C), like response 4, significantly affected (crossed the Bonferroni limit) the IVR at 12 h.

5.4.4 Analysis of Variance (ANOVA)

5.4.4.1 Response 1: Z-average

Table 5.6 shows the ANOVA for the selected model for the response Z-average. Based on such a high F-value (1694.27) and low p-value (<0.0001), it can be inferred that the selected model was significant. Model terms C, D, E, AB, AF, BF, and ABD were significant. R^2 of the model was 0.9999. Also, a good agreement between adjusted R^2 (0.9993) and predicted R^2 (0.9958) was observed (difference less than 0.2 between adjusted and predicted R^2).

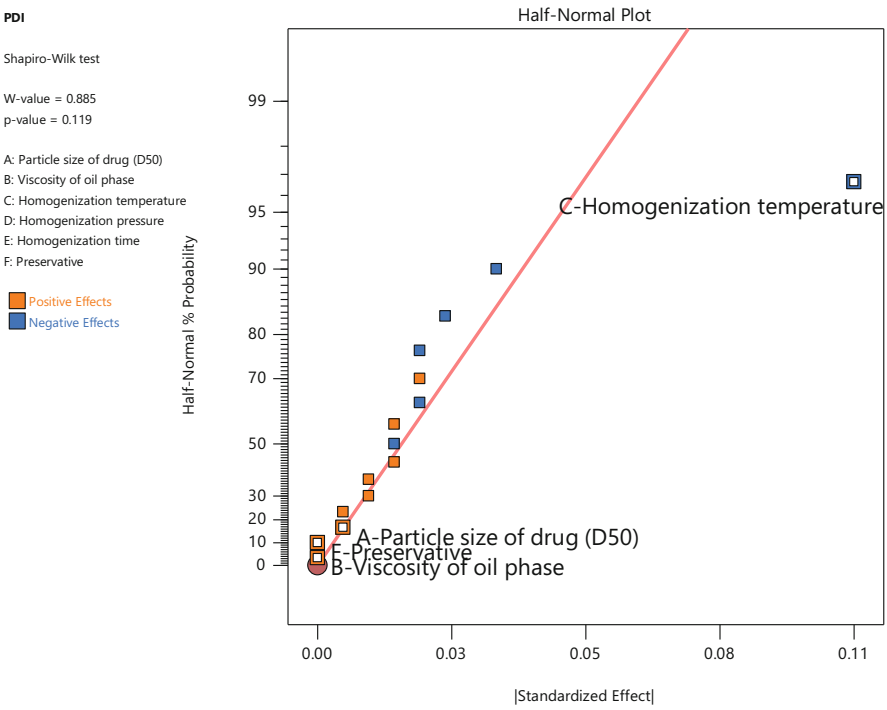

Fig. 5.4 Half-noraml plot for response 2 (PDI)

5.4.4.2 Response 2: PDI

ANOVA for response PDI shows that homogenization temperature (factor C) is significant. High F-value (7.69) and low p-value (0.0033) indicate that the model is significant (Table 5.7). The R^2 of the model was 0.7367. Also, a good agreement between adjusted R^2 (0.6409) and predicted R^2 (0.4429) was observed.

5.4.4.3 Response 3: IVR 1 h

Table 5.8 shows the ANOVA for the selected model for the response IVR 1 h. Based on F-value (14.86) and p-value (0.0002), the selected model was significant. Model terms C, E, and BF were significant. The R^2 of the model was 0.8814. Also, a good agreement between adjusted R^2 (0.8221) and predicted R^2 (0.6964) was observed.

5.4.4.4 Response 4: IVR 6 h

Homogenization temperature (factor C) was a significant term in the selected model (Table 5.9). The R^2 of the model was 0.5932. A good agreement between adjusted R^2 (0.5641) and predicted R^2 (0.4687) was observed.

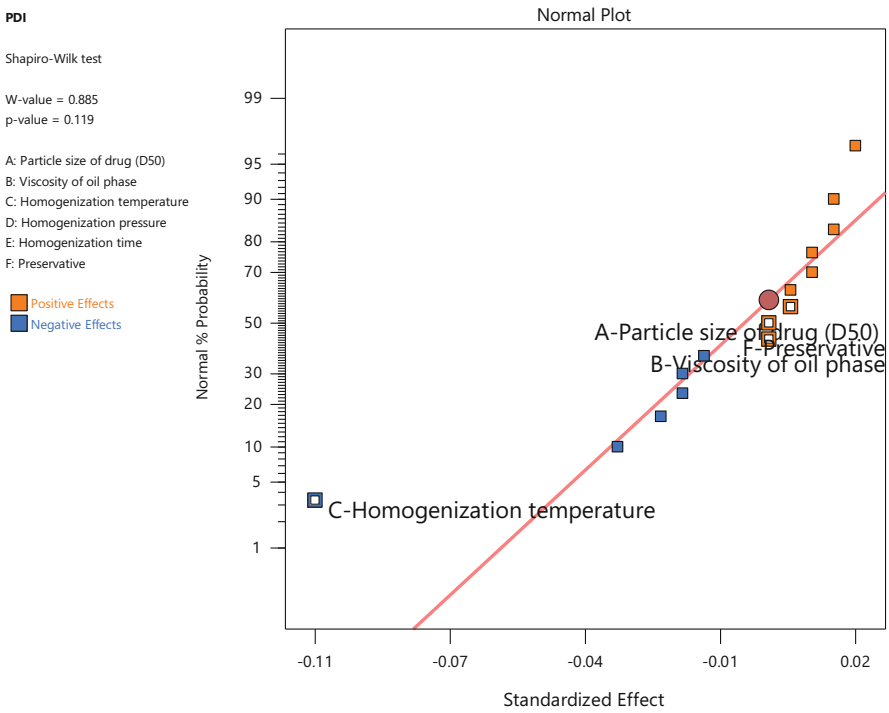

Fig. 5.5 Normal plot for response 2 (PDI)

5.4.4.5 Response 5: IVR 12 h

Like response 4, homogenization temperature (factor C) was a significant term in the selected model (Table 5.10). The R^2 of the model was 0.5780. A good agreement between adjusted R^2 (0.5479) and predicted R^2 (0.4488) was observed.

5.4.5 Diagnostics

Diagnostics play a vital role in verification, whether the selected regression model fits the data suitably and meet various assumptions. Various residual diagnostics and influence diagnostics are frequently used to test the appropriateness of the model. We will discuss the residual and influence diagnostics of the model selected for response 1 (Z-average) to avoid repetition. However, the diagnostic analysis should be performed for the models developed for all responses for practical purposes.

5.4.5.1 Residual Diagnostics

Analysis of residuals is an essential verification before concluding from the regression analysis. Diagnostics plots and residual analysis are used to detect problems associated with model analysis. The selected model, primarily linear, is reasonable if

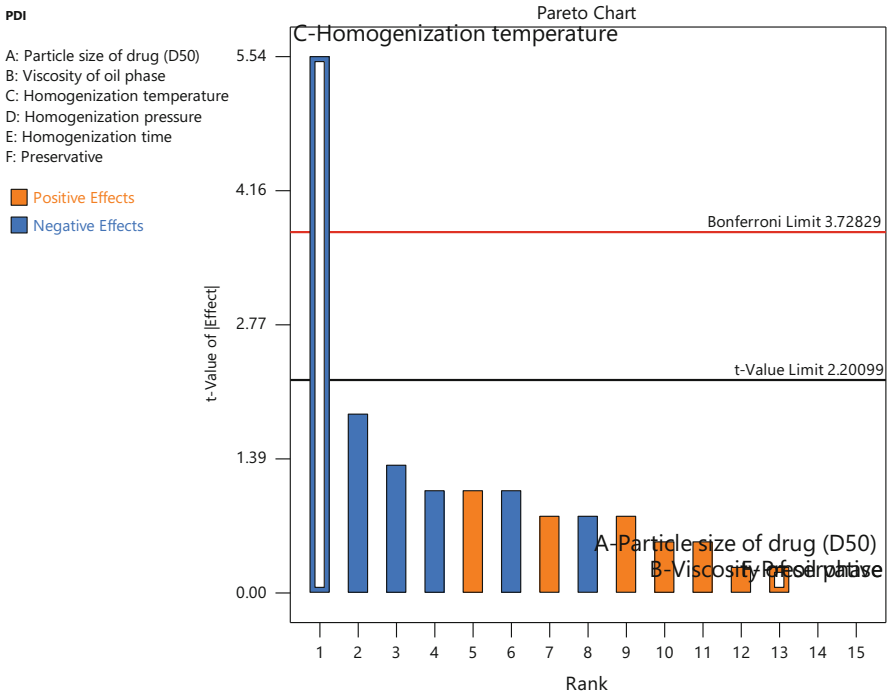

Fig. 5.6 Pareto chart for response 2 (PDI)

residuals have a normal distribution, constant variance, and independent of each other over time.

Normal Probability of Residuals

Figure 5.7 shows the normal distribution of the externally studentized residuals indicating that the selected model makes sense. Externally studentized residuals are used because of the higher sensitivity for the detection of problems. Moreover, each raw residual belongs to different populations and makes the interpretation difficult in both conditions (constant or variable variance). Studentized residuals calculation involves the deletion of an observation at a time and re-fitting the regression model on the remaining (n-1) observations followed by a comparison of observed and fitted values on the new model. Studentized residuals consider the standard deviation estimate and are thus more effective in detecting outliers. Normally distributed residuals follow a straight line. Any patterns in the normal probability plot of residuals suggest the superiority of alternative analysis, such as the transformation of the responses [27–30] and [35].

Table 5.6 ANOVA for response 1 (Z-average)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.654E+05	12	55452.10	1694.27	< 0.0001	significant
A-Particle size of drug (D50)	22.56	1	22.56	0.6894	0.4673	
B-Viscosity of oil phase	105.06	1	105.06	3.21	0.1711	
C-Homogenization temperature	4.183E+05	1	4.183E+05	12780.21	< 0.0001	
D-Homogenization pressure	80230.56	1	80230.56	2451.35	< 0.0001	
E-Homogenization time	30189.06	1	30189.06	922.39	< 0.0001	
F-Preservative	27.56	1	27.56	0.8421	0.4265	
AB	10764.06	1	10764.06	328.88	0.0004	
AD	27.56	1	27.56	0.8421	0.4265	
AF	6930.56	1	6930.56	211.75	0.0007	
BD	76.56	1	76.56	2.34	0.2236	
BF	1.134E+05	1	1.134E+05	3464.82	< 0.0001	
ABD	5365.56	1	5365.56	163.94	0.0010	
Residual	98.19	3	32.73			
Cor Total	6.655E+05	15				

Table 5.7 ANOVA for response 2 (PDI)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0442	4	0.0110	7.69	0.0033	significant
A-Particle size of drug (D50)	0.0001	1	0.0001	0.0696	0.7968	
B-Viscosity of oil phase	1.388E-17	1	1.388E-17	9.662E-15	1.0000	
C-Homogenization temperature	0.0441	1	0.0441	30.70	0.0002	
F-Preservative	0.0000	1	0.0000	0.0000	1.0000	
Residual	0.0158	11	0.0014			
Cor Total	0.0600	15				

Residual Vs. Predicted

Residual vs. predicted plot is used to test the assumption of the constant variance of residuals. The random scatter of the residuals indicates the constant range of residuals, whereas patterns such as megaphone suggest a transformation of the data. Figure 5.8 shows a random scatter pattern of the residuals, indicating a constant variance [31].

Table 5.8 ANOVA for response 3 (IVR 1 h)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	359.60	5	71.92	14.86	0.0002	significant
B-Viscosity of oil phase	1.0000	1	1.0000	0.2067	0.6591	
C-Homogenization temperature	292.41	1	292.41	60.44	< 0.0001	
E-Homogenization time	31.92	1	31.92	6.60	0.0280	
F-Preservative	0.0400	1	0.0400	0.0083	0.9293	
BF	34.22	1	34.22	7.07	0.0239	
Residual	48.38	10	4.84			
Cor Total	407.98	15				

Table 5.9 ANOVA for response 4 (IVR 6 h)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	444.16	1	444.16	20.41	0.0005	significant
C-Homogenization temperature	444.16	1	444.16	20.41	0.0005	
Residual	304.59	14	21.76			
Cor Total	748.74	15				

Table 5.10 ANOVA for response 5 (IVR 12 h)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	638.83	1	638.83	19.17	0.0006	significant
C-Homogenization temperature	638.83	1	638.83	19.17	0.0006	
Residual	466.42	14	33.32			
Cor Total	1105.24	15				

Residual Vs. Run

Residual vs. run is a plot of residuals with run order of experiments. It checks for the effect of lurking variables on the outcomes. The lurking variable is an extraneous variable that can have a positive or negative correlation with both the dependent variable and the independent variable. A specific trend in the plot indicates the existence of a lurking variable over time. Figure 5.9 shows a random scatter that means no interference of the lurking variable [31].

Predicted Vs. Actual

The plot of predicted vs. actual responses tests the ability of the model to predict the responses accurately. A good correlation is an indication of the ability of a model to predict close to the actual values (Fig. 5.10).

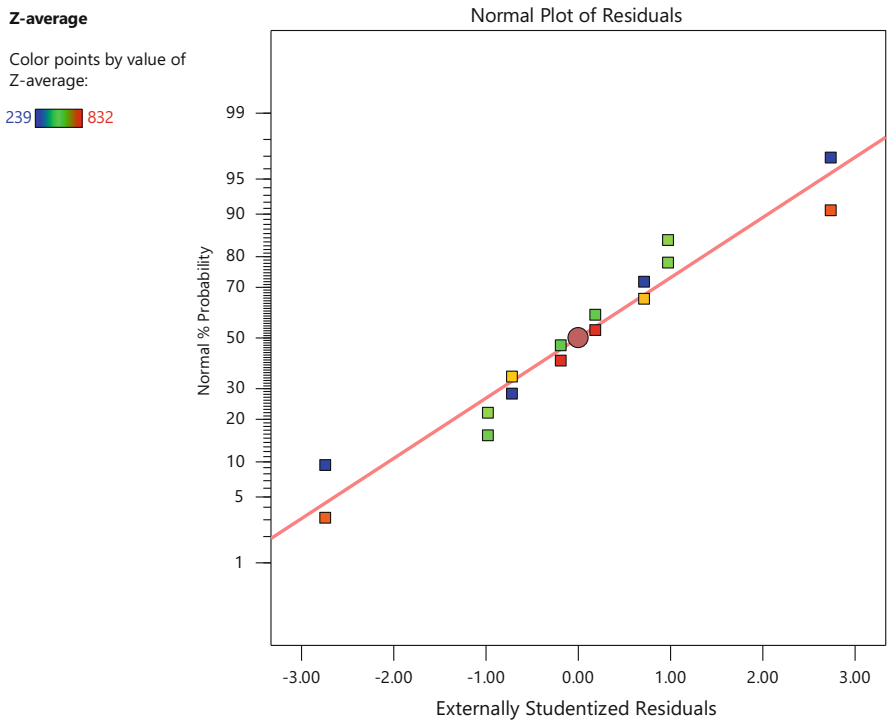

Fig. 5.7 Diagonastic plot and residual analysis: normal plot of residuals

Box–Cox Plot

A power transformation helps in reducing the anomalies such as non-normality and heteroscedasticity. Box–Cox transformation is a technique used for power transformations. Generally, statistical analysis and inference follow the assumption that data are normally distributed, have a common variance, and error structure is additive. However, if these assumptions are seriously violated, one may perform a power transformation and rebuild the model that has all essential aspects of the original model. Also, the new model satisfies all the assumptions. Box–Cox plot is a curve of the natural log of the sum of squares of residuals in which the minimum value indicates the lambda value. Power transformation is suggested based on lambda value. Lambda value of 1 (or any value for which 95% CI includes 1) does not require any transformation (Fig. 5.11). Other values of lambda such as 0.5 (square root), 0 (natural log), −0.5 (inverse square root), −1 (inverse), etc. suggest transformations [31, 32].

5.4.5.2 Influence Diagnostics

The identification of influential points is a critical aspect of regression diagnostics. It is essential to identify runs (observations) that have a high influence on the model

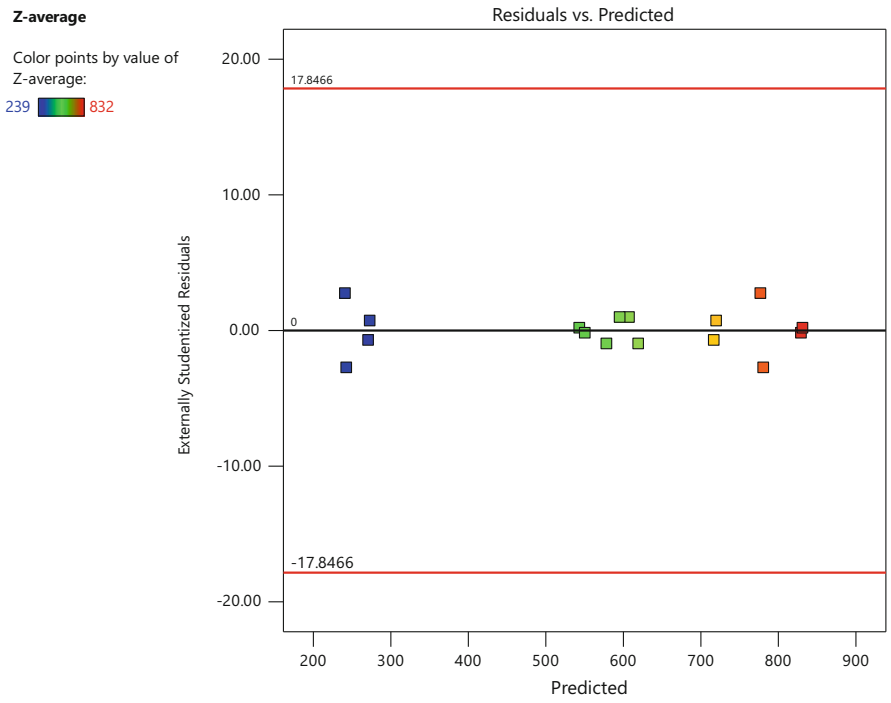

Fig. 5.8 Diagonastic plot and residual analysis: residual vs predicted

and the responses. Influence plots such as Cook's distance, leverage, DFFITS, and DFBETAS, provide a graphical measure of the influence of individual runs.

Cook's Distance or Cook's D (Di)

Cook's distance (Di) is used to identify the most influential run (or outlier) in regression analysis. A higher value of Di indicates a strong influence or a potential outlier. Generally, Di value more than one should be investigated, and value more than three represents an outlier. Di calculation includes rebuilding the regression model after removing i_{th} data point from the existing model and check for differences in predictions. Cook's distance within the limit indicates that no run is highly influential (Fig. 5.12) [33].

Leverage

Leverage is used to identify influential points and outliers by the distance of an observation point from the average predictor values. An observation point having a leverage of more than twice than the average is generally considered as high leverage. A high leverage point potentially has an impact on model fit; however, it does not necessarily mean that the point has a strong influence on the regression coefficient estimates. A higher distance from the predictor average for a point as compared to the other points can be situated in the same regression line. Therefore,

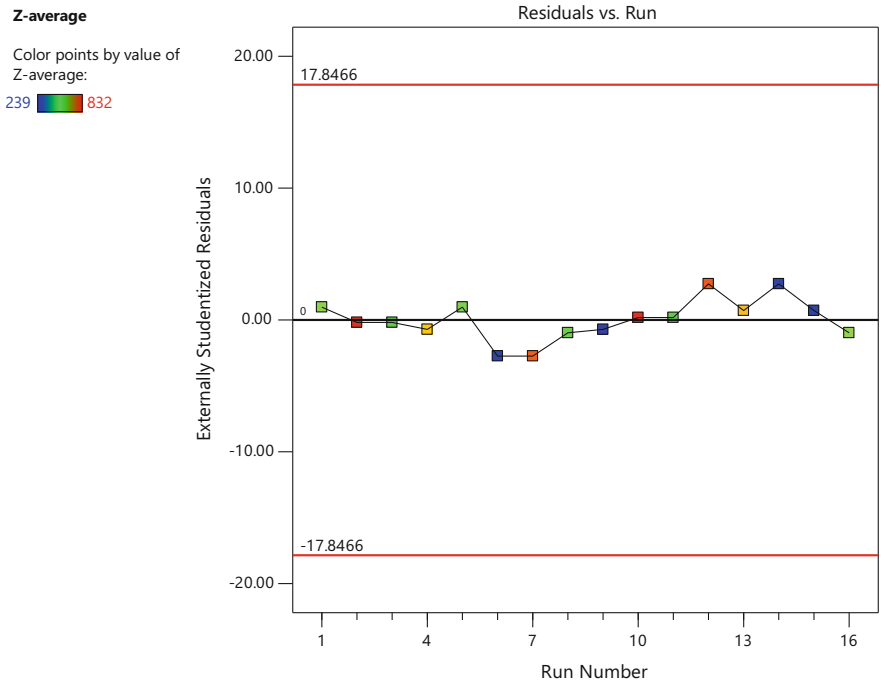

Fig. 5.9 Diagonastic plot and residual analysis: residual vs run

the evaluation of the discrepancy of the observation from other data might be helpful in addition to leverage value [34]. Figure 5.13 shows no high leverage run.

DFFITS, MDFFITS, and DFBETAS

In addition to the Cook's distance, several case-deletion diagnostics such as DFFITS, MDFFITS, and DFBETAS, are used in regression modeling. DFFITS measures the change in prediction value after removing the i_{th} point (i_{th} point not included in the model). MDFFITS is used when multiple points are removed. DFBETAS measures the change in coefficient estimate after removing the i_{th} point (i_{th} point not included in the model) [34].

Based on various residual and influence diagnostics, we can infer that the selected model is appropriate as residuals showed normal distribution, a constant variance, no transformation is required, and no high influence runs observed. Similar outcomes were observed for all the remaining models created for the other responses.

5.4.6 Summary of the Screening Design

The objective of the screening design of experiment was to identify critical factors affecting the critical quality attributes (CQA's) (responses) of the nanoemulsion. Six

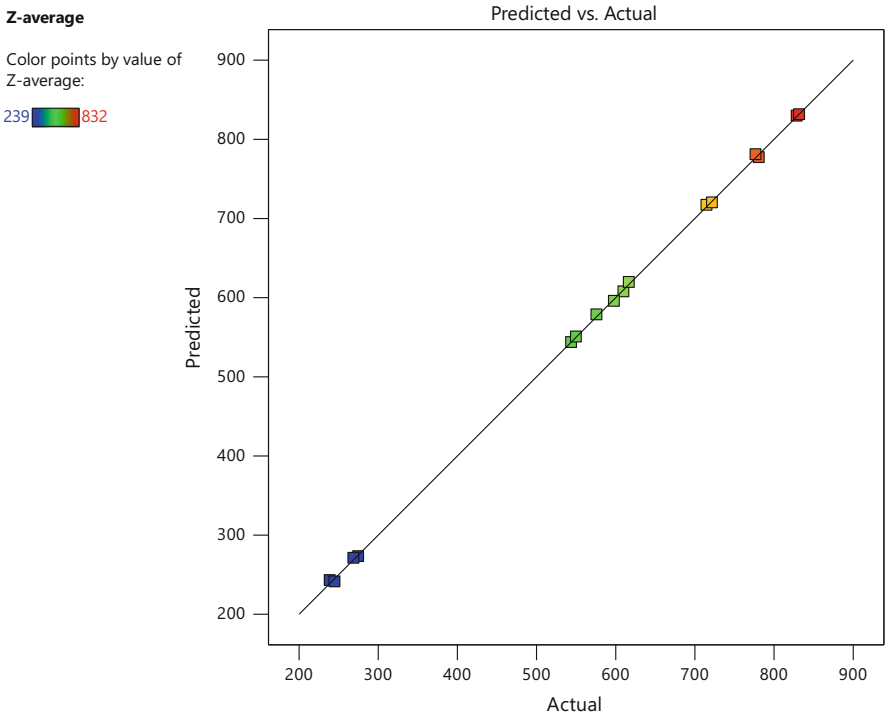

Fig. 5.10 Diagnostic plot and residual analysis: predicted vs actual

factors, namely, particle size of the drug, viscosity of oil phase, homogenization temperature, homogenization pressure, homogenization time, and preservative content were selected based on initial risk assessment. A fractional factorial design (2^{6-2}) of resolution IV was selected. Resolution IV design allows clean estimation of main effects. The two factors interactions confound with the other two factors interactions, but it might not be a concern for screening purpose. The factors can be prioritized for the optimization experiments based on the relative impact of the factors on responses. Globule size distribution (Z-average and PDI) and In vitro drug release (IVR) at 1 h, 6 h, and 12 h were selected as responses.

Based on the effects analysis and ANOVA for all responses, homogenization temperature (factor C) was found to be the most significant factor followed by homogenization time (factor E) and homogenization pressure (factor D). Also, a few interaction terms were significant. However, interaction terms were not given a due focus because the goal of screening design was to identify main factors affecting the CQA's of the nanoemulsion. Also, various residual and influence diagnostics showed that a reasonable regression model was built for all responses. Factors C, D, and E were selected for optimization studies. Optimization designs help define the design space (sweet spot), i.e., ranges of all factors that are suitable for achieving the desired qualities of the product.

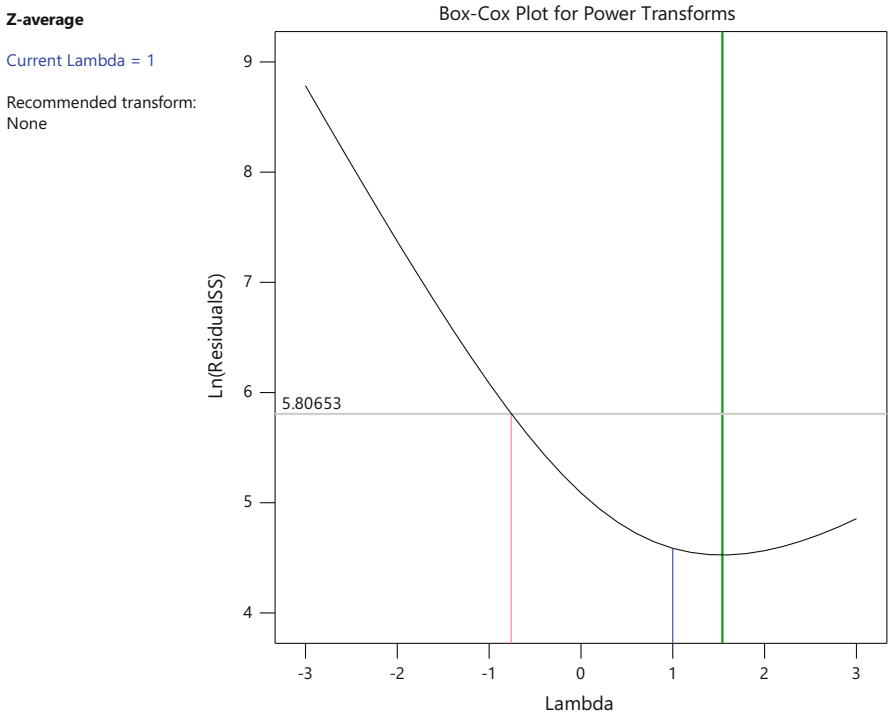

Fig. 5.11 Box-cox plot for power transforms

5.4.7 Connecting the Dots: The Science of Emulsion and Statistical Modeling

As we discussed earlier in the chapter, statistical modeling works best if complemented with sound scientific knowledge. Nanoemulsion can be defined as a kinetically stable system composed of oil and water, macroscopically homogeneous but heterogeneous at the microscopic scale. Based on type, oil (or water) droplets are distributed in the water (or oil) phase in o/w (or w/o) emulsion. The droplets of nanoemulsion, generally, have a submicron particle size (<1000 nm). The reduction and stabilization of particle size in the submicron size range require energy. The immiscibility of two phases is a result of high interfacial tension. Surfactants are used to reduce the interfacial tension and improve the stability of the emulsion. A negative change in Gibbs free energy upon mixing of two immiscible phases results in a stable emulsion. Gibbs free energy (ΔG) depends on enthalpy (ΔH), entropy (ΔS), temperature (T), interfacial tension (γ), and surface area (ΔA), given by the equation $\Delta G = \Delta H - T\Delta S + \gamma\Delta A$. Enthalpy change during the oil and water mixing is negligible. The entropy of mixing increases significantly with a decrease in particle size, i.e., $T\Delta S$ term dominates [35]. Higher temperature and

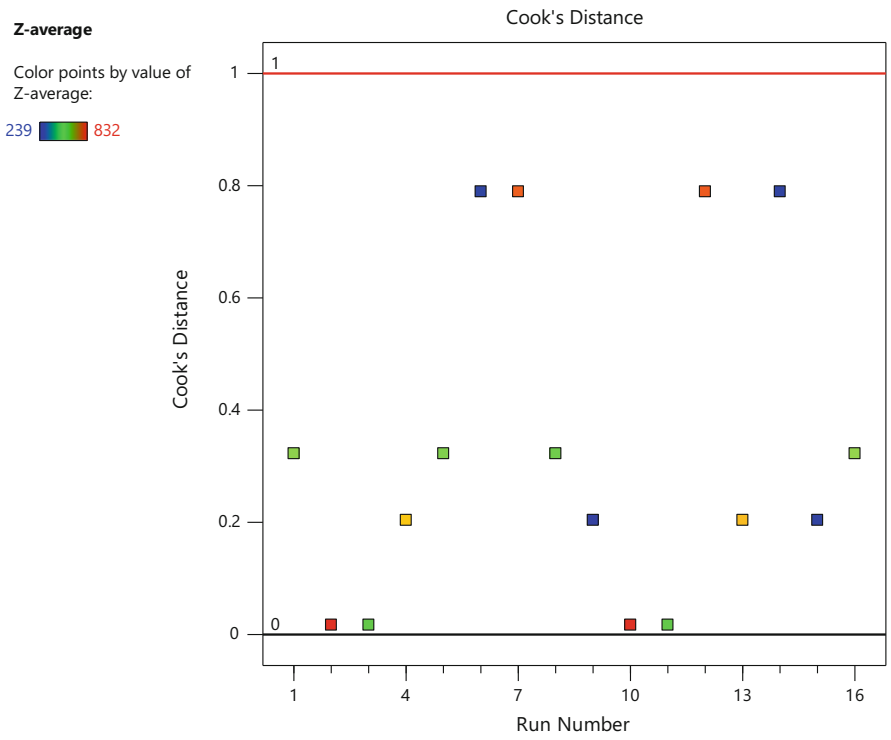

Fig. 5.12 Influence plots: Cook's distance

pressure during the homogenization process increase the energy input in the system and reduce the emulsion particle size. Moreover, the viscosity of the oil phase and surfactant decreases with an increase in temperature resulting in an efficient coating of surfactant on oil globules, making a stable emulsion. The screening design finds that parameters of the homogenization process significantly affected the quality of the nanoemulsion, which resonates with the theory of emulsion formation and stabilization.

5.5 Conclusion

DoE is an effective statistical tool to design the experiments in such a way that it ensures collecting the maximum information while minimizing the number of experiments. It helps in the analysis of the collected data and draws logical conclusions. DoE is used for screening and optimization purposes in various focus groups during injectable drug product development, including formulation, analytical, process scale-up, etc. DoE can detect and quantify interactions between factors. The traditional OFAT approach cannot detect interactions even with unlimited experiments. Interestingly, it has been observed many times during pharmaceutical

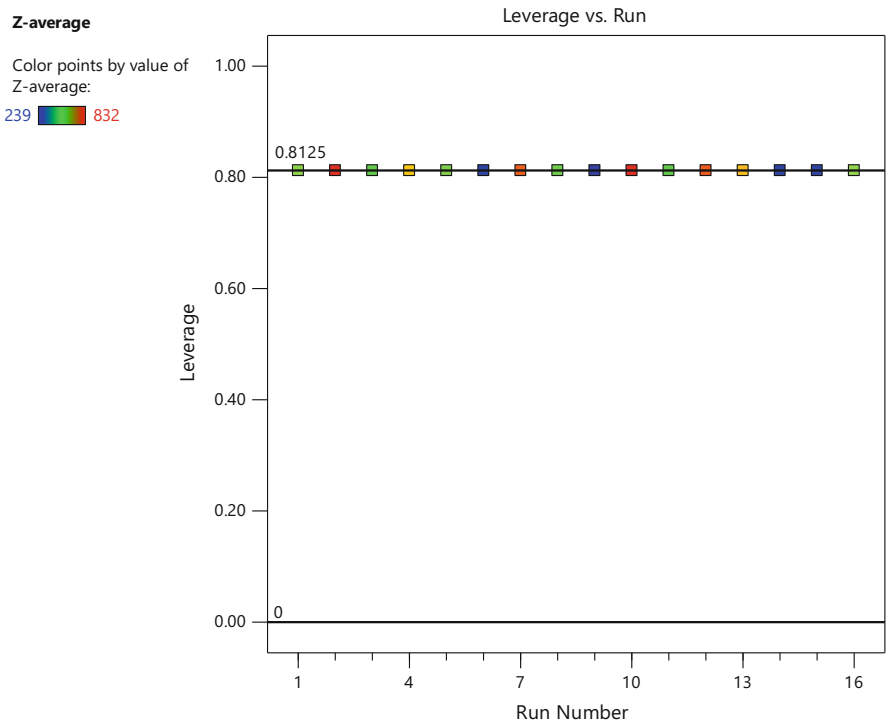

Fig. 5.13 Influence plots: Leverage vs Run

drug product development that interactions play a crucial role, sometimes exerting a more significant effect on responses than main effects. This chapter describes a few basic terminology and concepts which are used frequently in DoE. In addition, an outline of types of designs and criteria for selection of design were discussed. A case study of fractional factorial screening design for the development of a nanoemulsion product was discussed. Three out of six studied factors were found to be significant and considered for optimization studies.

Acknowledgment I want to thank Prof. Yoon Yeo for her support and suggestions in developing this book chapter.

References

1. Docarmo AM, Cunha-Filho MSS, Gelfuso GM, Gratieri T (2017) Evolution of quality on pharmaceutical design: regulatory requirement? Accred Qual Assur 22:199–205
2. Woodcock J, Woosley R (2008) The FDA critical path initiative and its influence on new drug development. Annu Rev Med 59:1–12
3. FDA (2020) Quality systems approach to pharmaceutical cGMP regulations

4. BEG S, Hasnain MS (2019) Pharmaceutical quality by design: principles and applications. Academic Press, Cambridge, MA
5. Chobisa D (2019) QbD-based development of pharmaceutical parenteral drug products: an overview. In: Pharmaceutical quality by design. Elsevier, Amsterdam
6. Anderson MJ, Whitcomb PJ (2016) DOE simplified: practical tools for effective experimentation. CRC Press, Boca Raton, FL
7. Fukuda IM, Pinto CFF, Moreira CDS, Saviano AM, Lourenço FR (2018) Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). Brazil J Pharm Sci 54:e01006
8. Stavros NP, Colombo P, Colombo G, Rekkas DM (2017) Design of experiments (DoE) in pharmaceutical development. Drug Dev Ind Pharm 43:889–901
9. Shivhare M, McCreath G (2010) Practical considerations for DoE implementation in quality by design. J BioProcess Int 8:22–30
10. Alferes VR (2012) Methods of randomization in experimental design. Sage Publications, Thousand Oaks, CA
11. Hall NS (2007) RA fisher and his advocacy of randomization. J Hist Biol 40:295–325
12. Suresh K (2011) An overview of randomization techniques: an unbiased assessment of outcome in clinical research. J Hum Rep Sci 4:8
13. Kirk RE (2012) Experimental design. Wiley, Hoboken, NJ
14. Anderson MJ, Whitcomb PJ (2000) Design of experiments. In: Encyclopedia of chemical technology. Wiley, Hoboken, NJ, pp 1–22
15. Seberry J (2017) Orthogonal designs. Springer, Cham
16. Wang Y, Liu H, Cai Z, Zhou Y (2007) An orthogonal design based constrained evolutionary optimization algorithm. J Eng Optim 39:715–736
17. Pyzdek T, Keller PA (2003) Quality engineering handbook. CRC Press
18. Grömping U (2018) R package DoE. Base for factorial experiments. J Stat Softw 85:1–41
19. Minitab (2020) What are confounding and alias structure? [Online]. Minitab: Minitab. https://support.minitab.com/en-us/minitab/18/help-and-how-to/modelingstatistics/doe/supporting-topics/basics/what-are-confounding-and-alias-structure/. Accessed 7 Jan 2020
20. NIST Sematech (2020) A glossary of DOE terminology. https://www.itl.nist.gov/div898/handbook/pri/section7/pri7.htm#:~:text=Alias%3A%20When%20the%20estimate%20of,be%20aliased%20(see%20confounding)
21. Design of Experiments (2020) APB Consultant. https://isoconsultantpune.com/design-of-experiments/. Accessed 7 Aug 2020
22. Comparison of Factorial Designs (2020) Design-Expert, Stat-Ease, Inc: StatEase. Accessed 7 July 2020
23. Chung JH, Lim YB (2019) A modified LGB method for detecting significant effects based on a half-normal probability plot. J Korean Stat Soc 48:568–577
24. Moral RA, Hinde J, Demétrio CGB (2017) Half-Normal plots and Overdispersed models in R: the hnp package. J Stat Softw 81
25. Hazir E, Erdinler ES, Koc KH (2017) Optimization of CNC cutting parameters using design of experiment (DOE) and desirability function. J For Res 29:1423–1434
26. Harvey HB, Sotardi ST (2018) The pareto principle. J Am Coll Radiol 15:931
27. Ranganai E (2016) On studentized residuals in the quantile regression framework. Springerplus 5:1231
28. Kozak M, Piepho HP (2018) What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J Agron Crop Sci 204:86–98
29. Vining G (2010) Technical advice: residual plots to check assumptions. Qual Eng 23:105–110
30. Studentized Residuals (2020) PennState Eberly college of science. https://online.stat.psu.edu/stat462/node/247/. Accessed 7 June 2020
31. Diagnostics Plots (2020) Design-Expert, Stat-Ease, Inc. Accessed 7 June 2020
32. Sakia RM (1992) The box-cox transformation technique: a review. J R Stat Soc: Ser D 41:169–178

33. Glen S 2016. *Cook's Distance / Cook's D: Definition, Interpretation* [Online]. https://www.statisticshowto.com/. Available: https://www.statisticshowto.com/cooks-distance/ [Accessed 7/6/2020 2020]
34. Liu X (2016) Residual and influence diagnostics. In: Methods and applications of longitudinal data analysis. Elsevier, Amsterdam
35. Moulik SP, Rakshit AK (2006) Physicochemisty and applications of microemulsions. J Surf Sci Technol 22:159

Design of Experiments for the Development of Inhalational Products

6

Al Sayed A. N. Sallam

Abstract

The pulmonary drug delivery systems (PDDSs) are considered complex systems because of having many variables related to formulations, processes, and devices. In general terms, a complex interaction of critical materials and formulation attributes (CMAs), device and critical process parameters (CPPs), and their effects on the critical quality attributes (CQAs) of PDDSs require an integrated strategic approach to design a drug product. This approach is fulfilled by implementing Quality by Design (QbD) through Design of Experiments (DoE). DoE can be applied with different purposes, which are identification of the main factors of CMAs, and CPPs affecting the CQAs in early product development of PDDSs; and subsequently determination of the factors that optimizing product and process performance. DoE represented by numerous multivariate statistical models such as factorial designs, fractional factorial designs, Plackett–Burman designs, central composite designs, Box–Behnken designs, optimal designs, mixture designs, and other models were used to screen, optimize, and validate various stages of PDDSs development.

Keywords

Design of experiments · DoE for DPIs · DoE for pMDIs · DoE and Pulmonary Delivery Systems · Applications of DoE

A. S. A. N. Sallam (✉)
TQ Pharma, Amman, Jordan
e-mail: a.sallam@tqpharma.com

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_6

6.1 Introduction

The pulmonary drug delivery systems (PDDSs) are delivering drugs for locally acting treatments of lung diseases such as asthma and chronic obstructive pulmonary diseases [1]. Moreover, PDDs are also used in delivering drugs systemically for treating systemic conditions such as diabetes, certain autoimmune diseases, lung infections, and some types of cancer [2–4]. PDDs of corticosteroids, bronchodilators or combinations are given in microgram doses, while cromolyn sodium is only given in milligram doses. However, treatment of lung infections and cystic fibrosis requires a much higher inhalation dose formulated as dry powder inhalers (DPIs) which accordingly require different powder formulation and engineering strategies, as well as different inhaler devices [5–7].

There are three major different types of PDDSs; nebulizers, DPIs, and the pressurized metered dose inhalers (pMDIs), in addition to soft mist inhalers [8, 9]. The formulations of PDDSs during development are dependent on the following factors [4, 8, 10–14]: (i) type of drugs either local or systemic action, chemical or biological macromolecule, low dose or high dose and frequency of administration; (ii) physicochemical characteristics of the drug substance, such as solubility at different pHs, pKa, Log P, particle size, polymorphic transformation and tendency of crystal growth, cohesiveness, and agglomeration tendency, morphology, surface characteristics and density; (iii) physicochemical characteristics of the excipient substances/additives; (iv) type of formulation (e.g., DPI, pMDI, or nebulizer) being selected to deliver the drug; (v) device design for compatibility with the formulation and the age of the targeted patient; and (vi) propellant nature and vapor pressure.

Formulation of pMDI is very complex whether in solution or suspension forms because of the presence of high vapor pressure and low dielectric constant propellants [12, 15, 16]. DPIs are formulated as a micronized drug (1–5 μm) in a blend with inactive excipient of greater size (40 μm). Because the micronized drug particles are cohesive in nature due to the high surface free energy, inactive excipient such as lactose, mannitol, and trehalose are used in order to reduce particulate surface energy and hence preventing the agglomeration of drug particles. Accordingly, both the powder blend and the DPI device must be well designed in order to ensure detachment of the micronized drug from the carrier excipient on inhalation [17].

Quality by Design (QbD) was originally related to the Quality Management and was recently used by pharmaceutical companies in order to achieve market and operational excellences [18].QbD is defined as a systematic approach to pharmaceutical development for drug products that begin with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [19]. Furthermore, QbD is achieved by identifying the critical material attributes (CMAs), which together with the critical process parameters (CPPs), both defined as inputs, will deliver through their proper selection and interaction the output [20–24]. This is the drug product with the desired quality obtained by the continuous optimization of its critical quality

attributes (CQAs) in order to meet the predefined quality target product profile (QTPP) and the patient's needs during the entire lifecycle of the product [25]. Recent reviews have discussed the application of the QbD approach in the development of PDDSs [4, 25–28]. QbD requires systematic investigations to be done in order to monitor how changes in CQAs of formulation, device, and manufacturing process influence main product performance parameters, such as delivered dose uniformity (DDU) and fine particle dose [29].

The US Food and Drug Administration (FDA) have provided its draft guidance and recommendations to industry on the development and manufacture of pMDIs and DPIs, updated to reflect current standards and requirements to enhance understanding of appropriate development approaches in order to ensure product quality and performance for these products consistent with the QbD approach [30]. Recently a new FDA's pharmaceutical quality assessment system which is called Knowledge-aided Assessment Structured Application (KASA) has emphasized on drug product design as one of its pillars [31]. It determines the ability to link input CMAs to output CQAs, so that input material attributes (e.g., drug substance, excipient, in-process material, process conditions, primary packaging material) can be controlled in order to minimize risks to the product quality [31, 32]. Furthermore, QbD requires using different tools such as Design of experiments (DoE), Risk assessment and Process analytical technology [33].

6.2 Design of Experiments

In general terms, a complex interaction of CMAs, device, and CPPs (Tables 6.1 and 6.2), and their effects on CQAs (Table 6.3) of pMDIs and DPIs require an integrated strategic approach to design a drug product [19, 34–36]. This approach is fulfilled by implementing QbD through DoE. DoE represented by numerous multivariate statistical models such as factorial designs, fractional factorial designs, Plackett–Burman designs, central composite designs, Box–Behnken designs, optimal designs, mixture designs, and other models are available to screen, optimize, and validate various stages of drug product development [34, 37, 38].

DoE is considered the most efficient tool to explore and quantitate the effects of the CMAs, CPPs, and their interactions on the product's CQAs. Such multidimensional combination and interaction of input variables (CMAs and CPPs) shall provide the assurance of quality which is referred to as the design space by the ICH Q8 [19]. Data collected from DoE applications are further treated by using response surface methodology (RSM), which is a statistical and mathematical techniques useful for development and optimization of product designs and process parameters and consequently optimizing the response [39]. These techniques are applied in order to reduce the number of evaluated parameters and determine the most relevant parameters required for optimization.

Table 6.1 Critical materials attributes and critical process parameters of pMDI

ICH Q8 (R2) pharmaceutical development	
pMDIs, design of experiments	
Critical materials attributes	Critical process parameters
1. Drug:	1. Size reduction process (milling, spray drying or others)
1.1 Drug content	
1.2 Purity	2. Mixing:
1.3 Polymorphism	2.1 Premixing (suspension)
1.4 Particle size and shape	2.2 Mixing order of drug and excipients (suspension)
1.5 Surface properties (suspension)	2.3 Mixing with propellants
1.6 Solubility, pka, log.P	2.4 Electrostatic charges
1.7 Moisture content	2.5 Amounts of concentrate and propellant
1.8 Aggregation/creaming, settling (suspension)	2.6 Deaggregation (suspension)
1.9 Drug density (suspension)	2.7 Homogeneity (suspension)
1.10 Adhesion to the device constituent part (suspension)	2.8 Temperature
2. Excipients:	2.9 Moisture and humidity
2.1 Propellants (level, density, hygroscopicity), Cosolvents, surfactants	2.10 Batch size
2.2 Surface properties/interparticulate interactions	3. Filling (pressure filling/cold filling):
2.3 Solubility	3.1 Dosator diameter
2.4 Moisture content	3.2 Filling speed
2.5 Purity	3.3 Total content/canister
2.6 Compatibility with drug	3.4 Crimp
2.7 Compatibility with closure container system (can coating, valve)	3.5 Leakage
3. Leachable materials	4. Device constituent part (design, geometry and dimensions)
4. Stability: (physical, Chemical and Microbial)	

6.2.1 Design of Experiments for pMDIs

Development of budesonide suspension pMDIs using hydrofluoroalkanes propellants and stabilizing agents such as oleic acid (OA) and sorbitan trioleate (STO) was done using DoE and factorial design method. The design investigated the effects of two factors (vapor pressure of the propellants and concentration of stabilizing agents) in three levels on pMDI formulations performance. Combinations of OA and STO were also investigated. A high level of OA was combined with a low level of STO, and a low level of OA was combined with a high level of STO. Mean, standard deviations, and statistical significance tests ($P < 0.05$) were used to assess the results. The conclusion was that when a propellant mixture with an intermediate vapor pressure was used and its density matched the density of the drug particles, the formulation of pMDI produced satisfactory aerosol properties and physical stability [40].

Table 6.2 Critical materials attributes and critical process parameters of DPI

ICH Q8 (R2) pharmaceutical development	
DPIs, design of experiments	
Critical materials attributes	Critical process parameters
1. Drug:	1. Size reduction process (milling, spray drying or others)
1.1 Drug content	2. Powder mixing:
1.2 Polymorphism	2.1 Type of mixer
1.3 Particle size and shape	2.2 Mixing order of drug and excipients
1.4 Surface properties/interparticulate interactions	2.3 Mixing time and speed
1.5 Flow properties	2.4 Electrostatic charges
1.6 Moisture content	2.5 Moisture and humidity
1.7 Cohesiveness and Agglomeration	2.6 Mixer volume and filling level
1.8 Solubility, pka, log.P	2.7 Batch size
2. Excipients:	2.8 Flowability
2.1 Drug/excipients ratio	2.9 Homogenity
2.2 Particle size and shape	3. Powder filling:
2.3 Surface properties/interparticulate interactions	3.1 Flowability
2.4 Moisture content	3.2 Dosator diameter
2.5 Flow properties	3.3 Powder layer depth
2.6 Deagglomeration tendency	3.4 Filling speed
2.7 Hygroscopicity	3.5 Humidity
2.8 Solubility	4. Sealing process: temperature, dwell time, machine speed
2.9 Purity	5. Device constituent part assembly
2.10 Compatibility with drug	
3. Stability: (physical, chemical and microbial)	

Selection of potential formulations of pMDI to be used for development at later stages is determined by running limited compatibility DoE in order to reduce testing requirements to an acceptable level. Hence, for determining a suitable pMDI system, a limited half to full factorial DoE may be performed focusing mainly on the numeric formulation variables, such as type of propellants, cosolvents, surfactants, and particle size distribution (PSD) for suspensions; along with categorical variables, such as valve type or can coating. Afterward, a response surface is estimated in terms of the main DoE factors, with the possibility to compare the other factor to the DoE center points via use of statistical tools such as an ANOVA. The limited compatibility DoE, therefore, aims to allow understanding of potential excipient combinations to progress to later development stages based on the CQAs of the required pMDI [41].

A pMDI batch manufacturing plan was developed by using variable levels of excipients (ethanol and oleic acid concentrations in suspension and solution

Table 6.3 ICH Q8 (R2) critical quality attributes

Attribute	DPI	pMDI
Identification	X	X
Assay	X	X
Aerodynamic particle size distribution (APSD)	X	X
Mass median aerodynamic diameter (MMAD)	X	X
Fine particle fraction	X	X
Powder flowability	X	For suspension
Electrostatic charges	X	For suspension
Net content (drug substance and excipients)	X	X
Content uniformity of dose	X	X
Delivered dose uniformity/ shelf life	Multi dose DPI	X
Foreign particulate matters	X	X
Leachable materials	Multi dose DPI	X
Device/ formulation compatibility	X	X
Cosolvent content	–	X
Water or moisture content	X	X
Moisture protection	X	X
Microbial limits	X	X
Physicochemical characteristics of solid particles (stability)	X	For suspension
Impurities and degradation products (stability)	X	X
Spray pattern and plume geometry	X	X
Dimensions of the device constituent	X	X
Product actuation force	–	X
Specific resistance to air flow	X	–
Device metering and robustness	X	X

formulations) and variable drug PSD (in suspension formulations) through a factorial DoE. The DoE plan for suspension (three-factor, three-level reduced factorial design) and solution (two-factor, three-level full factorial design) pMDIs was implemented. This design consisted of 22 suspensions and 9 solution pMDI formulations, and represented mid-points around which DoE were defined and manufactured. The DoE of pMDI batches using the most extreme combination of the factor levels (four for the suspension and two for the solution) were prepared first to confirm applicability of the DoE plan. The remaining batches were then prepared following the full randomized order as per the DoE plan. The CQAs of the suspension and solution pMDIs were then characterized according to total content per canister (drug and excipients), DDU and APSD performance. The plastic actuators of commercial suspension and solution pMDIs were used. The results from DDU and APSD testing were subjected to statistical two-way analysis of variance (ANOVA) with an a priori α level of 0.05, and compared with an available commercial suspension (salbutamol sulfate) and solution (beclomethasone dipropionate) pMDI products [42].

Full Factorial Design as a statistical technique in DoE was used to determine the effect of certain CPPs on the aerodynamic performance of a salmeterol xinafoate pMDI. The CPPs selected for evaluation in this investigation were based on knowledge obtained from previous small and large scale studies. The CPPs were the homogenization time (0–36 min), the mixer speed (100–200 rpm), and the vessel temperature (6–12 °C). The dependent parameters (CQAs) were identified as the fine particle dose, the emitted dose, and the total drug content. A multiple regression analysis was performed on the data and optimal CPP values were identified [43].

Effect of actuator design factors (orifice size, orifice length, and sump depth) as independent factors that influencing spray pattern and particle size of pMDI was studied. Experimental design was planned as factorial design having three factors, two levels, full factorial. Half-normal plots were analyzed for significant effects, and the sum of squares of the effects were used in the ANOVA analysis. The results showed that, spray patterns and APSD were significantly influenced by the actuator geometry features [44].

The actuator geometry features such as orifice diameter and sump volume have a significant effect on the atomization process of pMDIs. Their effects on pMDIs final performance were studied by applying a face-centered design. Fifteen responses were measured for each experiment, and data were treated by applying a Principal Component Analysis which allowed to understand the correlations among the responses and their relative weight. However, they showed limitation that the predicted values do not give any direct information about the single responses. Furthermore, multilinear regression was used to evaluate quantitatively the responses of the effect of CPPS (orifice diameter and sump volume) on CQAs (fine particle mass, fine particle fraction, MMAD, and APSD) [45].

Add-on devices such as valved holding chamber (VHC or Spacer) are used with pMDIs. VHC enhances droplet evaporation in the aerosol emanating from a pMDI by allowing the droplets more time to evaporate before being inhaled, producing smaller APSD and less mouth-throat deposition. In addition, if the distance from the pMDI nozzle to the back of the mouth-throat (the oropharynx) is increased, the velocity of the aerosol is reduced due to jet entrainment, again reducing impaction in the mouth-throat by lowering the Stokes number [46]. However, many factors may influence the performance of a metered dose inhaler when used with VHC [36]. A two-level, three-factor full factorial DoE was applied to determine the influences of VHC type, flow rate, and inhalation delay on a total of seven performance characteristics for each commercial pMDI product. The results showed the presence and type of VHC were the major influences on the emitted dose and respirable fraction. Furthermore, the inhalation delay had the most significant effect on the emitted dose, respirable particle dose and fraction, and fine particle dose and fraction. Thus, interchangeability of different VHCs and their effect on a given pMDI product cannot be assumed without in vitro testing [47].

In a veterinary administration of pMDI a set of simple experimental design was established to determine parameters which affected the efficiency of salbutamol pMDI delivery via equine spacer device. The first two sets of comparative experiments applied eight repetitions of the delivery of 10 X 100 μg actuations

(total 1 mg salbutamol) differing with respect to the direction of actuation within the spacer, while the third set of experiments examined the effect of rapid actuations. An optimal position relative to the spacer device was then selected to measure optimal delivery whereby drug retention within the spacer was also determined. Statistical analyses of significance ($P < 0.05$) using Mann–Whitney U-test for nonparametric data only in case of two experimental conditions were compared. Moreover, when more than two experimental parameters were compared, a Mann–Whitney U-test for nonparametric data was applied only if differences were confirmed by a Kruskal–Wallis analysis. Furthermore, the study demonstrated the difficulties in predicting the magnitude of drug delivery to the peripheral airways using a pMDI and equine spacer device [48].

6.2.2 Design of Experiments for DPIs

A mixed factorial design was used to study the effect of different process parameters of an Aerosizer® with the Aerodisperser®(a time-of-flight-based aerodynamic particle size analyzer), on the results of the size distribution of the formulations aerosolized from two commercially available DPIs; salbutamol sulfate (Ventilan Rotacaps™, GlaxoWellcome) and terbutaline sulfate (Bricanyl Turbohaler™, AstraZeneca). The different equipment variables were voltage applied to the detector, shear force, deagglomeration, feed rate, and pin vibration. In this mixed factorial design, a $4^1 \times 3^1 \times 2^3$ design was applied, where the exponents represent the equipment variables and the bases represent the levels of each variable in the investigation. Thus, the variables with three or four levels can be converted into two variables with two levels each (2^2), the lowest to the highest level. The study showed that the most significant factor was the applied voltage followed by the shear force, while pin vibration and feed rate were less significant. However, the interactions between variables were also responsible for significant changes in the responses [49].

Asymmetrical fractional factorial designs were used in the investigation of the effect of CMAs (drug alone and drug/fine lactose comicronization by jet milling) on enhancing aerosolization of fusafungine DPI systems. The respirable fraction and the emitted dose fraction of highly cohesive fusafungine powder, had increased significantly after comicronization with fine lactose [50].

Full factorial design was applied to investigate the effects of salbutamol sulfate load and lactose grade, capsule material, and capsule fill, and type of inhaler device on electrostatics and triboelectrification characteristics of DPI using electrical low pressure impactor (ELPI™). The selected five variables each at two levels/categories resulted in $2^5 = 32$ individual data points. Each setting (combination of five factors) was tested at least three times, and the experiments were divided into blocks of 16 actuations with randomized order. The study concluded that the choice of lactose grade, inhaler device, and capsule material had a strong effect on both magnitude and polarity of DPI triboelectrification [51].

In preparation of DPI for treatment of cystic fibrosis, unfractionated heparin was spray dried to produce spherical particles with particle size distribution in the range of 1–5 μm, using factorial design to optimize process performance with respect to pump speed, atomization spray flow rate, inlet temperature, and feed concentration [52].

Similarly, full factorial design was used to examine the impact of lactose particle size and the presence of fine fractions on the performance of DPI prepared from interactive mixtures containing 1% (w/w) micronized salbutamol sulfate in lactose. In this study DoE had enabled the evaluation of the effect of lactose size fractions on quality attributes of the DPI formulations [53].

For DPI formulation of high-dose drug azithromycin, a five-level, three-factor central composite rotatable design (CCRD) with response surface analysis was used to optimize physical and aerodynamic properties of inhaled drug powder. This sort of statistical design CCRD requires smaller number of tests than the full factorial design and proved to be sufficient to describe the majority of steady-state process responses [54].

Applications of RSM include different dosage forms which among of them optimization DPIs formulations [55–57]. Furthermore, screening designs such as Plackett–Burman and Taguchi techniques were used in order to reduce the number of evaluated parameters and determine the most relevant parameters required for optimization. For example, Taguchi design was used to assess the influence of combination of different factors such as environment, formulation, and device on powder aerosolization of micronized salbutamol sulfate DPI [58].

DoE was implemented using a half-factorial design involving five experimental variables (feed concentration, pH of nanosuspension medium, inlet temperature, feed rate, and atomizing gas flow rate) to optimize the spray drying production of aggregated biocompatible silica nanoparticles (hollow spherical aggregates with a narrow range between 2 and 4 μm) suitable for DPI photodynamic therapy. After the screening design, a full factorial design and central composite design (also known as Box–Wilson design) involving only the significant variables were used to optimize the DPI formulation parameters [59].

An investigation was done to study the effect of process parameters (inlet temperature, gas flow rate, feed solution flow rate, and feed concentration) of minispray dryer on powder characteristics of trehalose and raffinose powders (yield, particle size distribution and the specific surface area, residual solvent content), and process outlet temperature. Nanoporous microparticles were produced after optimization by using a randomized 2^4 full factorial design with two replicates and employed as carriers in preparation of DPI [60]. The impact of CMAs (three grades of lactose monohydrate (LMH) with particle sizes above and below the brittle-ductile transition) and CPPs of fluid air-jet milling (grinding pressure, injector pressure, and feed rate) on the micronization of LMH for DPI formulations were investigated using DoE with a full factorial design [61].

Response surface methodology with Box–Behnken statistical design (RSM/BBSD) in optimizing DPI was used to study the effect of six independent parameters: four CMAs (drug concentration, polyvinyl alcohol concentration,

poloxamer 188 concentrations and volume of the cosolvent), and two CPPs (stirring speed and stirring time) on the dependent parameter, the average particle size of alpha ketoglutarate nanoparticles. Furthermore, the individual and interactive effects of different process parameters were studied by conducting the process at different levels of all factors. Subsequently, the observed responses were simultaneously fitted to first order, second order, and quadratic models, where the best-fitted model was found to be the quadratic model. RSM/BBSD showed that two CMAs (drug concentration and stabilizer concentration) and one CPP (stirring rate) had significantly affected the average particle size of alpha ketoglutarate nanoparticles [62].

Spray-dried insulin DPI was prepared by applying QbD and implementing DoE with multivariate data analysis. The study investigated the effects of independent parameters: CMA (insulin concentration) and CPPs (nozzle, feed, and drying air flow rate and drying air temperature), on the dependent parameters (droplet size, geometric particle size, particle shape and surface morphology, APSD, yield, powder bulk and tapped densities, moisture content, outlet temperature, and physical and chemical stability). Principal component analysis was applied to explore correlations between dependent and independent parameters. The results indicated that the insulin concentration was found to be the most important parameter, followed by inlet drying air temperature and the nozzle gas flow rate. Furthermore, the physical and chemical stability of insulin was not affected [63].

The concept of the modular inhaler, DoE and evaluation of experiments were shown to be successful tools to develop a device for budesonide DPI. Multivariate statistical methods were used for evaluation of the results with statistical significance level defined as $P < 0.05$. Fractional factorial DoE with center point was applied while capsule and inhaler retention, induction port deposition, and the fine particle fraction were used as responses [64].

The inhalable cationic liposomal carrier for dry vaccine was prepared by spray drying technique. A QbD approach was applied to identify and link CPPS (aspirator %, outlet temperature, feedstock concentration, feed flow, and atomizing airflow) of the spray drying process to CQAs (liposome size, MMAD, relative moisture content, and %yield) using risk assessment and DoE, followed by identification of an optimal operating space. The DoE applied a central composite face-centered fractional factorial design which was followed by multiple linear regression analysis. This sort of DoE had systematically varied the factors taking into account the interactions between the factors, and thus evaluating simultaneously the effects of multiple variables [65].

An investigation was done to identify CMAs and CPPs of a dosator-nozzle for low dose capsule filling of a model drug in DPI product. Twelve carriers having a variable volume median diameter ranging from 1.6 to 160 μm and a variable density range between 0.15 and 0.74 g/ml were used. An initial screening DoE was done applying a D-optimal method and design statistics G-efficiency with three replicates. This DoE minimized the number of experiments and allowed obtaining the largest amount of information. Four process parameters of the capsule filling machine were examined: dosator diameter, dosing chamber length, powder layer depth, and capsule filling speed. Thereafter, multivariate data analysis via partial least squares was

used to study the correlations between the material attributes and process parameters and the capsule fill weight and weight variability [66].

DPI optimization of levofloxacin-loaded crosslinked chitosan microspheres was done as a dry inhalation therapy for treatment of pulmonary chronic Pseudomonas aeruginosa infections in Cystic Fibrosis patients. The microspheres were prepared by spray drying and according to a factorial DoE for the geometric particle size optimization. This experimental design included four independent variables (presence of levofloxacin, chitosan concentration, amount of cross-linking agent, and inlet temperature) and two coded levels (−1, +1). The dependent variable which was the response corresponded to the mean diameter of the microspheres derived from the volume distribution. The optimized microspheres showed satisfactory APSD for lung delivery as DPI and considered as an alternative to levofloxacin solution for inhalation [67].

Spray-dried amikacin sulfate powder for preparation of DPI was developed using DoE on two stages. The first stage was half-fractional factorial DoE in order to define the most critical parameters controlling the process and the experimental space. However, the second one was a face-centered composite design with three factors at three levels, and was designed in order to expand the experimental space. The independent parameters were three selected spray drying process and formulation parameters (drying temperature, feed rate, and ethanol content), while the dependent parameters were microparticles morphology, particle size distribution and density of powders and agglomerates, aerodynamic performance (emitted dose and fine particle fraction), and residual water content [68].

A DoE approach was adopted to determine the effect of pre-freezing conditions on the DPI respirability of rifampicin solid lipid nanoparticle formulations for an anti-TB inhalation treatment. Independent variables were lipid/cryoprotectant ratio, cryoprotectant type, particle suspension/water ratio and pre-freezing temperature. While dependent variables were circularity, drug loading, size, PDI, zeta potential, bulk density, tapped density, APSD, emitted dose and respirable fraction. The DoE adopted was a two-level full factorial design with no center points and three repetitions for each level. ANOVA test was applied and 3D response surface plots were constructed, where the slope of each line was related to the factor relevance. The 3D response surface plots, indicated significantly the dependence of bulk density, tapped density, respirability, and drug loading on the pre-freezing parameters; while the others were not [69].

An alternative to lyophilized vaccines, spray-dried vaccine formulations were proposed. A study was done utilized DoE for spray drying process to stabilize whole inactivated influenza virus vaccine (WIV) in order to be administered as DPI. The CPPs (inlet air temperature, nozzle gas flow rate, and feed flow rate) and their effect on WIV vaccine powder characteristics such as particle size, residual moisture content, percentage yield, and stability were investigated. Preliminary study as screening experiments was done using a full factorial design to determine the most relevant CPPs that affected the output process and product parameters. The model was further optimized using a reduced Central Composite Family design for optimization, consisting of in total 23 experimental runs. The study enabled the generation

of a thermostable, antigenic WIV vaccine powder suitable for pulmonary administration as DPI [70].

A fractional factorial DoE was used to study the effect of CPPs of supercritical CO_2-assisted spray drying (SASD) in the final properties of composite chitosan (CHT)/ibuprofen (IB) microparticles. Fractional factorial ANOVA was applied in order to evaluate statistical differences between DPI formulations. The factors were investigated using three levels, a higher level (1), a center level (0), and a lower level (−1). CPPs were CO_2 to liquid flow ratio, precipitator temperature and the co-atomization of CHT/IB, while the DPI formulations properties were the particle volumetric diameter, span and MMAD, fine particle fraction, geometric standard deviation, and emitted fraction. The results showed that porous CHT/IB composite microparticles with good aerodynamic properties suitable for DPI applications were prepared using SASD and DoE [71].

A spray drying technique was employed to prepare mannitol carrier particles having defined morphology and flow characteristics for DPI. Consequently, a design of experiments with rotation speed and drying temperature as CPPs was applied for the preparation of different mannitol particles suitable for DPI. It was found that drying parameters affected significantly particle size, particle shape, surface roughness, and flowability. Optimizing designs as face-centered central composite response surface design with three factors on three levels were implemented for this investigation. The model covers linear and quadratic effects because of three levels were considered for each factor [72].

DoE was applied in two stages in order to optimize DPI of budesonide-loaded large-porous microparticles. The first stage was a single factor screening DoE (one-factor-at-a-time method) which was carried to study the effect of budesonide loading, polymer type and its concentration on the characteristics of drug loaded large-porous microparticles. The second stage was an optimization DoE where a central composite design/response surface method (CCD/RSM) was applied. In CCD/RSM method the effects of independent parameters (O/W phase ratio, poly (vinyl pyrrolidone) and poly(vinyl alcohol) concentration) on the dependent (geometric particle size, fine particle fraction, MMAD, and drug encapsulation efficiency) were investigated. One-way ANOVA with Newman–Keuls post-test or t-test was used to check statistical significance ($P < 0.05$). Using this type of DoE allowed minimum number of experiments; 17 experiments with three center point to explore the experimental domain [73].

A full factorial DoE was applied to prepare composite DPI formulations of kanamycin (hygroscopic drug) and rifampicin (hydrophobic drug) using spray drying technique. The independent parameters were the drug ratio, cosolvent composition, and inlet temperature, while the dependent parameters were fine particle fraction (FPF), powder density, water content, particles morphology, crystallinity, emitted doses, surface composition, and the hydrophobic surface enrichment. The hydrophobic surface enrichment significantly improved the aerosolization of kanamycin as presented by the high percentage of FPF. Nested and balanced ANOVA were used to analyze results at a significance level of $P < 0.05$ [74].

A response surface randomized factorial design of experiment (DoE) was applied in order to prepare trehalose/leucine carriers for DPI by using spray dryer technique. Independent parameters were chosen as inlet temperature, spray dryer airflow rate, feed solution flow rate, aspiration setting and L-leucine concentration. Dependent parameters such as particle size, yield, residual moisture content, span, outlet temperature, and glass transition temperature of the product were determined. Each factor was studied at three levels: low (−1), center (0), and high (+1). The preliminary screening design for all the independent parameters was based on one-factor-at-a-time design representing the lower and upper limits of the parameter. Furthermore, the statistical model was constructed after choosing main effects and two-factor interactions that were the most likely to influence the response. Statistical analysis was done by ANOVA for determination of significance (P-value) and impact (F-value) between factors and their interactions. The study showed that a high leucine content (30%w/w) protected amorphous trehalose from moisture and hence prevented its transformation to a crystalline form. However, at higher concentration of leucine, a negative aerosolization performance was observed [75].

Optimization of supercritical CO_2-assisted spray drying technique of trehalose/leucine composite powder mixtures suitable for formulation of DPI was done using full factorial design DoE: eight points plus three central points (−1, 0, +1) to determine the process reproducibility. The independent parameters were selected as the static mixer pressure, inlet drying gas temperature and feed flow rate and their effect on process yield, powder morphological and physicochemical properties, particle size distribution, residual solvent content, fine powder fraction, and MMAD were evaluated. A statistical analysis was performed using a derived partial least squares regression model. This technique with its DoE method was successful to produce inhalation powders with a high yield up to 70% and fine powder fraction values as high as 86%, while trehalose was an amorphous powder and leucine as crystalline powder [76].

Sodium carboxymethylcellulose (SCMC), sodium alginate (SA), and sodium hyaluronate (HA) were used as biodegradable polymers to formulate inhalable sildenafil citrate (SC) spray-dried microparticles for the treatment of pulmonary arterial hypertension. A three-factor, degree 3 extreme vertices mixture design with nine runs was developed in order to screen the effect of CMAs of formulation components and identify their compositions that will provide spray-dried microparticles favorable for SC DPI product. It investigated small subportion of the composition within the overall formulations and consequently applied a limited number of experiments, making the design construction as well as model fitting easier over constrained region of interest. Accordingly, SCMC and SA were evaluated from levels of 0 to 1 and HA was evaluated between the levels of 0 and 0.5; while the other formulation components and the spray drying process parameters were kept constant. Furthermore, all individual as well as two and three factor interactions were studied within this design. CQAs such as product yield, particle size, particle size distribution, entrapment efficiency, and cumulative release after 24 h were determined. Different statistical significance tests such as Student t-test, one-way analysis of variance (ANOVA), Tukey's Honestly

Significant Difference post-hoc test, or Welch's ANOVA, whichever applicable were used [77].

Similarly, tadalafil nanocomposites as a DPI product for pulmonary arterial hypertension treatment was developed. A D-optimal design was applied to determine the effect of independent variables namely nanocrystal/sugar ratio, L-isoleucine/sugar ratio, and sugar type on three dependent variables, namely, yield percentage, fine particle fraction percentage based on fine particle fraction percentage of the emitted dose, and the ratio of nanoparticle size obtained after spray drying to the initial size before spray drying. The three experimental factors of interest were varied on three levels based on the experimental plan, that were chosen according to the results of the screening designs collected from previous studies and preliminary experiments. A general equation was obtained and represented the relationship between the main effects of the independent variables and their interactions with the experimental response. Statistical data analysis of each response by ANOVA and model validation were done and conformed model reliability and validity [78].

A combination of DoE/response surface methodology and simulation approaches were employed to optimize lung delivery as well as target the site of deposition in the lungs of individual patients. Accordingly, spray drying technique was developed and optimized for the preparation of DPI levofloxacin formulations. A three-level and three-factor central composite face-centered design was applied using inlet gas temperature, flow rate, and liquid precursor feed rate as CPPs, and the aerodynamic properties (emitted dose, fine particle fraction, extra-fine particle fraction) and the yield percentage of the dry powders as CQAs. Mathematical model applying second-order polynomial equation along with a three-dimensional response surface plot were generated in order to evaluate the collected data of 17 assigned spray drying runs. Furthermore, several numerical simulations were employed, including computational fluid dynamics (CFD) and discrete element method (DEM), for obtaining accurate knowledge and making predictions of the positions in lungs to which inhaled particles are delivered. It is known that CFD is used to calculate the transport and deposition of particles in the respiratory system; while DEM is used for the development of DPI formulations. However, a combined CFD and DEM when utilized are more efficient to evaluate the performance of DPI formulations and devices [79].

Optimization of high dose of lyophilized lysozyme (LL) with amino acids such as phenylalanine and leucine for DPI (LLDPI) using the combination of response surface methodology and time-of-flight measurement was reported. The DoE applied faced-central composite design with response surface methodology in order to be able to analyze the relationship between the independent parameters of the formulation (quantities of lysozyme, phenylalanine, and leucine) and the dependent parameters of the inhalation performance (the aerodynamic particle size distribution, the fine particle fraction, the volume median diameter, and the emission rate). The results showed that high-dose LLDPI formulations were prepared and the maximum formulable quantity of lysozyme with acceptable inhalation performance

was confirmed to be 3.0 mg/vial. The results suggested that LLDPI can be similarly employed for the preparation of the milligram-order proteins/peptides DPIs [80].

6.3 Conclusions

PDDSs being considered complex systems, hence DoE for such systems are usually fitted using second-order models that allow curvature rather than linear models, a factorial design should include at least three levels for each variable in order to enable the data to be fitted to a second-order response surface. However, this increases the number of experimental runs up to 27 runs or more for a three-factor experiment, which is not favorable in case of limited time and resources. An alternative design as shown above are available such as: factorial designs, fractional factorial designs, Plackett–Burman designs, central composite designs, Box–Behnken designs, optimal designs, mixture designs, and other models. These designs are able to extract the maximum amount of information while using a smaller number of experimental runs than that required in a full factorial design and could be used to screen, optimize, and validate various stages of PDDSs development.

References

1. Dekhuijzen PNR, Vincken W, Virchow JC, Roche N, Agusti A, Lavorini F, van Aalderen WM, Price D (2013) Prescription of inhalers in asthma and COPD: towards a rational, rapid and effective approach. Respir Med 107:1817–1821
2. Csoka I, Karimi K, Mukhtar M, Ambrus R (2019) Pulmonary drug delivery: role of antibiotic formulations for treatment of respiratory tract infections. APH 89:43–62
3. Magramane S, Papay Z, Turbucz B, Antal I (2019) Formulation and characterization of pulmonary drug delivery systems. APH 89:63–83
4. Sallam AA (2020) Pharmaceutical drug product development and process optimization. Apple Academic Press, Palm Bay, pp 217–249
5. Zhua B, Padronia M, Colombob G, Philips G, Crapper J, Young PM, Traini D (2016) The development of a single-use, capsule-free multi-breath tobramycin dry powder inhaler for the treatment of cystic fibrosis. Int J Pharm 514:392–398
6. Ashlee D, Brunaugha AD, Hugh DC, Smytha HDC (2018) Formulation techniques for high dose dry powders. Int J Pharm 547:489–498
7. Claus S, Weiler C, Schiewe J, Friess W (2014) How can we bring high drug doses to the lung? Eur J Pharm Biopharm 86(1):1–6
8. Hickey AJ, da Rocha SR (2019) Pharmaceutical inhalation aerosol technology, 3rd edn. CRC Press, Boca Raton, pp 1–2
9. Misik O, Lizal F, Asl VF, Belka M, Jedelsky J, Elcner J, Jicha M (2018) Inhalers and nebulizers: basic principles and preliminary measurements. EPJ Web Conf 180(02068):1–6. https://doi.org/10.1051/epjconf/201818002068
10. Adi H, Young PM, Chan HK, Stewart P, Agus H, Traini D (2008) Cospray dried antibiotics for dry powder lung delivery. J Pharm Sci 97(8):3356–3366
11. Thalberg K, Berg E, Fransson M (2012) Modeling dispersion of dry powders for inhalation. The concepts of total fines, cohesive energy and interaction parameters. Int J Pharm 427(2):224–233

12. Oliveira RF, Teixeira S, Teixeira JC, Silva LF, Antunes H (2012) Experiment and numerical advances in modeling of fluid dynamics. INTECH, New York, pp 255–292
13. Hou S, Wu J, Shu XH (2015) Practical, regulatory and clinical considerations for development of inhalation drug products. Asian J Pharm Sci 10:490–500
14. Jetzer M, Morrical BD (2019) Investigation of electrostatic behavior of dry powder-inhaled model formulations. J Pharm Sci 108(9):2949–2963
15. da Rocha SRP, Bharatwaj B, Heyder RS, Yang L (2019) Pharmaceutical inhalation aerosol technology, 3rd edn. CRC Press, Boca Raton, pp 427–453
16. Rangaraj N, Pailla SR, Sampathi S (2019) Insight into pulmonary drug delivery: mechanism of drug deposition to device characterization and regulatory requirements. Pulm Pharmacol Ther 54:1–21
17. Healy AM, Amaro MI, Paluch KJ, Tajber L (2014) Dry powders for oral inhalation free of lactose carrier particles. Adv Drug Deliv Rev 75:32–52
18. Beg S, Hasnain MS (2019) Pharmaceutical quality by design: principles and applications. Academic Press, New York
19. ICH Expert Working Group (2009) ICH harmonized tripartite guideline, Pharmaceutical development Q8(R2). International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use
20. Singh DJ, Jain RR, Soni PS, Abdul S, Darshana H, Gaikwad RV, Menon MD (2015) Preparation and evaluation of surface modified lactose particles for improved performance of fluticasone propionate dry powder inhaler. J Aerosol Med Pulm Drug Deliv 28(4):254–267
21. Singh B, Beg S (2014) Product development excellence and federal compliance via QbD. Chronicle Pharma Biz 15(10):30–35
22. Beg S, Akhter S, Rahman M, Rahman Z (2017) Perspectives of quality by design approach in nanomedicines development. Curr Nanomed 7:1–7
23. Beg S, Rahman M, Panda SS (2017) Pharmaceutical QbD: omnipresence in the product development lifecycle. Eur Pharm Rev 22(1):2–8
24. Beg S, Rahman M, Kohli K (2019) Quality-by-design approach as a systematic tool for the development of nanopharmaceutical products. Drug Discov Today 24(3):717–725
25. Buttini F, Rozou S, Rossi A, Zoumpliou V, Rekkas DM (2018) The application of quality by design framework in the pharmaceutical development of dry powder inhalers. Eur J Pharm Sci 113:64–76
26. Thorat SR, Meshram SM (2015) Review on quality by designing for metered dose inhaler product development. Sch Acad J Pharm 24(6):324–330
27. Cooper A (2016) A QbD method development approach for a generic pMDI. Pharm Tech 40 (5):30–36, 63
28. Sallam A, Nazzal S, AlKhatib HS, Darwazeh N (2015) Pulmonary drug delivery: advances and challenges. Wiley, Hobokan, pp 321–338
29. Sheth P, Sandell D, Conti DS, Holt JT, Hickey AJ, Saluja B (2017) Influence of formulation factors on the aerosol performance of suspension and solution metered dose inhalers: a systematic approach. AAPS J 19:1396–1410
30. Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Products (2018) Quality considerations guidance for industry. DRAFT GUIDANCE. U.S. Department of Health and Human Services Food and Drug Administration. Center for Drug Evaluation and Research (CDER)
31. Lawrence XY, Raw A, Wu L, Daniel CC, Zhang Y, Rosencrance S (2019) FDA's new pharmaceutical quality initiative: Knowledge-aided assessment & structured applications. Int J Pharm X 1(100010):1–4
32. Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, Woodcock J (2014) Understanding pharmaceutical quality by design. AAPS J. 16(4):771–783
33. Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance (2204) U.S. Department of Health and Human Services

Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Veterinary Medicine (CVM). Office of Regulatory Affairs (ORA), Pharmaceutical CGMPs
34. Grangeiaa HB, Silvaa C, Simõesa SP, Reisc MS (2020) Quality by design in pharmaceutical manufacturing: a systematic review of current status, challenges and future perspectives. Eur J Pharm Biopharm 147:19–37
35. Faulhammer E, Llusa M, Wahl PR, Paudel A, Lawrence S, Biserni S, Calzolari V, Khinast JG (2016) Development of a design space and predictive statistical model for capsule filling of low-fill-weight inhalation products. Drug Dev Ind Pharm 42:221–230
36. Stagner WC, Hickey AJ (2019) Quality by design considerations pharmaceutical inhalation aerosol technology, 3rd edn. CRC Press, Boca Raton, pp 511–522
37. Vinjamuri BP, Haware RV, Stagner WC (2017) Inhalable ipratropium bromide particle engineering with multicriteria optimization. AAPS PharmSciTech 18(6):1925–1935
38. Raula J, Seppälä J, Malm J, Karppinen M, Kauppinen EI (2012) Structure and dissolution of L-leucine-coated salbutamol sulphate aerosol particles. AAPS PharmSciTech 13(2):707–712
39. Myers R, Montgomery D, Anderson-Cook C (1995) Response surface methodology: process and product optimization using designed experiments. Wiley, Hoboken, pp 208–265
40. Sukasame N, Boonme P, Srichana T (2011) Development of budesonide suspensions for use in an HFA pressurized metered dose inhaler. Sci Asia 37:31–37
41. Slowey A (2014) Four pivotal steps in early stage MDI formulation. Pharm Manuf 12:1–4
42. Sheth P, Sandel D, Conti DS, Holt JT, Hickey AJ, Bhawana Saluja B (2017) Influence of formulation factors on the aerosol performance of suspension and solution metered dose inhalers: a systematic approach. AAPS J 19(5):1396–1410
43. Belotti S, Edwards M, Bryan S, Brown A (2015) A design of experiment applied to pMDI manufacture in order to explore the impact of the critical process parameters on product performance. Drug Deliv Lungs 26:1–4
44. Smyth H, Brace G, Barbour T, Gallion J, Grove J, Hickey AJ (2006) Spray pattern analysis for metered dose inhalers: effect of actuator design. Pharm Res 23(7):1591–1596
45. Copelli D, Bodria A, Magnani I, Militerno G, Ponticelli M, Usberti F, Leardi R (2016) Actuator performance comparison by an integrated multivariate approach. Pharm Anal Acta 7 (6):1000483, 1–5
46. Finlay WH (2019) The mechanics of inhaled pharmaceutical aerosols, 2nd edn. Academic Press, New York, pp 263–283
47. Liu X, Guo C, Chowdhury B, Starke P, Limb S, Peri P, Schroeder A, Doub W (2017) Valved holding chambers and in vitro metered dose inhaler performance: effects of flow rate and inhalation delay. J Aerosol Med Pulm Drug Deliv 30(6):399–410
48. Pirie RS, Mcgorum BC, Owen C, Carr C, Oakley H, Mcllachlan G (2016) Factors affecting the efficiency of aerosolized salbutamol delivery via a metered dose inhaler and equine spacer device. J Vet Pharmacol Therap 40(3):1–8
49. Mendes PJ, Raposo A, Sousa JMM, Pinto JF (2004) Sizing of powders in inhalers with an Aerosizer according to a mixed experimental factorial design. Journal of Aerosol Science 35:509–527
50. Giry K, Pean JM, Giraud L, Marsas S, Rolland H, Wüthrich P (2006) Drug/lactose co-micronization by jet milling to improve aerosolization properties of a powder for inhalation. Int J Pharm 321(1-2):162–166
51. Telko MJ, Kujanpaeae J, Hickey AJ (2007) Investigation of triboelectric charging in dry powder inhalers using electrical low pressure impactor (ELPI). Int J Pharm 336:352–360
52. Shur J, Nevell TG, Shute JK, Smith JR (2008) The spray drying of unfractionated heparin: optimization of the operating parameters. Drug Dev Ind Pharm 34:559–568
53. Guenette E, Barrett A, Kraus D, Brody R, Harding L, Magee G (2009) Understanding the effect of lactose particle size on the properties of DPI formulations using experimental design. Int J Pharm 380(1-2):80–88
54. Zhang Y, Wang X, Lin X, Liu X, Tian B, Tang X (2010) High azithromycin loading powders for inhalation and their in vivo evaluation in rats. Int J Pharm 395:205–214

55. Sultana S, Talegaonkar S, Ali R, Mittal G, Ahmad FJ, Bhatnagar A (2012) Inhalation of alendronate nanoparticles as dry powder inhaler for the treatment of Osteoporosis. J Microencapsul 29(5):445–454
56. Yas AA, Khalil YI, Mohamed MS (2013) Response surface methodology for development and optimization of theophylline pulmonary delivery system. Iraqi J Pharm Sci 22(1):65–81
57. Karner S, Urbanetz NA (2013) Triboelectric characteristics of mannitol based formulations for the application in dry powder inhalers. Powder Technol 235:349–358
58. Heng D, Lee SH, Kwek JW, Ng WK, Chan HK, Tan RBH (2012) Assessing the combinatorial influence of climate, formulation and device on powder aerosolization using the Taguchi experimental design. Powder Technol 226:253–260
59. Cheow WS, Li S, Hadinoto K (2010) Spray drying formulation of hollow spherical aggregates of silica nanoparticles by experimental design. Chem Eng Res Des 88:673–685
60. Amaro MI, Tajber L, Corrigan OI, Healy AM (2011) Optimisation of spray drying process conditions for sugar nanoporousmicroparticles (NPMPs) intended for inhalation. Int J Pharm 421:99–109
61. Shariare MH, de Matas M, York P, Shao Q (2011) The impact of material attributes and process parameterson the micronization of lactose monohydrate. Int J Pharm 408:58–66
62. Sultana S, Talegaonkar S, Ali R, Mittal G, Bhatnagar A, Ahmad FJ (2011) Formulation development and optimization of alpha ketoglutarate nanoparticles for cyanide poisoning. Powder Technol 211:1–9
63. Maltesen MJ, Bjerregaard S, Hovgaard L, Havelund S, van de Weert M (2008) Quality by design – spray drying of insulin intended for inhalation. Eur J Pharm Biopharm 70:828–838
64. Friebel C, Steckel H, Müller BW (2012) Rational design of a dry powder inhaler: device design and optimization. J Pharm Pharmacol 64(9):1303–1315
65. Ingvarsson PT, Yang M, Mulvad H, Nielsen HM, Rantanen J, Foged C (2013) Engineering of an inhalable DDA/TDB liposomal adjuvant: a quality-by-design approach towards optimization of the spray drying process. Pharm Res 30:2772–2784
66. FaulhammerE FM, Llusa M, Lawrence SM, Biserni S, Calzolari V, Khinast JG (2014) Low-dose capsule filling of inhalation products: critical material attributes and process parameters. Int J Pharm 473:617–626
67. Gaspar MC, Sousa JJS, Pais AACC, Cardoso O, Murtinho D, Serra MES, Tewes F, Olivier JC (2015) Optimization of levofloxacin-loaded crosslinked chitosan microspheres for inhaled aerosol therapy. Eur J Pharm Biopharm 96:65–75
68. Belotti S, Rossi A, Colombo P, Bettini R, Rekkas D, Politis S, Colombo G, Balducci AG, Buttini F (2015) Spray-dried amikacin sulphate powder for inhalation in cystic fibrosis patients: The role of ethanol in particle formation. Eur J Pharm Biopharm 93:165–172
69. Maretti E, Rustichelli C, Romagnoli M, Balducci AG, Buttini F, Sacchetti F, Leo E, Iannuccelli V (2016) Solid Lipid Nanoparticle assemblies (SLNas) for an anti-TB inhalation treatment—a design of experiments approach to investigate the influence of pre-freezing conditions on the powder respirability. Int J Pharm 51:669–679
70. Kanojiaa G, Willems GJ, Frijlink HW, Kerstena GFA, Soemaa PC, Amorij JP (2016) A design of experiment approach to predict product and process parameters for a spray dried influenza vaccine. Int J Pharm 511:1098–1111
71. Cabral RP, Sousa AML, Silva AS, Paninho AI, Temtem M, Costa E, Casimiro T, Ricardo AA (2016) Design of experiments approach on the preparation of dry inhalerchitosan composite formulations by supercritical CO2-assistedspray-drying. J Supercrit Fluids 116:26–35
72. Mönckedieck M, Kamplade J, Fakner P, Urbanetz NA, Walzel P, Steckel H, Scherließ R (2017) Spray drying of mannitol carrier particles with defined morphology and flow characteristics for dry powder inhalation. Dry Technol 35(15):1843–1857
73. Zhang L, Zhang X, Li J, Broichsitter MB, Muenster U, Wang X, Zhao J, Mao S (2019) Optimization of budesonide-loaded large-porous microparticles for inhalation using quality by design approach. J Drug Deliv Sci Technol 53:101140

74. Momin MAM, Tucker IG, Doyle CS, Denman JA, Das SC (2018) Manipulation of spray-drying conditions to develop dry powder particles with surfaces enriched in hydrophobic material to achieve high aerosolization of a hygroscopic drug. Int J Pharm 543:318–327
75. Focaroli S, Mah PT, Hastedt JE, Gitlin I, Oscarson S, Fahy JV, Healy AM (2019) A Design of Experiment (DoE) approach to optimise spray drying process conditions for the production of trehalose/leucine formulations with application in pulmonary delivery. Int J Pharm 562:228–240
76. Moura C, Casimiro T, Costa E, Ricardo AA (2019) Optimization of supercritical CO2-assisted spray drying technology for the production of inhalable composite particles using quality-by design principles. Powder Technol 357:387–397
77. Shahin HI, Vinjamuri BP, Mahmoud AA, Shamma RN, Mansour SM, Ammar HO, Ghorab MM, Chougulec MB, LipikaChablani L (2019) Design and evaluation of novel inhalable sildenafil citrate spray-dried microparticles for pulmonary arterial hypertension. J Control Release 302:126–139
78. Rad RT, Dadashzadeh S, Vatanara A, Alavia A, Ghasemian E, Mortazavi SA (2019) Tadalafil nanocomposites as a dry powder formulation for inhalation, a new strategy for pulmonary arterial hypertension treatment. Eur J Pharm Sci 133:275–286
79. Kadota K, Sosnowski TR, Tobita S, Tachibana I, Tse JY, Uchiyama H, Yuichi Tozuka Y (2020) A particle technology approach toward designing dry-powder inhaler formulations for personalized medicine in respiratory diseases. Adv Powder Technol 31:219–226
80. Ohori R, Kiuchi S, Sugiyama S, Miyamoto K, Akita T, Yamashita C (2020) Efficient optimization of high-dose formulation of novel lyophilizates for dry powder inhalation by the combination of response surface methodology and time-of-flight measurement. Int J Pharm 581:119255

Design of Experiments for the Development of Ophthalmic Products

7

Reshal Suri

Abstract

Ocular drug delivery has always been a thought-provoking area of investigation for the scientists around the globe due to the peculiar environment offered by the eye. The complex structure and functioning of this organ limit the access of therapeutics to the affected area in both anterior and posterior segment diseases. Thus, research directed towards the use of nanocarriers as vehicles for the delivery of encapsulated cargo to the target site has observed an exceptional rise in the last few decades. With many advantages offered by these nanoformulations, viz enhanced bioavailability, protection of cargo, improved stability and efficacy, etc., there are also present certain quality and safety concerns. Thus, preparation of nanocarriers is an imperative step that requires proper optimization of critical processes and parameters involved. Quality by Design (QbD) is one such systematic technique that ensures quality into the product and an important tool of QbD, called as Design of Experiments (DoE), generates statistical experimental designs in order to determine the sources of variation in the product and means to strategically combat them. This review summarizes the basic concepts of QbD and DoE and attempts to reflect upon various instances where this technique has been utilized by the formulators. The need of the same in optimization of ocular products is also highlighted as eye is a delicate organ, thus quality and safety of formulations employed in the treatment of ocular diseases can never be compromised.

Keywords

Ocular delivery · Optimization · QbD · DoE · Nanoformulation

R. Suri (✉)
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_7

7.1 Introduction

Delivery of therapeutics to the ocular tissues is one of the major challenges faced by the researchers and formulators worldwide owing to the protective anatomy, physiology, and environment of the eye that forms an impervious barrier for the entry of foreign moieties. The use of conventional delivery systems like eye drops, suspensions, and ointments is limited due to their failure of maintaining an optimal drug concentration at the target site for desired duration and hence, low therapeutic efficacy. Drug delivery to the posterior segment of the eye is yet another task due to the presence of static, dynamic, and metabolic barriers [1]. Furthermore, the constraints offered by the ocular routes of administration, viz topical, systemic, and intravitreal, also add to the problem [2]. This has led to the manipulation of matter in the nanosize range and development of novel drug delivery systems such as polymeric nanoparticles, micelles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), liposomes, dendrimers, etc., that can circumvent the formidable barriers without causing any permanent damage to the ocular tissues. These nanocarriers are proved to protect the payload from degradation and improve the ocular bioavailability.

The small size of these nanocarriers that offers unparalleled advantage also poses detrimental effects. For instance, smaller particles with large surface area interact intimately with ocular membranes and induce cellular toxicity and systematic immune responses. Shape and surface charge also influence nanoparticle toxicity, for example, fiber-shaped nanoparticles and positively charged nanoparticles are more toxic than spherical and negatively charged ones. Nevertheless, concentration of excipients and different solvents employed (or residual solvents) in nanoparticles preparation also determines their toxicity profile [3]. As a result, the need of hour is to build safety and quality characteristics in the nanocarriers at the initial step itself via optimizing the critical parameters and processes involved in the preparation stage that unwaveringly affects the shelf life, stability, and efficacy of the final product. In-depth knowledge about the unique opportunities, regulatory considerations, and technical challenges offered by the ophthalmic products defines their concept to clinic translation and commercial success. Eye, being a highly sensitive organ, demands rigorous design and testing of the ocular formulations in terms of risk: benefit ratio before being marketed and so, only a few ocular formulations have achieved regulatory approval like Visudyne® (Liposome) and Restasis® (Micellar) [4].

Consequently, a technique called Quality by Design (QbD) has become an indispensable part of formulation optimization. Quality pioneers like Sir Ronald Fisher and Dr. Joseph M. Juran emphasized the facts that statistical analysis should be applied during the planning stages of research rather than at the completion of experiment phase [5] and focus should be directed towards building the quality into a product [6], i.e. mere increase in testing does not necessarily improve the product quality. Their notions thus, led to the introduction of the concept of Quality by Design (QbD), a process centered culture, which is still an integral part of regulatory framework governing the design and development of pharmaceutical products

including ophthalmic formulations. This technique focuses on achieving meaningful product quality specifications by enhancing process capability and reducing product variability which directly affects the pharmaceutical development and manufacturing efficiencies in a positive manner. In addition, QbD improves cause–effect analysis and regulatory flexibility. This review attempts to summarize the basic concepts and elements of QbD and Design of experiments (DoE), which is one of the vital tools for QbD implementation. This review also highlights the application of DoE in design and development of various ophthalmic products.

7.2 Quality by Design (QbD)

Over the years, pharmaceutical QbD has evolved with the issuance of ICH Q8 (R2) (Pharmaceutical Development), ICHQ9 (Quality Risk Management), and ICHQ10 (Pharmaceutical Quality System) and has laid a foundation for the science-based and risk-based regulatory processes (http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html). Hence, QbD, a regulatory-driven technique, which ensures predefined product specifications, has directed both industry and Food and Drug Administration (FDA) towards a more scientific and practical approach that gives due consideration to risk assessment during pharmaceutical product development [7]. QbD takes into account certain elements such as quality target product profile (QTPP), identification of critical material attributes (CMAs) and critical process parameters (CPPs), control strategy, process capability, and continual improvement.

A well-defined QTPP avoids wasting of time and resources. As far as ophthalmic formulations are concerned, QTPP includes, intended use in a clinical setting (anterior or posterior segment disease), route of administration (topical, oral, intravitreal, periocular, systemic), dosage form (conventional or novel), strength, dosage container closure system, therapeutic moiety release, attributes affecting pharmacokinetic characteristics (e.g., dissolution), and drug product quality criteria such as identity, purity, sterility, and stability in order to deliver the therapeutic benefit promised on label in a reproducible manner for products intended to be marketed [7]. For the design and development of a robust product with desirable QTPP, considering the biopharmaceutical properties of the drug substance is mandatory. These characteristics are called as critical quality attributes (CQAs) and include physical (particle size distribution/morphology, polymorphism, aqueous solubility), chemical (pKa, chemical, photolytic, and oxidative stability), biological/microbiological property (partition coefficient, membrane permeability, bioavailability, microbial limits), or characteristic of an output material including finished drug product that should be within an appropriate limit to ensure the desired product quality. Criticality of an attribute is decided on the basis of degree of harm it can cause to the patient. Therefore, list of safety limits of excipients is provided in the FDA's inactive ingredients database. Product design defines whether the product is capable in meeting the needs of the patient and maintains its performance through its shelf life, which is determined with clinical and stability studies. Furthermore,

there are certain process parameters called as critical process parameters (CPPs), the variability in which has an impact on a CQA of the product and should be monitored to ensure that the process produces the desired quality. For preparing an ophthalmic formulation, these may include input operating parameters (speed, flow rate) or process state variables (temperature, pressure) [7].The mathematical relationships of the CPPs and critical material attributes (CMAs) with the CQAs that have been proofed to provide assurance of quality, safety, and efficacy define a multidimensional Design Space (DS). For the formulators of novel ophthalmic products, these concepts are highly valuable [4] as movement out of the design space is considered to be a change and is subject to regulatory assessment and approval. The scale and equipment may alter the design space; hence, the design space obtained at laboratory scale may need justification if used at the commercial scale. Process capability measures the inherent variability of a stable process that is in a state of statistical control in relation to the established acceptance criteria. Timely identification and justification of potential sources of common cause variation should be detected via the control strategy through product and process understanding gained during QbD development. As it is not possible for a formulation scientist to investigate the impact of all the variables on CQA during the formulation optimization studies, risk assessment would identify the variables that warrant further study to make sure that the available limited resources are used effectively and efficiently [8]. There are three essential elements in risk assessment: viz. risk identification, risk analysis, and risk evaluation.

Cost efficiency and simplicity of manufacturing process are the main advantages of QbD approach. Several tools are utilized to make QbD system easily applied to pharmaceutical field, namely multivariate data analysis (MVDA), design of experiment (DoE), and process analytical technology (PAT) that correlate the complicated multifactorial relationship among formulation parameters, process variables, and product quality attributes. Design of Experiments (DoE) is the main component of the statistical toolbox to deploy Quality by Design which is discussed in the next section.

7.3 Design of Experiments (DoE)

Development of an ophthalmic product is a process-based and quality-oriented task which mostly cannot be accomplished satisfactorily using the traditional approach of one variable at a time (OVAT) that does not evaluate the interaction between all factors, therefore, leading to an insufficient formulation optimization. A typical ophthalmic product development is influenced by a complicated matrix of input and output parameters like CPPs and CQAs as discussed earlier that may or may not be interlinked. Changes in raw materials, facilities, or equipment additionally provide a source of variability, which affects quality of the final product in a way which is impossible to interpret completely. This demands for a need of a rational, structured, and efficient system, for optimization of products and/or processes, capable of evaluating all the potential factors simultaneously, systematically and in

a time and cost-effective manner (few number of experiments). This is where DoE comes into play. DoE methods involve the use of statistical experimental designs, generation of mathematical equations and graphic outcomes, that portrays a complete picture of variation of the response(s) as a function of the factor(s) [9].

Through DoE, the formulators globally are able to manipulate factors systematically as per pre-specified design, hence determining the relationships between input factors (xi—independent variables) affecting one or more output responses (y—dependent variables), through mathematical model interpretation ($y = f(xi)$). The controlled input factors can be varied in order to observe their effects on the output responses allowing the elucidation of the most important input factors leading to optimized output responses, and also determining the interactions between input factors. Assessment of the effects of these changes on a predefined output is then made. DoE has various advantages over traditional univariate approach.

- It is a formal way to determine how factors jointly affect the output responses.
- It is a tool to maximize information from a minimum number of resources/experiments by using probability and statistics.
- Strategically studies the effects individually by simultaneously varying all parameters.
- Considers different sources of variations.
- Characterizes optimal conditions and acceptable ranges of CMAs, CPPs, contributing to identification of a design space, which provides "assurance of quality."

Moreover, choosing an appropriate experimental design is an important point while employing DoE methods. The success of the study depends upon the design employed which in turn is dependent on various aspects like whether the nature of study is screening, optimization, or robustness, how many factors to be studied, interaction between the factors, and availability of resources like time, cost, and labor. Therefore, a thorough product and process understanding enabled via DoE method leads to proper estimation of these variations and hence improves the quality, safety, and efficacy of the product [10]. An overview of different experimental designs is given in Fig. 7.1.

Usually, the analysis of the designs is carried out using Design Expert Software (StatEase, version 9.0.1, Minneapolis, MN). Design, analysis, and optimization are the three major components of DoE which can be easily carried out by Design-Expert while generating useful information. DoE, quality risk management (QRM), and process analytical technologies (PATs), together maintain good formulation control and consistency to assure the quality of the drug products [11].

ICH Q8 (R2) encourages the use of PAT to ensure that the process remains within an established design space and the application of PAT be part of the control strategy to monitor/control CMA and CPP within the expected limits. In-process testing, CMAs, or CQAs can also be measured on-line or in-line with PAT detecting more failures than end-product testing alone. Application of PAT involves four key components as follows: Multivariate data acquisition and analysis, process analytical

A. Response surface designs
1. Factorial designs (FD)
2. Central composite designs (CCD) or Box–Wilson design (Face centered design, Center of gravity designs)
3. Box–Behnken designs (BBD)
4. Equiradial designs (EqD)
5. Mixture designs
6. Optimal designs
7. Star designs

B. Screening designs
1. Fractional factorial designs (FFD)
2. Plackett–Burman designs (PBD
3. Taguchi designs (TgD)
4. Cotter designs
5. Rechtschaffner design

Fig. 7.1 Overview of different experimental designs

chemistry tools, process monitoring and control, continuous process optimization and knowledge management. The FDA's PAT says that "Continuous learning through data collection and analysis over the lifecycle of a product is important. These data can contribute to justifying proposals for post-approval changes. Approaches and information technology systems that support knowledge acquisition from such databases are valuable for the manufacturers and can also facilitate scientific communication with the Agency." [7] In light of aforementioned importance of QbD, Table 7.1 summarizes various ocular nanoformulations that have been prepared using QbD approch.

7.4 Conclusion

The perpetual problems of ophthalmic product developers can be unquestionably solved by Quality by Design (QbD), successful implementation of which depends upon understanding of the basics and applying the correct methods and tools. Among the vast toolkit, the most preferable tool, Design of Experiments (DoE) helps understand the importance of critical process parameters and gives the flexibility to create in-control operating space near the center of the Design Space without compromising the quality, where an optimum and robust formulation can be designed. Less time to market, less out-of-specification results, and increased cost efficiency with regulatory flexibility are some of the strengths of DoE. In conclusion, knowledge management and statistical thinking applied in ophthalmic drug products development can consistently promote operational excellence within the QbD framework.

Table 7.1 Ocular nanoformulations prepared using QbD

Carrier	Factor/independent variables	Design	References
Nanoparticles	Concentration of polymer, amount of AOT, % drug loading	CCD	[12]
Self-assembled liquid crystalline nanoparticles	Heating temperature, duration, homogenization heating, number of cycles, pressure	Fractional factorial design	[13]
SLN	Concentration of solid lipid, surfactant, and drug/lipid ratio	BBD	[14]
Nanoparticles	Amount of bioadhesive polymers and drug	BBD	[15]
Nanoparticles	Chitosan and polyvinyl alcohol concentration, PLGA content, sonication time	BBD	[16]
NLC	Amount of castor oil, Precirol® ATO 5, span® 80, and high-pressure homogenization (HPH) time	BBD	[17]
NLC	Surfactant/lipid ratio (S/L), liquid lipid percentage, and Transcutol percentage	2 [3] factorial design	[18]
Nanomatrix	PVA concentration, sonication time, EAP: OP ratio, and % thymoquinone	CCD	[19]
Cubic liquid crystalline nanoparticle	Sonication time, sonication amplitude, sonication depth, and premixing time	CCD	[20]
SLN	Amount of GMS, phospholipid, and surfactant	BBD	[21]
Chitosan-SLNs	Amount of methazolamide, phospholipid, GMS, co-emulsifier, and chitosan	Orthogonal design, BBD	[22]
NLC	Concentration of Nepafenac, liquid lipid, and CRE/SOY ratio	CCD	[23]
In-situ gel	Concentration of P407, P188, and chitosan	D-optimal	{Krtalić, 2018 #66}
In-situ gel	Amount of poloxamer 407 and HPMC	CCD	[24]
Emulsion	Mixing order method, phase volume ratio, and pH adjustment method, temperature of primary and raw emulsion formation, microfluidizer pressure, and number of pressure cycles	Hunter screening design matrix	[25]
Nanosuspension	Percentage of Pluronic® F68 and tween® 80	Full factorial	[26]
Ointment	Quantity of API and mineral oil, stirring rate, temperature, time, cooling temperature, mixing rate	Plackett–Burman screening design	[27]
In-situ gel	Concentration of Gelrite and Hypromellose METHOCEL E 15 premium LV	3 [2] factorial design	[28]

(continued)

Table 7.1 (continued)

Carrier	Factor/independent variables	Design	References
Eye drops	Concentration of polymer and Mucin, type of Carbopol, sonication	Full factorial	[29]
Nanoparticles	Concentration of CMTKP and calcium chloride	CCD	[30]
Nanoparticles	Concentration of PF, PVA, PLGA, and aqueous phase pH	CCD	[31]
Thermosensitive gel	Concentration of P407 and P188	CCD	[32]
Liquid crystalline nanoparticles	The amount of TET, glyceryl monoolein, and the ratio of poloxamer 407 to glyceryl monoolein	CCD	[33]
Chitosan nanoparticles	Concentration of chitosan and NaTPP and volume of NaTPP	BBD	[34]
Nanoparticles	Concentration of polylactide acid, solvent to non-solvent ratio and Pluronic-F68 concentration	BBD	[35]
SLN	Lipid mix concentration, poloxamers-188, and sodium-taurocholate	BBD	[36]
Ultrasound engineered NLC	% FB, % tween 80, % SA with regard total lipid, storage temperature	2 [4]full factorial design	[37]
NLC	Liquid lipid concentration in the total lipid phase, surfactant concentration, and drug concentration	CCD	[38]
Chitosan-dextran nanoparticles	Concentration of CS, DS and amount of drug	BBD	[39]
SLN	Concentration of lipid and surfactant and sonication frequency	BBD	[40]
Nanoparticles	Concentration of PVA, PLGA, chitosan, and sonication time	BBD	[41]
Nanoparticles	Concentration of FX and CH	CCD	[42]
PEGylated PLGA nanospheres	pH, DXI, and PVA concentrations	CCD	[43]
In-situ gel (nanosuspension)	Chiller temperature, high-pressure homogenization pressure, and HPH cycles	CCD	[44]
Nanoparticles	Concentration of polymer, calcium chloride, and sonication time	BBD	[45]
Thermosensitive in-situ gels	Concentration of poloxamer P407 and P188	RSM plus CCD	[46]
SLN	Amount of lipid and drug, stirring speed, and stirring time	CCD	[47]
Nanoparticles	Concentration of PLGA, chitosan, and PVA	BBD	[48]
Self-assembled nanostructures	Effects of pH and drug to polymer ratio	RSM	[49]

(continued)

Table 7.1 (continued)

Carrier	Factor/independent variables	Design	References
Lipid nanoparticles	Amount of Softisan® 100, Poloxamer 188, and lecithin	Three-level full factorial design	[50]

Acknowledgements Author is thankful to Indian Council of Medical Research (ICMR45/51/2018-NAN-BMS), New Delhi, India for providing Senior Research Fellowship.

References

1. Suri R, Beg S, Kohli K (2020) Target strategies for drug delivery bypassing ocular barriers. J Drug Deliv Sci Technol 55:101389
2. Subrizi A, del Amo EM, Korzhikov-Vlakh V, Tennikova T, Ruponen M, Urtti A (2019) Design principles of ocular drug delivery systems: importance of drug payload, release rate, and material properties. Drug Discov Today 24(8):1446–1457
3. Zhu S, Gong L, Li Y, Xu H, Gu Z, Zhao Y (2019) Safety assessment of nanomaterials to eyes: an important but neglected issue. Adv Sci 6(16):1802289
4. Havel H, Finch G, Strode P, Wolfgang M, Zale S, Bobe I, Youssoufian H, Peterson M, Liu M (2016) Nanomedicines: from bench to bedside and beyond. AAPS J 18(6):1373–1378
5. Hald A (1998) A history of mathematical statistics from 1750 to 1930. Wiley, Hoboken, NJ
6. Beg S, Rahman M, Panda SS (2017) Pharmaceutical QbD: omnipresence in the product development lifecycle. Eur Pharm Rev 22:58–64
7. Beg S, Hasnain MS (2019) Pharmaceutical quality by design: principles and applications. Academic Press, New York
8. Beg S, Hasnain MS, Rahman M et al (2019) Chapter 1 - Introduction to quality by design (QbD): fundamentals, principles, and applications. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic Press, Cambridge, MA, pp 1–17
9. Beg S, Al Robaian M, Rahman M, Imam SS, Alruwaili N, Panda SK (2020) Pharmaceutical drug product development and process optimization: effective use of quality by design. CRC Press, Boca Raton, FL
10. Beg S, Swain S, Rahman M et al (2019) Chapter 3 - application of Design of Experiments (DoE) in pharmaceutical product and process optimization. In: Beg S, Hasnain MS (eds) Pharmaceutical quality by design. Academic Press, New York, pp 43–64
11. Singh B, Raza K, Beg S (2013) Developing "optimized" drug products employing "designed" experiments. Chem Ind Dig 23:70–76
12. Yadav M, Ahuja M (2010) Preparation and evaluation of nanoparticles of gum cordia, an anionic polysaccharide for ophthalmic delivery. Carbohydr Polym 81(4):871–877
13. Achouri D, Hornebecq V, Piccerelle P, Vr A, Sergent M (2015) Self-assembled liquid crystalline nanoparticles as an ophthalmic drug delivery system. Part I: influence of process parameters on their preparation studied by experimental design. Drug Dev Ind Pharm 41(1):109–115
14. Hao J, Fang X, Zhou Y, Wang J, Guo F, Li F, Peng X (2011) Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a box-Behnken design. Int J Nanomed 6:683
15. Motwani SK, Chopra S, Talegaonkar S, Kohli K, Ahmad FJ, Khar RK (2008) Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and in vitro characterisation. Eur J Pharm Biopharm 68(3):513–525

16. Pandit J, Sultana Y, Aqil M (2016) Chitosan-coated PLGA nanoparticles of bevacizumab as novel drug delivery to target retina: optimization, characterization, and in vitro toxicity evaluation. Artif Cells Nanomed Biotechnol 45(7):1397–1407
17. Patil A, Lakhani P, Taskar P, Wu KW, Sweeney C, Avula B, Wang YH, Khan IA, Majumdar S (2018) Formulation development, optimization, and in vitro-in vivo characterization of Natamycin-loaded PEGylated Nano-lipid carriers for ocular applications. J pharm Sci 107 (8):2160–2171. https://doi.org/10.1016/jxphs201804014
18. Sharif Makhmal Zadeh B, Niro H, Rahim F, Esfahani G (2018) Ocular delivery system for propranolol hydrochloride based on nanostructured lipid carrier. Sci Pharm 86(2):16
19. Verma D, Thakur P, Padhi S, Khuroo T, Talegaonkar S, Iqbal Z (2017) Design expert assisted nanoformulation design for co-delivery of topotecan and thymoquinone: optimization, in vitro characterization and stability assessment. J Mol Liq 242:382–394
20. Verma P, Ahuja M (2016) Cubic liquid crystalline nanoparticles: optimization and evaluation for ocular delivery of tropicamide. Drug Deliv 23(8):3043–3054
21. Wang F, Chen L, Jiang S, He J, Zhang X, Peng J, Xu Q, Li R (2014) Optimization of methazolamide-loaded solid lipid nanoparticles for ophthalmic delivery using box-Behnken design. J Liposome Res 24(3):171–181
22. Wang F-Z, Zhang M-W, Zhang D-S, Huang Y, Chen L, Jiang S-M, Shi K, Li R (2018) Preparation, optimization, and characterization of chitosan-coated solid lipid nanoparticles for ocular drug delivery. J Biomed Res 32(5):411–423
23. Yu S, Tan G, Liu D, Yang X, Pan W (2017) Nanostructured lipid carrier (NLC)-based novel hydrogels as potential carriers for nepafenac applied after cataract surgery for the treatment of inflammation: design, characterization and in vitro cellular inhibition and uptake studies. RSC Adv 7(27):16668–16677
24. Patel N, Thakkar V, Metalia V, Baldaniya L, Gandhi T, Gohel M (2016) Formulation and development of ophthalmic in situ gel for the treatment ocular inflammation and infection using application of quality by design concept. Drug Dev Ind Pharm 42(9):1406–1423
25. Rahman Z, Xu X, Katragadda U, Krishnaiah YSR, Yu L, Khan MA (2014) Quality by design approach for understanding the critical quality attributes of cyclosporine ophthalmic emulsion. Mol Pharm 11(3):787–799
26. El-Feky GS, Zayed G, Farrag AR (2013) Optimization of an ocular nanosuspension formulation for acyclovir using factorial design. Int J Pharm Pharm Sci 5:213–219
27. Xu X, Al-Ghabeish M, Rahman Z, Krishnaiah YSR, Yerlikaya F, Yang Y, Manda P, Hunt RL, Khan MA (2015) Formulation and process factors influencing product quality and in vitro performance of ophthalmic ointments. Int J Pharm 493(1):412–425
28. Wagh DG, Shahi SR, Bora S, Dabir PD, Deore SV (2016) QBD approach in formulation and evaluation of gelrite based in situ ophthalmic gel of Nepafenac. World J Pharm Res 6 (1):638–654
29. Ceulemans J, Ludwig A (2002) Optimisation of carbomer viscous eye drops: an in vitro experimental design approach using rheological techniques. Eur J Pharm Biopharm 54 (1):41–50
30. Kaur H, Ahuja M, Kumar S, Dilbaghi N (2012) Carboxymethyl tamarind kernel polysaccharide nanoparticles for ophthalmic drug delivery. Int J Biol Macromol 50(3):833–839
31. Abrego G, Alvarado HL, Egea MA, Gonzalez-Mira E, Calpena AC, Garcia ML (2014) Design of nanosuspensions and freeze-dried PLGA nanoparticles as a novel approach for ophthalmic delivery of pranoprofen. J Pharm Sci 103(10):3153–3164
32. Hao J, Wang X, Bi Y, Teng Y, Wang J, Li F, Li Q, Zhang J, Guo F, Liu J (2014) Fabrication of a composite system combining solid lipid nanoparticles and thermosensitive hydrogel for challenging ophthalmic drug delivery. Colloids Surf B: Biointerfaces 114:111–120
33. Liu R, Wang S, Fang S, Wang J, Chen J, Huang X, He X, Liu C (2016) Liquid crystalline nanoparticles as an ophthalmic delivery system for Tetrandrine: development, characterization, and in vitro and in vivo evaluation. Nanoscale Res Lett 11(1):254

34. Chopra AK, Marwaha RK, Kaushik D, Dureja H (2014) Box-Behnken designed fluconazole loaded chitosan nanoparticles for ocular delivery. J Pharm Drug Deliv Res 3(1):2
35. Ibrahim HM, Ismail HR, Lila AE (2012) Formulation and optimization of ocular poly-D, L-lactic acid nano drug delivery system of amphotericin-B using box behnken design. Int J Pharm Pharm Sci 4(2):342–349
36. Kalam MA, Sultana Y, Ali A, Aqil M, Mishra AK, Aljuffali IA, Alshamsan A (2013) Part I: development and optimization of solid-lipid nanoparticles using box-Behnken statistical design for ocular delivery of gatifloxacin. J Biomed Mater Res A 101(6):1813–1827
37. Gonzalez-Mira E, Egea MA, Garcia ML, Souto EB (2010) Design and ocular tolerance of flurbiprofen loaded ultrasound-engineered NLC. Colloids Surf B: Biointerfaces 81(2):412–421
38. Gonzalez-Mira E, Egea MA, Souto EB, Calpena AC, García ML (2010) Optimizing flurbiprofen-loaded NLC by central composite factorial design for ocular delivery. Nanotechnology 22(4):045101
39. Kaskoos RA (2014) Investigation of moxifloxacin loaded chitosan-dextran nanoparticles for topical instillation into eye: in-vitro and ex-vivo evaluation. Int J Pharm Investigation 4(4):164
40. Khames A, Khaleel MA, El-Badawy MF, El-Nezhawy AOH (2019) Natamycin solid lipid nanoparticles as sustained ocular delivery system of higher corneal penetration against deep fungal keratitis: preparation and optimization. Int J Nanomed 14:2515
41. Khan N, Zafar A, Khanna K, Bhatnagar A, Ahmad F, Ali A (2018) Chitosan coated PLGA nanoparticles amplify the ocular hypotensive effect of forskolin: statistical design, characterization and in vivo studies. Int J Biol Macromol 116:648–663
42. Mittal N, Kaur G (2019) Investigations on polymeric nanoparticles for ocular delivery. Adv Polym Technol 2019:14
43. Sánchez-López E, Egea MA, Cano A, Espina M, Calpena AC, Ettcheto M, Camins A, Souto EB, Silva AM, García ML (2016) PEGylated PLGA nanospheres optimized by design of experiments for ocular administration of dexibuprofen in vitro, ex vivo and in vivo characterization. Colloids Surf B: Biointerfaces 145:241–250
44. Khurana LK, Singh R, Singh H, Sharma M (2018) Systematic development and optimization of an in-situ gelling system for Moxifloxacin ocular Nanosuspension using high-pressure homogenization with an improved encapsulation efficiency. Curr Pharm Des 24(13):1434–1445
45. Ali J, Khan N, Ali A (2014) Development and optimization of Carteolol loaded Carboxymethyl tamarind kernel polysaccharide nanoparticles for ophthalmic delivery: box-Behnken design, in vitro, ex vivo assessment. Sci Adv Mater 6(1):63–75
46. Ju FG, Zhu ZJ, Wang LJ, Ma LL (2009) Optimization and evaluation of ophthalmic thermosensitive in situ gels of diclofenac sodium [J]. Chin J Hospital Pharm 7
47. Shah M, Agrawal YK, Garala K, Ramkishan A (2012) Solid lipid nanoparticles of a water soluble drug, ciprofloxacin hydrochloride. Indian J Pharm Sci 74(5):434
48. Zafar A, Khan N, Alruwaili NK, Bukhari SNA, Alsuwayt B, Afzal M, Akhtar S, Yasir M, Elmowafy M, Shalaby K (2020) Improvement of ocular efficacy of levofloxacin by bioadhesive chitosan coated PLGA nanoparticles: box-behnken design, in-vitro characterization, antibacterial evaluation and Scintigraphy study. Iran J Pharm Res 19(1):292–311
49. Afify EAMR, Elsayed I, Gad MK, Mohamed MI, Afify AE-MMR (2018) Enhancement of pharmacokinetic and pharmacological behavior of ocular dorzolamide after factorial optimization of self-assembled nanostructures. PLoS One 13(2):e0191415
50. Fangueiro JF, Andreani T, Egea MA, Garcia ML, Souto SB, Silva AM, Souto EB (2014) Design of cationic lipid nanoparticles for ocular delivery: development, characterization and cytotoxicity. Int J Pharm 461(1-2):64–73

Design of Experiment for the Development of Vesicular Drug Products

8

Poonam Negi, Chetna Hemrajani, and Shweta Agarwal

Abstract

The chapter presented deals with the importance of Design of Experiments (DoE) in formulation development of vesicular drug products. The important vesicular systems like liposomes, niosomes, transferosomes, ethosomes have been briefly discussed along with literature citations of experimental designs (EDs) that have been used for their formulation development. Broad classification of EDs into screening and response surface designs has been given and some important designs along with their key terminologies like independent and dependent variables, design matrix, levels, constraints, etc., have been presented. Relevance of DoE in vesicular has been outlined and various methods for selection of EDs like graphical, numerical, and point prediction have been introduced. The information presented would provide basic understanding of application of DoE in research and development of vesicular drug delivery systems and help researchers in taking sound, scientifically guided decisions during product design and development.

Keywords

Design of experiments · Vesicular carriers · Localized delivery · Topical products · Liposomes · Ethosomes · Transfersomes

P. Negi (✉) · C. Hemrajani
School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh, India

S. Agarwal
L.R. Institute of Pharmacy, Solan, Himachal Pradesh, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_8

8.1 Introduction to Vesicular Drug Delivery Systems

Lately, a lot of attention is being paid to the development of novel drug delivery systems (NDDS) for two reasons—for delivering the drug in the right concentration at the right site (tissue or organ). Vesicular drug delivery systems can be classified as a type of NDDS comprising concentric bilayers of self-assembling amphiphilic molecules in the presence of water (Fig. 8.1). They possess the capability of localization of drug at the target site, thus resulting in targeted drug delivery. Their superiority over conventional drug delivery systems also includes providing stability to the drugs, protection of labile drugs from the harsh environment of gastrointestinal tract, aid in improvement of bioavailability, encapsulation of both hydrophilic and lipophilic drugs, reduction in toxicity of some drugs [1].

Different kinds of vesicular drug delivery systems are available based on the chief constituent present in their composition. Some of the important systems have been discussed below.

1. Liposomes—They are spherical usually nanosized vesicular structures of one or more bilayers of natural or synthetic lipids enclosing an aqueous compartment. The drug can be encapsulated in the aqueous compartment or lipid bilayer depending on drug's characteristics. Phospholipids (especially phosphatidylcholine) and cholesterol are important constituents of liposomes which make them imitate the biophysical model of cells. Therefore liposomes occupy an important place in NDDS because of their ability to target sites either actively or passively. Targeting potential of liposomes can be modified by manipulating their main constituent, phospholipids to suit the desired applications. Other characteristics that can be modified are number of bilayers, curvature, fluidity of bilayer membrane. The characteristics of liposomes also depend on the method of preparation to a great extent and manipulation of process variables may yield products with different characteristics and applications [2, 3]. Some methods that can be used

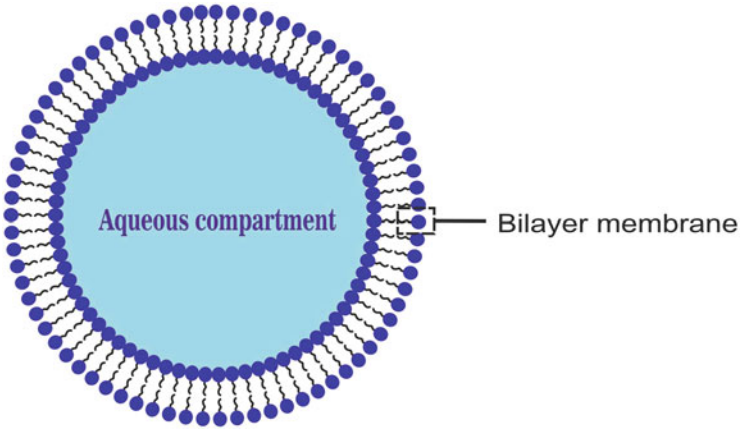

Fig. 8.1 General structure of vesicular systems

for preparation of liposomes are as follows—lipid film hydration, sonication, ether injection, ethanol injection, French pressure cell, membrane extrusion, reverse phase evaporation method.

2. Niosomes—To overcome the major limitation of stability and cost of liposomes, niosomes were introduced. They are also self-assembling microscopic vesicular drug delivery systems made up of non-ionic surfactants like Tweens, Spans, Brij, etc., and cholesterol in an aqueous environment with the input of some kind of energy like heat, stirring, sonication, etc. In niosomes, non-ionic surfactants are responsible for the formation of bilayer lamellae which may be one or multiple like in liposomes [4]. Other advantages of niosomes over liposomes include less leakiness and greater encapsulation efficiency. Similar to liposomes, niosomes are helpful in targeting the drug, prolonged release, reduction in dose and toxicity by increase in bioavailability. The properties of niosomes depend on the composition of the bilayer and the method of preparation. Various methods can be used for the preparation of niosomes like ether injection, bubble, reverse phase evaporation, microfluidization, supercritical carbon dioxide fluid, transient pH-gradient, heating, lipid injection methods.
3. Ethosomes—They are also known as "Ethanolic liposomes" and are counterparts of liposomes possessing a high content of alcohol (ethanol or isopropyl alcohol) which improves their penetrability through skin. The content of alcohol may be as high as 50% along with the presence of phospholipids (main constituent for forming vesicles), glycol, cholesterol, and water. The greater permeation of ethosomes across the skin may be attributed to the ethanol effect suggesting that ethanol tends to disturb the lipid bilayer arrangement of cell membrane and also of intercellular lipids causing an increase in their fluidity and thus enhancing drug penetration. Also the high alcohol content in the vesicles causes the vesicle membrane to be less tightly packed making this vesicular system more malleable and flexible and easy to penetrate the stratum corneum. On the flip side this high alcohol content increases the leakiness of the vesicle, thus lowering its entrapment efficiency but keeping its stability similar to liposomes. Change in composition of ethosomes like alteration in alcohol:water ratio or alcohol-polyol:water ratio alters drug delivery. Alteration in the ethanol content may also alter the charge on the vesicles taking it towards the negative side on increasing alcohol content, thus influencing the stability and vesicle–skin interactions. The type and content of edge activators also affects the characteristics of ethosomes. Various methods can be used for the preparation of ethosomes like the classical cold method, ethanol injection-sonication, hot method, thin film hydration, reverse phase evaporation, trans-membrane pH-gradient method. The choice of method affects the size and lamellarity of ethosomes formed consequently affecting their use [5, 6]. Ethosomes are primarily used for transdermal delivery of drugs because of enhancement in their penetrability through skin due to presence of high content of alcohol. They can be incorporated in gels, creams, patches for transdermal delivery. Even large molecules like peptides can be delivered by this vesicular system.

4. Transferosomes—Transfersomes possess the advantage of good permeability and deformability over liposomes and niosomes. They can squeeze through pores of skin much smaller than their own size. They are made up of an aqueous core surrounded by a bilayer of amphiphilic lipid like phospholipids along with surfactants in the membrane (called edge activator) which provides flexibility to the vesicles, being single chained surfactants. They are capable of carrying large molecules of drugs as well through the skin like peptide and proteins. Methods that can be used for formulation are rotary fill evaporation, reverse phase evaporation, vortexing-sonication, ethanol injection, and freeze-thaw method. They can be used for topical and transdermal delivery of drugs [5]. Their entrapment efficiency is very high for hydrophilic drugs.

   The methods used for preparation have variables which on changing yield vesicular systems with different characteristics, uses, targeting potential, circulating time, encapsulation efficiency, and stability. Thus, formulation and process variables and their levels play a key role in producing vesicular drug products with desired characteristics and utility and should be selected with utmost care. Experimental designs can, therefore, be successfully applied for optimization of vesicular systems by screening important variables, bringing about systematic changes in variables and their levels and for studying the cause–effect relationships giving a thorough understanding of the formulation of these systems.

8.2 Introduction to Design of Experiments

Design of Experiments (DoE) is a tool of Quality by Design (QbD) introduced as a result of ICH Q8 guidelines "as a systematic approach to pharmaceutical development, beginning with predefined objectives and emphasizing on product and process understanding and control based on sound science and quality risk management," pharmaceutical industry being a strictly regulated industry due to its impact on human health [7].

DoE encompasses the principles of optimization techniques introduced by British statistician Sir Ronald Fisher as early as 1925. These principles were used to replace the OVAT (one variable at a time) approach or the trial and error approach for optimization as they gave a suboptimal product [8]. The concept of building quality in a product by design rather than by testing was given by J.M Juran, an American engineer and quality analyst in the 1970s and was implemented in healthcare sector in the 1990s in producing medical devices but their adoption in the pharmaceutical industry happened quite late in the twenty-first century [9].

DoE, being an integral part of QbD in developing and designing optimized products and processes has been used in Formulation by Design (FbD) for providing holistic development of drug formulations. The five elements of FbD are [9]:

(a) Defining the quality target product profile (QTPP).
(b) Identification of critical quality attributes (CQAs).
(c) Critical formulation attributes (CFAs).
(d) Critical process parameters (CPPs).
(e) Selection of appropriate experimental design for defining design and control space through DoE for development of an optimized product.

DoE comprises use of experimental designs, generation of mathematical equations, representation of outcomes graphically for showcasing a complete picture of effect of variation of process/formulation variables on the response. Formulation and process variables that influence CQAs of product and are under the control of product development scientists are considered as input or independent variables like amount of drug, lipids, surfactants, composition of polymers, percentage of penetration enhancers, hydration volume, temperature, stirring speed, etc [10]. These can be qualitative or quantitative variables. Quantitative variables have a numerical value, for example, time, speed, volume, weight, etc., whereas qualitative variables (categorical) are the different types of polymers, lipids, diluents, and other excipients used. The values given to these variables (factors) are termed as their levels and the restrictions imposed on the levels are called constraints [11]. The quality attributes, traits, or characteristics of the product affected by the input variables are regarded as dependent or response variables like entrapment efficiency, particle size, polydispersity index, zeta potential, drug release profile, drug loading and are used to assess the result of the experiments. They are usually directly affected by any change in the independent variables. Orthogonality in a design means that the estimated effects are solely because of the main factor and are not due to the presence of interactions.

Experimental designs are used to organize, conduct, and interpret results of experiments statistically in an efficient manner ensuring derivation of maximum useful information by performance of a small number of trials or experimental runs.

The layout of experimental runs in a matrix form for the chosen experimental design by using a multidimensional combination and interaction of chosen input variables at various levels is called the design matrix [11]. The formulations are prepared in accordance with the design matrix and the results of the response variables so obtained are studied and analyzed statistically. The result data obtained is modeled to produce mathematical relationship between the independent and response variables in the form of an algebraic equation as given in Eq. (8.1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \tag{8.1}$$

where.

β_o is the intercept.
$\beta_1$and β_2 are the coefficients of main effect.
β_{12} is the coefficient of interaction effect.
β_{12} and β_{22} are the coefficients of second-order quadratic effect.

The mathematical models used can be linear, quadratic, or cubic correlating the response variable with input variables. The graphical representation of the mathematical relationship is called response surface graphs. Response surface graphs can be 3D or 2D representation of relationship between two independent variables and one response variable. Some techniques that can be used for modeling factor-response relationships are—Multiple linear regression analysis (MLRA), partial least squares analysis (PLSA), and principal component analysis (PCA) [9]. Model diagnostic plots such as perturbation, outlier, Box–Cox, leverage, Cook's Distance plots help in understanding interaction among input variables.

Response surface modeling is a statistical technique in which the results of experiments are analyzed with respect to response and the factors affecting that response in a pictorial or graphical form. Designs which permit assessment of main, interaction, and quadratic effects are called response surface designs [12].

Optimal solution can be determined graphically, numerically, or when a target value is to be achieved by point prediction [12]. This has been discussed in detail in further sections.

Validation of optimization technique is important as it indicates the predictive quality and efficiency of the model used and gives assurance about the reliability of the model to give optimized formulation. Validation is usually done by checkpoint analysis or by performing confirmatory runs in the several suggested formulations by the software [13]. Formulations are prepared and evaluated as done in original experiment at least in triplicate and residuals calculated. Low values of residuals for predicted and observed results confirm the reliability of the model. Model fit parameters like R^2, R^2_{adj}, and PRESS (Predicted residuals sum of squares of the model) are also used to ascertain the predictive ability of the model.

8.3 Need of DoE in Development of Vesicular Drug Products

Vesicular drug products are the newer kind of drug delivery systems developed with the aim of targeted drug delivery, time-dependent drug delivery, or both. Their development involves use of complex processes and excipients with many variables affecting their design. There could be interaction between some of the variables used for optimization of these formulations which cannot be taken into account while using the one variable at a time (OVAT) optimization approach resulting in suboptimal product [8]. Moreover, due to the presence of a large number of variables affecting the development of an optimized product, changing of one variable at a time would result in a large number of trial runs making the optimization very expensive, cumbersome, and time-consuming, moreso because the excipients and equipment used in the formulation of novel drug delivery systems especially the vesicular systems are high priced.

Use of DoE in optimization helps in studying the interaction between variables and cause–effect relationship for certain changes. Optimization by DoE gives systematic and holistic development of the product with thorough understanding

of the process and product. DoE is capable of providing reasoning behind every change in the process development and provides a complete picture of variation in product with respect to changes in input variables. Thus the formulation and process variables can be systematically varied to obtain a product with desired properties [7].

DoE is capable of providing various combinations of input variables with predicted results to achieve the objective. The model used for analysis of results can also be validated statistically to be sure of the entire process making it a very reliable and efficient approach for optimization of vesicular systems. Problems occurring during the design and development of vesicular systems can be easily traced and rectified with the help of DoE approach of optimization. There are screening designs in DoE which assist in segregating the important input variables and the not-so-important input variables. With the help of DoE, performance and characteristics of a product can be predicted even before formulating and evaluating it, making it an important tool in formulation development.

The following steps are involved in development of vesicular systems by DoE [8]:

1. Definition of objectives of study and desired characteristics.
2. Screening of factors to determine important input variables and defining of range of those variables by experimentation.
3. Selection of an appropriate experimental design based on objectives of the study, number and type of input variables, their levels and response variables. This step also involves generation of a design matrix for choosing optimal formulation.
4. Preparation of the formulations using the levels of input variables given in the design matrix and analysis of responses so obtained.
5. Analysis of experimental data obtained in step 4 by using a suitable mathematical model and determination of statistical significance of the proposed model is done. Optimal formulation is ascertained by graphical, numerical, or point prediction method.
6. Checkpoint analysis is carried out for validating the response prediction ability of the model used.
7. Scaling up of the process is done in an industrial environment and the process is made ready for production.

8.4 Types of Experimental Designs

Experimental designs can be classified based on the objectives of the study like:

1. Screening designs—These are used for identification of significant main effects rather than interaction effects. Therefore, they are also called main effect designs or orthogonal arrays. They are first-order designs with low resolution. Their main purpose is identification of influential input variables or factors having significant main effects. The number of experiments to be performed in screening designs is

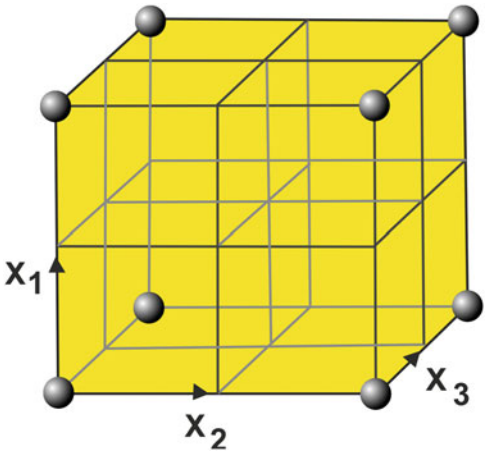

Fig. 8.2 Pictorial illustration of 2^3 full factorial design

small. Some examples of screening designs are—Plackett–Burman, Taguchi, fractional factorial design.
Factors are usually screened by varying them at two levels.

2. Response surface designs—They are used after identification of important influential factors for their optimization and for analysis of their effect on response variables. These factors are usually varied at three levels. The experimental data is fitted to obtain a mathematical model which can be represented graphically for visual representation of the influence of factors on selected responses. Hence, these designs allow calculation of main, interaction, and quadratic effects and give an idea of the shape of response surface. These can be used for maximization or minimization of a response, reducing variation in characteristics of a product, making a robust process and for hitting a target. Some examples of response surface designs are central composite design, Box–Behnken design, full factorial design, and Mixture design.

Some of the experimental designs used in formulation development of vesicular products have been discussed below [7, 8, 10]:

1. Full factorial design (FFD)—In this design all levels of a given factor are taken with each level of every other factor, thus studying the effect of all factors including interaction effect. The number of experiments in FFD is x^k where x is the number of levels and k is the number of factors. FFDs can be symmetrical, when the number of levels are the same for all factors, and asymmetrical when the number of levels are different for different factors. They are the most popular response surface designs. Figure 8.2 gives the pictorial representation of full factorial design.
2. Fractional factorial design—When a full factorial design is reduced by a fixed fraction, it becomes a fractional or partial factorial design. This is usually done

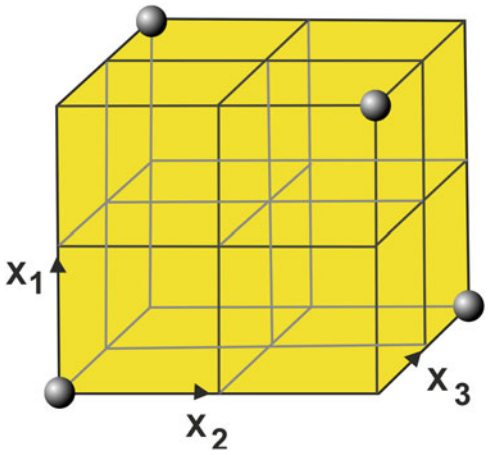

Fig. 8.3 Pictorial illustration of 2^{3-1} fractional factorial design

when use of large number of factors makes full factorial design unmanageable due to the sheer number of experiments required. Fractional factorial designs are economical as they require lesser number experiments but their ability to recognize some factor effects is compromised making them of a lesser resolution. Fractional factorial design has been diagrammatically represented in Fig. 8.3.

3. Plackett–Burman design (PBD)—This design can be called a special case of two-level FFD, recognized as a screening design. This is usually used for screening a large number of factors with minimum number of runs. In this only the main effects of factors are considered important. The number of experimental runs in this is in multiples of 4. PBD is also called Hadamard or reduced design or non-geometric design.
4. Central composite design (CCD)—These are second-order designs and possess the advantage of factorial design or FFDs and star designs. They have factorial, axial, and central points. The star points account for curvature of this design. The symbol α denotes the distance of axial point from central point. CCD may be circumscribed (having five levels for each factor bearing a circular, spherical, or hyperspherical symmetry), inscribed with each factor level divided by α, also having five levels of each factor and face centered (having three levels of each factor). Face-centered CCD has been illustrated in Fig. 8.4. They contain embedded factorial or full factorial design. Circumscribed and inscribed CCD are rotatable.
5. Box–Behnken design (BBD)—BBD is an independent quadratic design and requires three levels for each factor. It is considered economical to CCD as in CCD each factor is taken at five levels. This design is rotatable or nearly rotatable. It is widely used for optimization of various drug delivery systems. BBD has less capacity for orthogonal blocking than CCD.
6. Mixture designs (MDs)—MDs are designs useful for formulations with a large number of excipients in which characteristics of the formulations depend on the

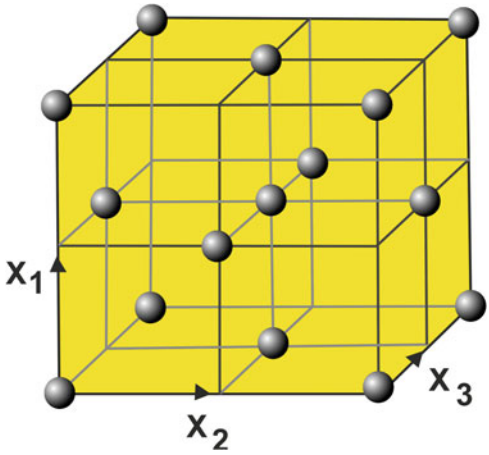

Fig. 8.4 Pictorial illustration of face-centered central composite design

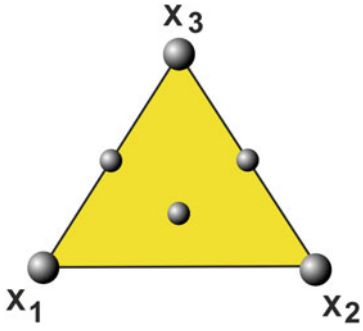

Fig. 8.5 Pictorial illustration of quadratic mixture design

proportion of excipients but not on their quantity and the sum of all proportions cannot exceed 1. Therefore in this design the components should be varied keeping in mind that the total cannot exceed 1. Thus these designs are suitable for formulation optimization and not for process optimization. Figure 8.5 has an illustration of mixture design.

7. Taguchi design (TD)—This design is used to develop robust products and processes to counteract natural variability. This design divides system variability according to sources and finds control factor setting that can generate acceptable responses. TD uses signal and noise factors. Signal factors are system controlled inputs. The response variable in this design is S/N ratio. In this the factors affecting the S/N ratio most strongly are reset for maximizing, minimizing, or targeting a given limit or range. Thus, TD is used for screening of influential variables and also for response surface modeling when the number of factors is very large.
8. Optimal designs (ODs)—ODs are non-classical custom designs and unlike other classic designs discussed above do not require much prior information. They are

used when experimental domain is irregular in shape. ODs can be based on several optimality criteria like D, A, G, I, and V. These optimality criteria are used for comparison of Fisher information matrix (FIM), which contains a summary of information of a design. The most commonly used optimal design criterion is D-criterion which is based on the principle of minimization of variance and covariance of parameters. These designs can be used along with factorial, CCD, and mixture designs and can also be used for screening factors [14].

8.5 Selection of Experimental Design

1. If the experimental objective is to select few important main effects from unimportant ones, screening designs may be used.
2. If estimation of interaction of various factors to get the shape of response is desired, response surface methodology may be used.
3. If factors are proportions of a mixture and we desire to know the best proportions to maximize or minimize, a response mixture designs may be used.
4. If mathematical function response of few continuous factor is desired, then regression design may be used.
5. No of factors also effect the choice of design, e.g. if the number of factors are 2–4, CCD or BBD may be used. For factors above 5, Plackett–Burman design may be used.
6. Resources available and degree of control over wrong decisions that the experimenter desires influence choice of design.
7. A design that requires fewer runs than the budget of the experimenter so that backup resources can be utilized for re-runs if need arises.
8. As a general rule FDs (full or fractional), PBDs, or Taguchi designs are usually are categorized as simpler designs for screening, for non-linear responses usually complex designs are desired [15].

8.6 Methods for Selection of Optimized Batch

To achieve best value of a response, appropriate experimental conditions may be determined. Optimum response could be minimum or maximum or if a single optimum value is desired to be achieved, then optimum zone may be defined within the experimental region. Some of the various methods are

(a) Graphical method: displays the area of feasible response values in the factor space. According to the relative importance of the objectives, it requires trading off of one objective over other.

(b) Numerical optimization: When there are multiple responses then mathematical model may be considered to find a feasible region [16].

8.7 Illustration of Optimization by DoE for Vesicular Systems

8.7.1 Liposome

Liposome with one or more phospholipid layer is extensively used in numerous scientific disciplines and offers the advantage of nanosize, sustained release, biocompatibility, and biodegradability. Table 8.1 highlights the key factors that influence the end user response such as encapsulation efficiency, drug loading capacity, particles' biologics, structural, and physicochemical properties.

8.7.2 Niosomes

They are composed of non-ionic surfactant and are able to encapsulate large amounts of materials in a small volume of vesicles, can entrap wide range of chemicals (hydrophilic, lipophilic, and amphiphilic drugs), and provide controlled and sustained release of drugs. Niosomes claim to fame is its higher chemical stability and lower cost. The vital attributes that impact the final product characteristics have been listed in Table 8.2.

8.7.3 Ethosome

These are malleable and elastic vesicles that offer advantage of greater penetration, effective release across various layers of the skin. They are primarily composed of phospholipids, ethanol (up to 45%), glycerol, and water. Table 8.3 details the variables that impacts the final ethosomal composition of the formulations.

8.7.4 Transferome

These are also known as modified liposomes. It consists of edge activator that imparts vesicle fluidity. High entrapment efficiency and greater penetrability are the benefits offered by them. Table 8.4 offers us a view of the interplay between important variable factors and final robust product objectives.

Table 8.1 DoE optimization of liposome

Drug	Factor/(s)	Design	Response/(s)	Year
Prednisolone	Drug concentration, phospholipid ratio	D-optimal design	%EE (Percent entrapment efficiency), size	2018 [17]
Pingyangmycin	Glycerophosphate disodium content, chitosan content, drug content	3 Factorial BBD	% drug release in 1 day, 9 day, rate constant	2018 [18]
TerbinafineHCl	Drug to lipid ratio, lipid to cholesterol molar ratio, temperature of rotary evaporator, speed of rotary evaporator, film rehydration, fluid volume, rehydration time, amplitude of sonication, and sonication time	BBD	Size, PDI (Polydispersity index), zeta potential, % EE	2018 [19]
Temozolomide	Phospholipid molar ratio, organic phase	3^2 Factorial design	Size, % EE	2018 [20]
Pravastatin	Phospholipid: Cholesterol ratio, phospholipid molar concentration, drug concentration, temperature used for hydration of lipid film, temperature used in extrusion step, rotation speed at hydration of film	D-optimal design	Size, PDI, %EE, zeta potential, residual moisture content, glass transition temperature, drying time, macroscopic cake appearance	2017 [21]
Doxorubicin and curcumin	Phospholipid concentration, phospholipid: cholesterol molar ratio, curcumin concentration, doxorubicin concentration, working temperature, and pH of the buffer	2^{6-2} Factorial design	Encapsulated drug concentration, % EE, size, zeta potential	2017 [22]
Coenzyme Q10- and D-panthenyl triacetate	Cholesterol to phosphatidylcholine ratio, each drug to phosphatidylcholine ratio	CCD-RSM	Entrapment efficiency of coenzyme Q10 and of D-panthenyl triacetate	2017 [23]
Simvastatin	PEG proportion, cholesterol	RSM	Liposomal size, drug concentration in the	2017 [24]

(continued)

Table 8.1 (continued)

Drug	Factor/(s)	Design	Response/(s)	Year
	concentration, cryoprotectant to phospholipids molar ratio, number of extrusions through 100 nm polycarbonate membranes and freezing conditions prior lyophilization		freeze-dried product, encapsulated simvastatin retention, residual moisture content, and change in the phospholipid's transition temperature	
Methotrexate	Drug lipid, drug cholesterol ratio, drug surfactant ratio	CCD	Drug entrapment	2016 [25]
Clodronate	Phosphatidylcholine to cholesterol ratio, lipid component to active substance ratio, and sonication time	BBD-RSM	Drug encapsulation efficiency and size	2016 [26]
Paclitaxel and lapatinib	Drug to phospholipid ratio, cholesterol content, phospholipid type	D-optimal design-RSM	% EE and size	2015 [27]
Quercetin	Temperature during preparation, rotation speed of rotary evaporator	3-level factorial-RSM	Drug release, mean particle size diameter, entrapment efficiency	2014 [28]
–	Variation in the lipid content	Simplex centroid design	Size, transition temperature, z-potential, fluidity, and entrapment efficiency (calcein)	2012 [29]
Paeonol	Cholesterol concentration, molar ratio of lipid/drug, and the polymer concentration	BBD	Drug encapsulation efficiency, flux and viscosity of the gels	2012 [30]
Peptide	Peptide concentration, lipid concentration, number of freeze-thawing cycles, and mixing time	CCD-face centered	Encapsulation efficiency	2010 [31]

Table 8.2 DoE optimization of niosomes

Drug	Factor/(s)	Design	Response/(s)	Year
Nevirapine	Cholesterol and surfactant content, hydration time, and temperature	BBD-RSM	Size, %EE, PDI, drug release at 48 h	2019 [32]
Zolmitriptan	Different ratio of surfactant	BBD	Size, %EE, PDI, zeta potential, release after 4 h	2019 [33]
Brimonidine tartrate	Amount of surfactant, ratio of surfactant: cholesterol, type of surfactant	D-optimal design	Size, %EE, zeta potential, PDI, % drug release after 2 h, 8 h, 24 h	2019 [34]
Natamycin	Amount of surfactant, cholesterol, drug concentration	BBD	Size, %EE, zeta potential	2019 [35]
BuspironeHCl	Concentration of surfactant, cholesterol	3^2 Factorial design	Size, %EE	2018 [36]
Diacerein	Amount of salt in hydration medium, lipid amount, and number of surfactant parts	CCD	EE%, size, PDI, zeta potential	2018 [37]
NefopamHCl	Cholesterol: Surfactant ratio and surfactant type	4^2 Full factorial design	EE%, size, cumulative percent released after 8 h, cumulative amount of drug permeated after 24 h per 1 cm^2 of nasal mucosa, permeation coefficient of drug across nasal mucosa	2018 [38]
Pregabalin	Water required for film hydration, surfactant: cholesterol molar ratio	Full factorial design	Size, drug release, and entrapment efficiency	2017 [39]
Methotrexate	Amount of cholesterol, surfactant, short chain alcohol	BBD	Size, %EE, zeta potential	2017 [40]
Lacidipine	Surfactant, cholesterol concentration, hydration time, sonication time	BBD	Size, %EE, flux	2017 [41]
Acyclovir	Surfactant ratio, cholesterol: lecithin ratio	3^2 Factorial design	Vesicle size, EE%, % drug accumulated in the stratum corneum	2016 [42]
Diacerein	Cholesterol, surfactant, hydration time	BBD	Size, %EE, PDI	2016 [43]
Ursolic acid	Cholesterol, surfactant, phospholipid	BBD	Size, %EE, transflux	2015 [44]

(continued)

Table 8.2 (continued)

Drug	Factor/(s)	Design	Response/(s)	Year
Methotrexate	Drug concentration in hydration medium, total weight of niosomal components, and surfactant: cholesterol ratio	BBD	Encapsulation efficiency percent, particle size	2015 [45]
Morin hydrate	Amount of drug, surfactant, cholesterol, diacetyl phosphate	Taguchi orthogonal array (TOA)	Size, %EE, zeta potential	2013 [46]
Sumatriptan succinate	Drug amount, surfactant type, surfactant: cholesterol ratio, hydration time, stearyl amine amount	Taguchi	Vesicle size, zeta potential, and drug entrapment.	2012 [47]
Carvedilol	Cholesterol content, weight of proniosomes, and amount of drug	2^3 Full factorial design	% EE, size, microscopic examination	2010 [48]

Table 8.3 DoE optimization of ethosome

Drug	Factor/(s)	Design	Response/(s)	Year
Thymosin β-4	Surfactant concentration, ethanol concentration, hydration speed, hydration temperature, hydration time, water injection speed	Orthogonal design	%EE, size	2019 [49]
Vismodegib	Concentration of phospholipid, cholesterol, isopropyl alcohol/total alcohol content	BBD	% EE, vesicle size, % release, and steady-state flux	2019 [50]
Etodolac	Amount of lipid, amount of cholesterol	3^2 Factorial design	Size, in vitro drug release, % EE	2019 [51]
Fisetin	Phospholipid 90G, ethanol, propylene glycol	BBD	Size, %EE, flux	2019 [52]
Paeonol	Amount of cholesterol, ethanol, and phosphatidylcholine	CCD	% EE, zeta potential, PDI, size, overall desirability	2018 [53]
Paeoniflorin	PC mass, mass ratio of drug and PC, water phase pH	Orthogonal design	Size, %EE, zeta potential, PDI, morphology	2018 [54]

(continued)

Table 8.3 (continued)

Drug	Factor/(s)	Design	Response/(s)	Year
TropisetronHCl	Concentration of phosphatidylcholine, ethanol, and phosphatidylcholine type	3×2^2 Full factorial design	Size, %EE, zeta potential, PDI	2017 [55]
Eletriptan hydrobromide	Concentration of soya lecithin and ethanol	3^2 Factorial design	Size, %EE	2016 [56]
Zolmitriptan	Concentration of soy lecithin and ethanol	3^2 Factorial design	Size, %EE	2016 [57]
Methoxsalen	Amount of phospholipid and ethanol	CCD	Size, % PDE (Percent drug entrapment), %PDL (Percent drug loading), flux, and skin deposition	2015 [58]
Tramadol	Phospholipon 90G, ethanol, sonication time	BBD	Size, %EE, flux	2015 [59]
Diclofenac	PC (Phosphatidyl choline): cholesterol ratio, ethanol concentration	4×5 full factorial design	Size, zeta potential, % EE, elasticity	2014 [60]
Clotrimazole	Cyclodextrin concentration, lecithin concentration	3^2 Factorial design	Size, %EE, zeta potential, PDI	2012 [61]
Repaglinide	Phosphatidylcholine, ethanol, and water concentration	3^2 Factorial design	Size, %EE, ex vivo permeation	2012 [62]

Table 8.4 DoE optimization of transferosome

Drug	Factor/(s)	Design	Response/(s)	Year
Resveratrol	Ratio of PC: penetration enhancer (PE), ratio of PC and PE to surfactant, type of surfactant, penetration enhancer	3^4 definitive screening design	Size, %EE, in vitro release	2019 [63]
Lidocaine	Lipid type, surfactant type, lipid: surfactant ratio	Taguchi design	Size, %EE, zeta potential, PDI	2019 [64]
Felodipine	Edge activator, its molar ratio to phosphatidylcholine, and presence or absence of cholesterol	$2^2 \times 4$ full factorial design	Entrapment efficiency, size, polydispersity index, zeta potential, and percent drug released after 8 h	2018 [65]

(continued)

Table 8.4 (continued)

Drug	Factor/(s)	Design	Response/(s)	Year
Loratadine	Ratio of lipid: edge activator, sonication time	Simplex centroid design	Size, %EE	2017 [66]
Sildenafil	Drug: phospholipid molar ratio, phospholipid: Surfactant ratio, HLB balance, hydration medium, time, temperature	Plackett–Burman design	Size, %EE	2014 [67]
BuspironeHCl	Concentration of lipid, ethanol	3^2 Factorial design	Size, %EE, PDI, zeta potential	2013 [68]
Insulin	Ratio of lipids, Ratio of lipids: Surfactants, and ratio of surfactants	2^3 Factorial design	Size, %EE, in vitro permeation flux	2012 [69]
Valsartan	Amount of phospholipid 90G, drug, surfactant sonication time	BBD	Size, %EE, flux	2012 [70]

References

1. Jain S, Jain V, Mahajan SC (2014) Lipid based vesicular drug delivery systems. Adv Pharm 2014:574673
2. Zylberberg C, Matosevic S (2016) Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv 23(9):3319–3329
3. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S (2015) Advances and challenges of liposome assisted drug delivery. Front Pharmacol 6:1–13
4. Bhardwaj P, Tripathi P, Gupta R, Pandey S (2020) Niosomes: a review on niosomal research in the last decade. J Drug Deliv Sci Technol 56:101581–101594
5. Singh D, Pradhan M, Nag M et al (2014) Vesicular system: versatile carrier for transdermal delivery of bioactives. Artif Cells Nanomed Biotechnol 43:282–290
6. Abdulbaqi IM, Darwis Y, Khan NAK et al (2016) Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Int J Naomed 11:2279–2304
7. Djuris J (2013) Computer-aided applications in pharmaceutical technology. Woodhead Publishing, New Delhi
8. Singh B, Kumar R, Ahuja N (2005) Optimizing drug delivery systems using systematic "design of experiments." part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst 22(1):27–105
9. Bhoop BS (2014) Quality by Design (QbD) for holistic pharma excellence and regulatory compliance. Pharma times 46:26–35

10. Pramod K, Tahir MA, Charoo MA et al (2016) Pharmaceutical product development: A quality by design approach 6:129–139
11. Lewis GA, Mathieu D, Phan-Tan-Luu R (eds) (1999) Pharmaceutical Experimental Design. Marcel Dekker, New York
12. Myers RH, Montgomery DC (eds) (1995) Response Surface Methodology: Process and Product Optimization using Designed Experiments. Wiley, New York
13. Bolton S, Bon C (2004) Pharmaceutical statistics: practical and clinical applications. Marcel Dekker, New York
14. Shahmohammadi A, McAuley KB (2019) Sequential model-based A- and V-optimal design of experiments for building fundamental models of pharmaceutical production processes. Computer Chem Engg 129:1–15
15. Singh B, Kumar R, Ahuja N (2005) Optimizing drug delivery systems using ``Design of Experiments.'' Part I: Fundamental aspects. Crit Rev Ther Drug Carrier Sys 22:27–105
16. Singh B, Bhatowa R, Tripathi CB et al (2011) Developing micro-/nanoparticulate drug delivery systems using "design of experiments. Int J Pharmaceutical Investigation 1(2):75–89
17. Sylvester B, Porfire A, Muntean DM, Vlase L, Lupuț L, Licarete E, Sesarman A, Alupei MC, Banciu M, Achim M, Tomuță I (2018a) Optimization of prednisolone-loaded long-circulating liposomes via application of quality by design (QbD) approach. J Liposome Res 28(1):49–61
18. Zhang L, Chen F, Zheng J, Wang H, Qin X, Pan W (2018) Chitosan-based liposomal thermogels for the controlled delivery of pingyangmycin: design, optimization and in vitro and in vivo studies. Drug Deliv 25(1):690–702
19. Shah VH, Jobanputra A (2018) Enhanced ungual permeation of terbinafineHCl delivered through liposome-loaded nail lacquer formulation optimized by QbD approach. AAPS PharmSciTech 19(1):213–224
20. Vanza J, Jani P, Pandya N, Tandel H (2018) Formulation and statistical optimization of intravenous temozolomide-loaded PEGylated liposomes to treat glioblastoma multiforme by three-level factorial design. Drug Dev Ind Pharm 44(6):923–933
21. Sylvester B, Porfire A, Achim M, Rus L, Tomuță I (2018b) A step forward towards the development of stable freeze-dried liposomes: a quality by design approach (QbD). Drug Dev Ind Pharm 44(3):385–397
22. Tefas LR, Sylvester B, Tomuta I, Sesarman A, Licarete E, Banciu M, Porfire A (2017) Development of antiproliferative long-circulating liposomes co-encapsulating doxorubicin and curcumin, through the use of a quality-by-design approach. Drug Des Devel Ther 11:1605
23. Çelik B, Sağıroğlu AA, Özdemir S (2017) Design, optimization and characterization of coenzyme Q10-and D-panthenyl triacetate-loaded liposomes. Int J Nanomedicine 12:4869
24. Porfire A, Muntean DM, Rus L, Sylvester B, Tomuță I (2017) A quality by design approach for the development of lyophilized liposomes with simvastatin. Saudi Pharm J 25(7):981–992
25. Vanaja K, Shobha Rani RH, Narendra C (2016) Application of statistical designs in the formulation of lipid carriers of methotrexate. J Drug Res Dev 2(2):2470–1009
26. Ailiesei I, Anuta V, Mircioiu C, Cojocaru V, Orbesteanu AM, Cinteza LO (2016) Application of statistical design of experiments for the optimization of clodronate loaded liposomes for oral administration. Rev Chim 67(8):1566–1570
27. Ravar F, Saadat E, Kelishadi PD, Dorkoosh FA (2016) Liposomal formulation for co-delivery of paclitaxel and lapatinib, preparation, characterization and optimization. J Liposome Res 26 (3):175–187
28. Jangde R, Singh D (2016) Preparation and optimization of quercetin-loaded liposomes for wound healing, using response surface methodology. Artif Cells Nanomed Biotechnol 44 (2):635–641
29. Maherani B, Arab-Tehrany E, Kheirolomoom A, Reshetov V, Stebe MJ, Linder M (2012) Optimization and characterization of liposome formulation by mixture design. Analyst 137 (3):773–786

30. Shi J, Ma F, Wang X, Wang F, Liao H (2012) Formulation of liposomes gels of paeonol for transdermal drug delivery by Box-Behnken statistical design. J Liposome Res 22(4):270–278
31. Ducat E, Brion M, Lecomte F, Evrard B, Piel G (2010) The experimental design as practical approach to develop and optimize a formulation of peptide-loaded liposomes. AAPS PharmSciTech 11(2):966–975
32. Witika BA, Walker RB (2019) Development, manufacture and characterization of niosomes for the delivery for nevirapine. Die Pharmazie 74(2):91–96
33. Mohamed MI, Abdelbary AA, Kandil SM, Mahmoud TM (2019) Preparation and evaluation of optimized zolmitriptan niosomal emulgel. Drug Dev Ind Pharm 45(7):1157–1167
34. Emad Eldeeb A, Salah S, Ghorab M (2019) Proniosomal gel-derived niosomes: an approach to sustain and improve the ocular delivery of brimonidine tartrate; formulation, in-vitro characterization, and in-vivo pharmacodynamic study. Drug Deliv 26(1):509–521
35. Verma A, Sharma G, Jain A, Tiwari A, Saraf S, Panda PK, Katare OP, Jain SK (2019) Systematic optimization of cationic surface engineered mucoadhesive vesicles employing design of experiment (DoE): a preclinical investigation. Int J Biol Macromol 133:1142–1155
36. Mathure D, Madan JR, Gujar KN, Tupsamundre A, Ranpise HA, Dua K (2018) Formulation and evaluation of niosomal in situ nasal gel of a serotonin receptor agonist, buspirone hydrochloride for the brain delivery via intranasal route. Pharm Nanotechnol 6(1):69–78
37. Aziz DE, Abdelbary AA, Elassasy AI (2018) Implementing central composite design for developing transdermal diacerein-loaded niosomes: ex vivo permeation and in vivo deposition. Curr Drug Deliv 15(9):1330–1342
38. Abou-Taleb HA, Khallaf RA, Abdel-Aleem JA (2018) Intranasal niosomes of nefopam with improved bioavailability: preparation, optimization, and in-vivo evaluation. Drug Des Devel Ther 12:3501
39. Arafa MG, Ayoub BM (2017) DOE optimization of nano-based carrier of pregabalin as hydrogel: new therapeutic & chemometric approaches for controlled drug delivery systems. Sci Rep 7:41503
40. Zidan AS, Mokhtar Ibrahim M, Megrab NA (2017) Optimization of methotrexate loaded niosomes by Box–Behnken design: an understanding of solvent effect and formulation variability. Drug Dev Ind Pharm 43(9):1450–1459
41. Qumbar M, Imam SS, Ali J, Ahmad J, Ali A (2017) Formulation and optimization of lacidipine loaded niosomal gel for transdermal delivery: in-vitro characterization and in-vivo activity. Biomed Pharmacother 93:255–266
42. Jacob S, Nair AB, Al-Dhubiab BE (2017) Preparation and evaluation of niosome gel containing acyclovir for enhanced dermal deposition. J Liposome Res 27(4):283–292
43. Moghddam SR, Ahad A, Aqil M, Imam SS, Sultana Y (2016) Formulation and optimization of niosomes for topical diacerein delivery using 3-factor, 3-level Box-Behnken design for the management of psoriasis. Mater Sci Eng C 69:789–797
44. Jamal M, Imam SS, Aqil M, Amir M, Mir SR, Mujeeb M (2015) Transdermal potential and anti-arthritic efficacy of ursolic acid from niosomal gel systems. Int Immunopharmacol 29 (2):361–369
45. Abdelbary AA, AbouGhaly MH (2015) Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: application of Box–Behnken design, in-vitro evaluation and in-vivo skin deposition study. Int J Pharm 485(1–2):235–243
46. Waddad AY, Abbad S, Yu F, Munyendo WL, Wang J, Lv H, Zhou J (2013) Formulation, characterization and pharmacokinetics of Morin hydrate niosomes prepared from various non-ionic surfactants. Int J Pharm 456(2):446–458
47. González-rodríguez ML, Mouram I, Cózar-bernal MJ, Villasmil S, Rabasco AM (2012) Applying the Taguchi method to optimize sumatriptan succinate niosomes as drug carriers for skin delivery. J Pharm Sci 101(10):3845–3863

48. Aboelwafa AA, El-Setouhy DA, Elmeshad AN (2010) Comparative study on the effects of some polyoxyethylene alkyl ether and sorbitan fatty acid ester surfactants on the performance of transdermal carvedilol proniosomal gel using experimental design. AAPS PharmSciTech 11 (4):1591–1602
49. Fu X, Shi Y, Wang H, Zhao X, Sun Q, Huang Y, Qi T, Lin G (2019) Ethosomal gel for improving transdermal delivery of thymosin β-4. Int J Nanomedicine 14:9275
50. Sayed OM, El-Ela FI, Kharshoum RM, Salem HF (2020) Treatment of basal cell carcinoma via binary Ethosomes of Vismodegib: in vitro and in vivo studies. AAPS PharmSciTech 21(2):51
51. Madhavi N, Sudhakar B, Reddy KN, Ratna JV (2019) Design by optimization and comparative evaluation of vesicular gels of etodolac for transdermal delivery. Drug Dev Ind Pharm 45 (4):611–628
52. Moolakkadath T, Aqil M, Ahad A, Imam SS, Praveen A, Sultana Y, Mujeeb M, Iqbal Z (2019) Fisetin loaded binary ethosomes for management of skin cancer by dermal application on UV exposed mice. Int J Pharm 560:78–91
53. Ma H, Guo D, Fan Y, Wang J, Cheng J, Zhang X (2018) Paeonol-loaded ethosomes as transdermal delivery carriers: design, preparation and evaluation. Molecules 23(7):1756
54. Cui Y, Mo Y, Zhang Q, Tian W, Xue Y, Bai J, Du S (2018) Microneedle-assisted percutaneous delivery of paeoniflorin-loaded ethosomes. Molecules 23(12):3371
55. Abdel Messih HA, Ishak RA, Geneidi AS, Mansour S (2017) Nanoethosomes for transdermal delivery of tropisetronHCl: multi-factorial predictive modeling, characterization, and ex vivo skin permeation. Drug Dev Ind Pharm 43(6):958–971
56. Shelke S, Shahi S, Jadhav K, Dhamecha D, Tiwari R, Patil H (2016a) Thermoreversible nanoethosomal gel for the intranasal delivery of Eletriptan hydrobromide. J Mater Sci Mater Med 27(6):103
57. Shelke S, Shahi S, Jalalpure S, Dhamecha D (2016b) Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: formulation, optimization, evaluation and permeation studies. J Liposome Res 26(4):313–323
58. Garg BJ, Garg NK, Beg S, Singh B, Katare OP (2016) Nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: formulation optimization, in vitro evaluation and preclinical assessment. J Drug Target 24(3):233–246
59. Ahmed S, Sarim Imam S, Zafar A, Ali A, Aqil M, Gull A (2016) In vitro and preclinical assessment of factorial design based nanoethosomes transgel formulation of an opioid analgesic. Artif Cells Nanomed Biotechnol 44(8):1793–1802
60. Jain S, Patel N, Madan P, Lin S (2015) Quality by design approach for formulation, evaluation and statistical optimization of diclofenac-loaded ethosomes via transdermal route. Pharm Dev Technol 20(4):473–489
61. Akhtar N, Pathak K (2012) Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. AAPS PharmSciTech 13 (1):344–355
62. Bodade SS, Shaikh KS, Kamble MS, Chaudhari PD (2013) A study on ethosomes as mode for transdermal delivery of an antidiabetic drug. Drug Deliv 20(1):40–46
63. Salem HF, Kharshoum RM, Abou-Taleb HA, Naguib DM (2019) Nanosized transferosome-based intranasal in situ gel for brain targeting of resveratrol: formulation, optimization, in vitro evaluation, and in vivo pharmacokinetic study. AAPS PharmSciTech 20(5):181
64. Bnyan R, Khan I, Ehtezazi T, Saleem I, Gordon S, O'Neill F, Roberts M (2019) Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic. J Pharm Pharmacol 71(10):1508–1519
65. Kassem MA, Aboul-Einien MH, El Taweel MM (2018) Dry gel containing optimized felodipine-loaded transferosomes: a promising transdermal delivery system to enhance drug bioavailability. AAPS PharmSciTech 19(5):2155–2173
66. Elkomy MH, El Menshawe SF, Abou-Taleb HA, Elkarmalawy MH (2017) Loratadine bioavailability via buccal transferosomal gel: formulation, statistical optimization, in vitro/in vivo characterization, and pharmacokinetics in human volunteers. Drug Deliv 24(1):781–791

67. Ahmed TA (2015) Preparation of transfersomes encapsulating sildenafil aimed for transdermal drug delivery: Plackett–Burman design and characterization. J Liposome Res 25(1):1–10
68. Shamma RN, Elsayed I (2013) Transfersomal lyophilized gel of buspironeHCl: formulation, evaluation and statistical optimization. J Liposome Res 23(3):244–254
69. Malakar J, Sen SO, Nayak AK, Sen KK (2012) Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. Saudi Pharm J 20(4):355–363
70. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M, Ali A (2012) Formulation and optimization of nanotransfersomes using experimental design technique for accentuated transdermal delivery of valsartan. Nanomedicine 8(2):237–249

Design of Experiments for the Development of Nanoparticles, Nanomaterials, and Nanocomposites

9

Md Noushad Javed, Faheem Hyder Pottoo, Athar Shamim, Md Saquib Hasnain, and Md Sabir Alam

Abstract

Due to the promises of nanoparticles, nanomaterials, and nanocomposites, the development of such materials emerges as an extensive area of research for material scientists. However, conventional manufacturing processes of these materials at the nanoscale dimensions for industries are usually irreversible, highly expensive, and having many critical process affecting parameters. Subsequent to this, empirical approaches which were extensively exploited to test quality performances of the finished products are inefficacious; as well as failed to maintain predefined qualities, which are however critical requisite.

Hence, systemic integration of design of experiment (DoE) based approach to design experiments and to control process parameters yield optimized final products to help researchers to maintain predefined product quality, in a very scientific manner. Different statistical parameters and response surface method (RSM) based approaches are important characteristics and serve as assistive tools to enable modeling complexed relationships into some mathematical formulae. So, modeling of these independent process variables within the domain of "Design space" enable products with quality characteristics; with minimal efforts, money, and inter-prouct variations in performances.

M. N. Javed (✉) · A. Shamim
Quality Assurance Lab, Department of Pharmaceutics, SPER, Jamia Hamdard, New Delhi, India
e-mail: noushad.sch@jamiahamdard.ac.in

F. H. Pottoo
Department of Pharmacology, College of Clinical Pharmacy, Imam Abdul Rahman Bin Faisal University, Dammam, Saudi Arabia

M. S. Hasnain
Department of Pharmacy, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

M. S. Alam
SMAS, KR Mangalam University, Gurgram, Haryana, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_9

This chapter discusses the introductory concepts of DoE principles in relationship to nanoparticles, nanomaterials and nanocomposites.

Keywords

Nanotechnology · Quality by Design QbD · Optimization · Nanoparticles · Design of Experiment DoE · Nanocomposite · Experimental design

9.1 Introduction to Nanotechnology

"Nano" is Greek equivalent of English word "dwarf", and Feynman was the first person who introduced concepts of nanotechnology to broach the possibility of manipulating materials at the individual atomic or molecular levels [1]. However, Professor Norio Taniguchi was the first person who defined nanotechnology as a method for processing, separation, consolidation, and deformation of materials; at the level of atomic or molecular scales [2]. In another way, we may formally define nanotechnology as engineered manipulation of functional systems at atomic and molecular scale levels [3]. Modern approaches which are required for the development of functionalized components with desired attributes regardless of process by which these are assembled into different type of systems [4]. Usually, interdisciplinary researchers exploit the unique physical and electronic properties of these nanomaterials to create unique functional attributes within newly fabricated systems [5]. With the advancement, these nanotechnology-based approaches yield to propose an important agenda to inquiry progress, not only in the domain of devices and implants however most promising applications include drug delivery and biomedical purposes [6].

9.1.1 Types of Nanotechnology-Based Systems

Nanomaterials are being developed by processing bulk form of materials at the nanoscale dimension, before their fabrication into conventional systems, into single independent constituting block units [7]. So, development of particulated type of capsular or matrix-based systems at nanoscale dimension, lead to emergergence of concepts for nanoparticles [8]. Subsequently, in 1961, Blumstein further conceptualized nanocomposites as heterogeneous multiphase solid materials which are processed at the nanoscale levels, before their actual reinforcing into composites [9]. To improve thermal stability related properties, Blumstein developed polymethyl methacrylate (PMMA)–layered silicate nanocomposites in 1965, via polymerization of methyl methacrylate (MMA) with silica through exploitation of free radical based reaction mechanism [10]. Nature has designed many biological systems in such a well-planned and synchronized ways that their chemical forces and physical attributes would serve essential characteristics towards structures and functions of in living organisms [11]. Hence, efforts are being made by scholars to

discover the purpose of nature's towards productions of some specific self-assembled units and small clusters of structures; in well-defined manners [12]. Such systemic processes are commonly inscribed as bioinspired systems, while such materials are inscribed as bioinspired materials [13]. Meanwhile, the concepts of nanobiotechnology also emerged due to integration of nanotechnology aspects either with biological materials or processes. Here, emphasis is given to elucidate how nanotechnology processed materials behave with living systems [14]. Another important term biomimetics also came into existence, with the advancement of understanding about phenomena and purposes of biomaterials in biological systems. Followed by this; bulk form of materials are judiciously processed at nanoscale dimensions in such a way that these would ultimately lead into emergence of unique and promising attributes as desired functions in biological systems [15]. Although biomimetic systems are not equivalent to mere copying of natural architects, rather these systems integrate inbuilt principles of nature within the processed materials and systems to mimick biomaterials [16]. In contrast to bulk form of materials; processes of synthesizing nanomaterials would ultimately favor improved physical, chemical, and biological capabilities; so being exploited as a diagnostic aid to study cellular biomarkers [17, 18]. Hence, such desired modulations in attributes and performances are bearing promising applications in electronic devices for biomedical applications and implants. Along with above; these are also serving important roles in advanced drug delivery applications, especially for the management of neurological disorders which are highly cross-linked and utilize rationalized combinations [19–22].

9.2 Concept of Quality Systems in Nanotechnology

"Quality can be built into the product rather than periodic testing" [23]. So to popularize the culture of implementing quality practices into the manufacturing process of developing final finished products; American engineer J.M. Juran (1970s) popularized principle of "Quality Trilogy" in his very famous book "Juran on Quality by Design" [24].

In pharmaceutical industries, due to issues of improper understanding on product attributes which affect final performances, substandard quality of raw materials, poor insights on process variables, lack of information on impacts of unit operations; process for delivering of final products and their performances are facing severe challenges [25]. Quality in some of pharmaceutical finished products and their manufacturing standards are so poor that these products are often reported with disturbing incidents of withdrawal from market. Vary famous *The Wall Street Journal* quoted (Sept 2002) "although the pharmaceutical industry has a little secret as it invents futuristic new drugs, yet its manufacturing standards are lag far behind the potato chips and laundry soap makers" [26]. Subsequently, due to continuous facing of criticism by the inefficient regulatory agencies over poor maintenance of quality standard in finished pharmaceutical products, United States Food and Drug Administration (USFDA) started emphasizing on quality as a

prime vision [27]. So, in the year of 2004, paradigm shifted with the publication of a concept literature "Pharmaceutical cGMP for 21st Century," which revolutionized and motivated industries to integrate quality concepts within the final pharmaceutical products for forever [26]. However, although well before integration of QbD in pharmaceutical sectors, it was quite interesting to ascertain fact that the concept of QbD was already, as early as of 1990, integrated with manufacturing operation successfully as a principle by leading medical device manufacturers, [28]. Furthermore, over the time concepts of QbD in pharmaceutical industries not merely emerged as a alternative and efficient tool; rather popularized as a preferred choice, in exponential manners [29]. In subsequent year, i.e., in the year of 2005; another attempt was made to integrate values of enabling a culture of quality systems with the released guidances of USFDA. Along with this, "Quality Systems Approach to Pharmaceutical CGMP Regulations" further emphasized on different aspects such as, how chemistry, manufacturing process control as well as their level if not controlled, would impact those attributes of final products which are relevant to both safety and efficacy of final finished products [30]. Hence, establishment and percolation of QbD concepts in different manufacturing sectors and technology-driven areas such as telecommunication and aviation industries already yielded many high-quality products and services, so neither DoE nor QbD a buzz or alien to pharmaceutical industries nowdays [31].

9.3 DoE Approached QbD Methodology

Performances meeting quality standards can only be integrated within the final products by judicious integration of scientifically developed plans, not by the chance [32]. Furthermore, global pharmaceutical industries being a highly regulated sector, are always striving hard to enable qualities within finished products to match predefined standards, i.e. minimal variability within finished products [33]. While these final finished products of such industries are intended for use in human. While, any variability from quality standard severely affect performances, in terms of pharmacotherapeutic benefits [34]. Subsequently, such issues lead into deviations in final performance of developed products. These concerns are more critical especially with the drugs of low potency as well as narrow therapeutic indexes. A poorly process control would develop products which would failed to manage diverse range of ailments [35]. So, regulatory agencies such as ICH and USFDA conceptualized the word "QbD" to achieve goals by implementation of judicious strategies, during the process of product development [36]. Hence, QbD already abled to gain sufficient attentions of global pharmaceutical industries because such developed pharmaceutical products best match with inbuilt quality standards, owing to Design of Experiment (DoE) based tools and robust design-based control over the process [37]. Briefly, DoE-based approaches integrate associated critical risk factors of QbD using appropriately chosen architects of design with optimal resolutions [38].

9.3.1 Philosophy of DoE in Nanotechnology

Although QbD is a holistic methodology that is being mainly achieved by identifying associated risk factors, followed by the selection of suitable experimental design of good degree of resolution, so QbD is often being interchanged with DoE, as well [15]. DoE is an inevitable tool to implement the methodology of QbD in the pharmaceutical industry [31]. DoE-based approaches enable better understanding of risk factors and their impacts on those attributes which lead to achieve predefined objectives of QbD *i.e.,* zero-quality defects and insignificant quality crisis. Such approaches also provide thoughtful philosophical flexibility to researchers for the development of a quality products, using conscious intent as well as by equipping them with intellects to execute manufacturing operations [30]. Ultimately DoE-based products by improving quality sense within the final products, improvement of overall inbuilt values as well as reduced consumer skepticism on products [26].

9.3.2 Concept of DoE in Quality Systems of Nanotechnology

In nanotechnology industry, among the major approaches to fabricate materials at nanotechnology scale levels, first approach is bottom-up approach, where different types of chemical or physical forces are used to process and fabricate materials in such a way that assembling of basic units led into production of newly synthesized materials *i.e.*, nanoscale structures [39]. If any bottom-up approach is being inspired from nature or similar to the functioning of biological systems, then such process is often pronounced as bioinspired nanotechnology-based process [13]. In other words, if source of inspiration in bottom-up approaches, where materials are being manipulated at nanoscale levels by associating their units, is already present in nature or part of any biological system; then being called bioinspired nanotechnology-based process [16]. In contrast to bottom-up process which requires assembling of units; the second approach *i.e.*, top-down approach is a well patterned and built in the same place; where bulky materials generate and replicate different structures at nanoscale levels, in a fashion which is similar to "carving" smaller objects. For example, fabrication methods are similar to process where short-wavelength light-mediated lithographic pattern techniques involved [11]. Purpose of integrating of DoE-based approaches is to implement strategies for better understanding of risk factors and their impacts on attributes. Such approaches lead into achievement of predefined objectives of quality within the products *i.e.*, minimal quality of defects and quality crisis incidences [17]. DoE-based outcomes also provide thoughtful philosophical flexibility to researchers for the development of a quality product through conscious intents as well as equipping them with intellects to execute manufacturing operations [15]. However, any DoE-based approach would serve best purposes, only if associated risk factors were already accessed thoroughly before [35]. If in any design emphasis is given toward low-risk factors rather than high or critical risk factors, then even attempts to control process with

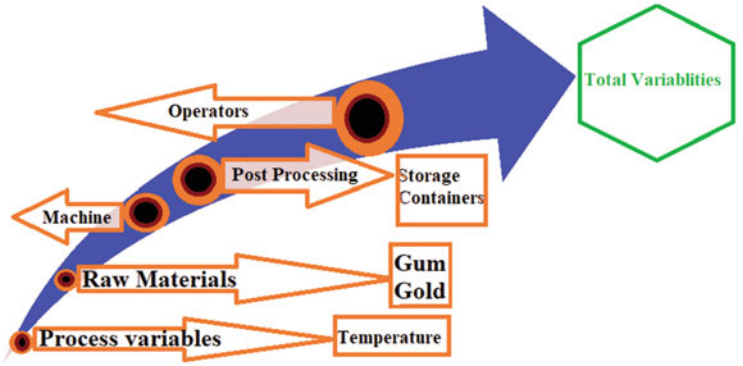

Fig. 9.1 Total source of variability in the synthesis of gold nanoparticles

higher-resolution design would give improper results [16]. DoE strategies include improvement of manufacturing standards as well as the establishment of quality-centered work environment [27]. DoE-based products by the way of improved quality sense within final products are ultimately improved overall in-built performance values as well as reduce consumer skepticism on products [39] (Fig. 9.1).

9.4 Fundamental of Quality Principles in Nanotechnology

Inconsistent quality related issues in products and robustness challenges with different processes are major concerns which may affect achieving pre-defined objectives in final products [40]. As per ICH Q8-10 guidelines which act in tandem manners; variability issues with final products consequence into high rate of failures to match pre-defined objectives [23]. Such failures to match objectives or deviations in final performances would ultimately lead an undesired high degree of variability in the final performances. Briefly, reliability of the QbD processes usually depend upon how well different sources of variability in different unit operations were being considered as a associated risk factor, along with efforts made to estimate their impacts at different levels [41]. So, different processes are required to frame objectives for the development of QbD based methodology using DoE-based approach for optimal performing final products [42]. Exercise of DoE hence involves understanding of key elements as well as judicious integration of risk-based scientific approaches to address complicated product variability issues in simplified manners [43]. Such efforts ultimately potentiate level of trust within the product about their quality as well as performances [44]. Quality of the product should be defined in terms of their abilities for delivering intended performances, in very consistent and predicted manners [45]. So, ICH Q8 guides pharmaceutical industries to focus on various aspects of quality product designs as well as on manufacturing processes to enable quality within products [46]. ICH Q8 guides

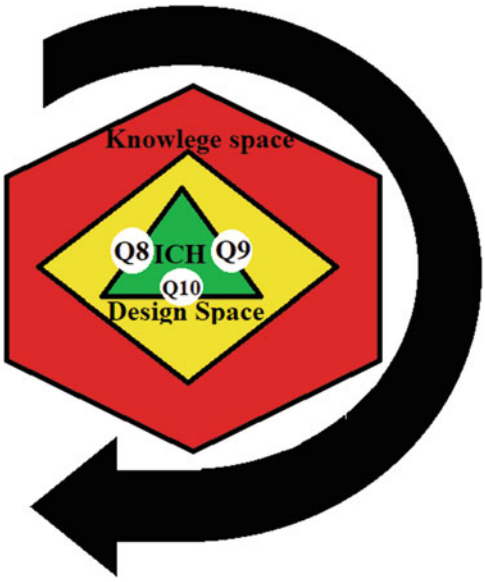

Fig. 9.2 Relationship of design space in pharmaceutical quality system related guidelines

the pharmaceutical industry for the selection of only critical attributes, out of all probable attributes, preferably by exploiting empirical evidences as well as previously generated experimental data [47]. Many attributes are bearing no significant impacts or some minor deviations in the final performances so focusing on insignificant attributes would rather would complicate the overall situation [48]. So, screening of critical and non-critical attributes are very important because controlling of significant and non-significant attributes, through DoE-based approaches, are not making any rationale sense [49]. Any manufacturing process would ultimately offers qualities within the products only if process would able to identify involved risk factors, both qualitatively and quantitatively; by estimating probability of occurrences as well as the severity of risk factors [50]. Hence, concepts of "Quality Risk Management (QRM): was established in ICH Q9. Different architects of experimental design in DoE-based approaches serve the purpose of estimating both qualitative and quantitative impacts of critical process parameters and critical material attributes, in terms of designating them as a risk factor [51].

In complement to previously mentioned guidelines under ICH Q8 and Q9, ICH Q10 guidelines focus on "Pharmaceutical Quality System" (Fig. 9.2) as a holistic approach to improve final product quality; on the principle of "International Standards Organization (ISO) on "Good Manufacturing Practice (GMP)" [52].

9.5 Trends of DoE-Based Principles in Nanotechnology

Rather than just a one-time concept on QbD, USFDA always get itself engaged in the timely release of important guidances and encourage participant sponsors to focus on the scientific basis for the phenomena or reasons which may cause variability in product performances [53]. So with advanced scientific innovations as well as the emergence of a new understanding of processes; domain of QbD start evolving every day with the continuous improving of process throughout the lifecycle of products [54]. All these characteristics hence provide better options to chemists to control and tune product qualities with optimal performances in minimal efforts [55]. Such emerging interests of pharmaceutical industries towards QbD provides better insights to regulatory bodies, with a high degree of regulatory flexibilities in the approach, to approve submitted documentary evidences related to product qualities and process controls [56]. Furthermore, pharmaceutical industry sometimes requires a minor change in controls of process, due to several reasons. However, without previously integration of QbD principles with the process, it was however quite challenging for industry to demonstrate that such minor changes wouldn't leading to significant impacts on product performances [57]. Integration of QbD methodology in product development phases, reduces burdens on regulatory agencies as well as may facilitate increased trusts about efficacy, safety as well as efficiency products [58].

9.5.1 Conceptualization of DoE in Nanotechnology

Although the basic philosophy of DoE-based approaches is to minimize inter-product variations and to achieve desired functionalities by estimating impacts of individual risk factor [59]. Subsequently, every design architect offers same promising approaches but due to different level of resolution in such designs, their end performances differ, in terms of predictability and trust towards the systems to yield a quality product [60]. Briefly, as a rule of thumb, a more complex (higher resolution) design architect encodes more detailed information on the impacts and interactions of different process variables in any system or process [35]. Usually architects of low level or low-resolution designs such as Plackett–Burman design higher-level design is preferred for qualitative estimation of risk factors impacts on final attributes [16]. While high-level design such as Box–Behnken design is preferred only for quantitative estimation of risk factors, owing to their low resolution [35]. For example, Javed et al. identified important risk factors and attempted to the optimized level of these risk factors by adopting a design architect Plackett–Burman with the process of Monoolein-Poloxamer integrating unique cuboidal shaped Limicubes, which were developed as a delivery system of Rosuvastatin [16]. Although attempts are also made towards optimization of different levels of these process variables in low resolution of Plackett–Burman design architect but these face significant chances of poor control over process [16]. With the increased in complexity or level of design, DoE-based optimized products give relatively more

robust controls with a lesser incidences of inter-product variability [17]. To achieve better control of process variables during the synthesis of gold nanoparticles; Alam et al. integrated the design architect of Box–Behnken to understand how different process variables affected size, shape, and surface charge of gold nanoparticles in the hydrothermal based synthesis of gold nanoparticles [15]. Subsequently, different statistical tools such as p-value, F-statistics, and R^2 value were used in this approach to translate impacts into an ideal mathematical model of the fitting [16]. However, these mathematical fittings may also bear some limitations and restrictions towards their further implementations, such as should be vaild under the boundary limits of design space only [35]. Within the boundry limits, these mathematical models serve most important feature in DoE to achieve objective of multi-goal optimization [17].

9.5.2 Components of DoE

As discussed above, DoE based approaches are being implemented in a judiciously systematized way, during the process of synthesizing nanomaterials by integrating principles of chemistry, arts of formulation as well as statistics in a suitable manner [61]. DoE enable establishment of relationships between input-process variables and that of their output responses; for the modeling of cause-and-effect relationships in mathematical terms [62].

Briefly, here "input or independent variables" are those independent controls of any process which if being changed would ultimately affect attributes in significant manners.

While, those factors which are being affected by the change in the level of independent variables are called "output or dependent or response variables."

Only those attributes are suitably chosen which may either directly determine the end performance of products else or may work as an indirect parameter to predict end performances [63].

For example, in green synthesis of gold nanoparticles; changing in the level of three independent variables such as gum, gold, and temperature would determine size and surface charge of metallic nanoparticles.

Here, although size is directly addressing end performances but surface charge serve the purpose of an independent parameter to determine the stability of these nanoparticles [39]. So, attributes such as charge and size of nanoparticles directly affect end performance such as antimicrobial or anticancer properties of nanoparticles.

9.5.3 Type of Designs in DoE

Subsequently, resolution of different design models which are being used to optimize the process affects optimal performances. Of-late, many attempts are being made towards adaptation of some judicious approaches for the selection of appropriate architects with adequate resolution [17]. A higher resolution design

Table 9.1 Table showing D-optimal design summary

D-optimal design summary				
Design	D-optimal			
Type	Factorial			
Total Runs	4			
Center Points	0			
Design Model	2FI			
Factor	**Unit**	**Low**	**High**	**Levels**
A: Solid Lipid	mg	40	60	2
B: Liquid Surfactant	mL	3	7	2
Response	**Name**	**Units**	**Obs**	**Analysis**
Y1	Particle size	nm	0	Factorial

Table 9.2 Table showing Plackett–Burman design summary

Design summary of Plackett–Burman			
Study Type	Factorial	**Runs**	13
Initial Design	Plackett–Burman	**Blocks**	No Blocks
Center Points	1		
Design Model	Main effects		
Factor	**Units**	**Low**	**High**
A Solid Lipid	mg	−1.00	1.00
B. Liquid Surfactant	mL	−1.00	1.00
C. Liquid Lipid	mL	−1.00	1.00
D. Temperature	Celcius	−1.00	1.00
E. Stirring speed	rpm	−1.00	1.00
F. Bead shape	–	−1.00	1.00
G. Water	mL	−1.00	1.00
H. Polymer	mg	−1.00	1.00
I. Beaker size	mL	−1.00	1.00
J. Stirrer model	–	−1.00	1.00
K. Bead size	–	−1.00	1.00
	Response	**Units**	
Y1	Particle size	Nm	

although offers better control of process and achievement of optimal solutions. While at the same time, it involves relatively more complicated architects, consumption of more money, resources, efforts, and time [15]. For example, due to relatively fewer numbers of design points required in the architect of Taguchi and Plackett–Burman based designs; such designs provide relatively more flexible controls over the process variables so are inadequate to achieve desired outputs precisely [16]. While, due to the relative requirement of higher numbers of required runs (design points) in the architect of full factorial, Box–Behnken, and central composite designs, these would enable more strict control over the process variables for optimal performance [35] (Tables 9.1, 9.2, and 9.3, Figs. 9.3, 9.4, and 9.5).

Table 9.3 Table showing Box–Behnken design summary

Design summary of Box–Behnken design			
Study Type	Response surface	**Runs**	17
Initial Design	Box–Behnken	**Blocks**	No Blocks
Design Model	Quadratic		
Factor	**Units**	**Low**	**High**
A. Solid Lipid	mg	−1.00	1.00
B. Liquid Surfactant	mL	−1.00	1.00
C. Liquid Lipid	mL	−1.00	1.00
	Response	**Units**	
Y1	Particle size	Nm	

	Std	Run	Block	Factor 1 A:Solid Lipid mg	Factor 2 B:Liquid Surfac ml	Response 1 Particle size nm
	2	1	Block 1	60	3	
	3	2	Block 1	40	7	
	4	3	Block 1	60	7	
	1	4	Block 1	40	3	

Fig. 9.3 Figure showing the design architect of the D-optimal model

	Std	Run	Block	Factor 1 A:Solid Lipid mg	Factor 2 B:Liquid Surfa	Factor 3 C:Liquid Lipid ml	Factor 4 D:Temperature Celcius	Factor 5 E:Stirring speed rpm	Factor 6 F:Bead shape -	Factor 7 G:Water ml	Factor 8 H:Polymer mg	Factor 9 J:Beaker size ml	Factor 10 K:Stirrer model -	Factor 11 L:Bead size -	Response 1 Particle size nm
	3	1	Block 1	1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	
	7	2	Block 1	1.00	-1.00	-1.00	-1.00	1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	
	12	3	Block 1	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	
	11	4	Block 1	1.00	-1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	1.00	-1.00	1.00	
	8	5	Block 1	1.00	1.00	-1.00	-1.00	-1.00	1.00	-1.00	1.00	1.00	-1.00	1.00	
	13	6	Block 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	10	7	Block 1	-1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	1.00	-1.00	1.00	1.00	
	9	8	Block 1	1.00	1.00	1.00	-1.00	-1.00	-1.00	1.00	-1.00	1.00	1.00	-1.00	
	2	9	Block 1	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	1.00	
	5	10	Block 1	-1.00	-1.00	1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	-1.00	
	6	11	Block 1	-1.00	-1.00	-1.00	1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	
	4	12	Block 1	-1.00	1.00	-1.00	1.00	1.00	-1.00	1.00	1.00	1.00	-1.00	-1.00	
	1	13	Block 1	1.00	1.00	-1.00	1.00	1.00	1.00	-1.00	-1.00	-1.00	1.00	-1.00	

Fig. 9.4 Figure showing design architect of Plackett–Burman design

Subsequently, after the selection of appropriate design, it requires various statistical tools to identify the best mathematical model of fit for input variables and desired outputs independently [43]. After this, a best fitting mathematical model as well as RSM (response surface-based methodology) approach for chosen experimental design architect, predict not only desired outcomes from the selected level of inputs but also enable to identify desired input levels for achievement of multi-objective goals [47] (Figs. 9.6 and 9.7).

	Std	Run	Block	Factor 1 A:Solid Lipid mg	Factor 2 B:Liquid Surfa ml	Factor 3 C:Liquid Lipid ml	Response 1 Particle size nm
	12	1	Block 1	0.00	1.00	1.00	
	10	2	Block 1	0.00	1.00	-1.00	
	17	3	Block 1	0.00	0.00	0.00	
	3	4	Block 1	-1.00	1.00	0.00	
	16	5	Block 1	0.00	0.00	0.00	
	8	6	Block 1	1.00	0.00	1.00	
	7	7	Block 1	-1.00	0.00	1.00	
	4	8	Block 1	1.00	1.00	0.00	
	11	9	Block 1	0.00	-1.00	1.00	
	2	10	Block 1	1.00	-1.00	0.00	
	5	11	Block 1	-1.00	0.00	-1.00	
	15	12	Block 1	0.00	0.00	0.00	
	9	13	Block 1	0.00	-1.00	-1.00	
	13	14	Block 1	0.00	0.00	0.00	
	1	15	Block 1	-1.00	-1.00	0.00	
	6	16	Block 1	1.00	0.00	-1.00	
	14	17	Block 1	0.00	0.00	0.00	

Fig. 9.5 Figure showing design architect of Box–Behnken design

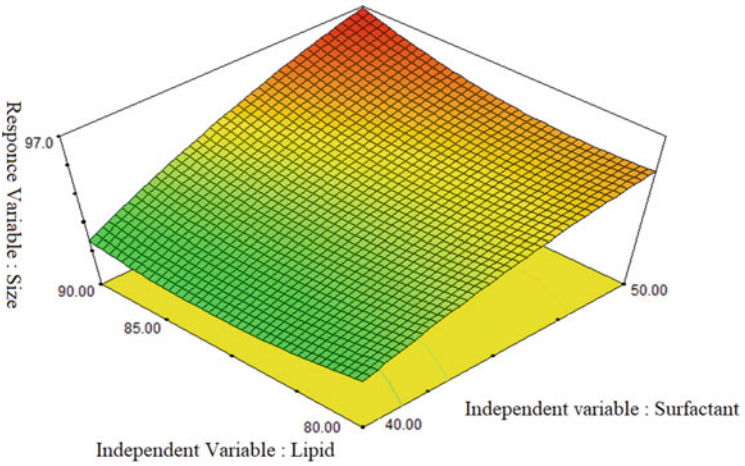

Fig. 9.6 Figure showing the impacts of lipid and surfactant on particle size in RSM graph of Box–Behnken design. Here Color wavelength proportion to size, *i.e.* violet area shows minimum size, while the red zone depicts maximum size

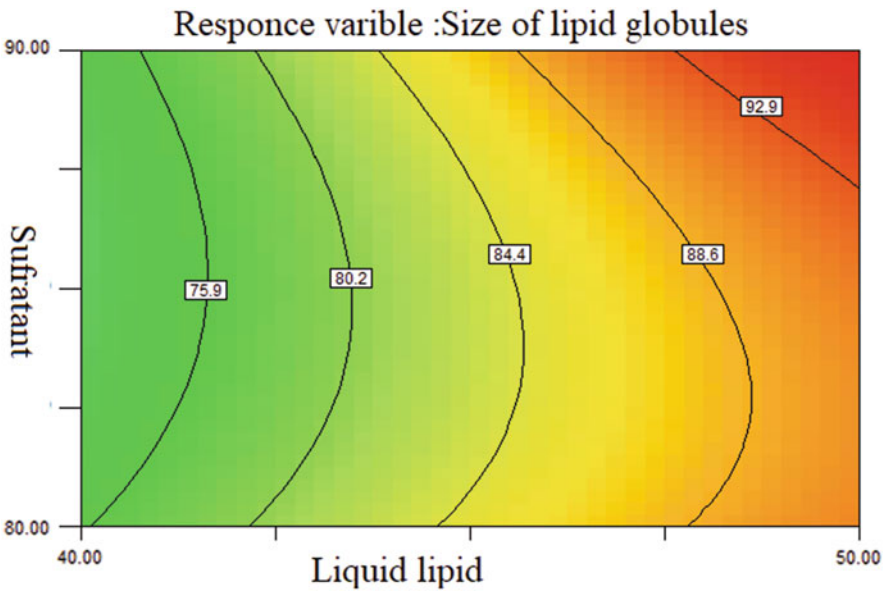

Fig. 9.7 Figure showing the impacts of liquid lipid and surfactant on globule size of emulsion in the contour graph of Box–Behnken design. Here color wavelength proportion to size, *i.e.* violet area means minimum size, while red portion depicts the maximum size

9.6 DoE Practices with Nanomaterials

In parallel to above, nanomaterials are some specific category of materials because of their unique physical and chemical attributes, hence are being used in different industries, ranging from space sectors to healthcare device manufacturers [17]. These materials, owing to their nanoscale dimensions, are usually exhibiting size-dependent performances and applications in various purposes. Subsequently, apart from their sizes even different geometry of nanoparticles are offering entirely different purposes [15]. Hence, effective regulation of size and shape of these nanoparticles is very critical because change in the dimension of nanoparticles affects detecting and sensing abilities for different biological signals, in many of applications [17]. In complicated neuronal disorders, signals for detecting changes in the level of neurotransmitters are very low to be estimated by conventional biochemical approaches [21]. Furthermore, conventional biochemical approaches which are exploited to detect such neurotransmitter types of signals are insensitive at low level of analyses; poor specificity towards neurotransmitters [18, 19]. So, attempts were made towards development of antibody-enzyme linked systems for site-specific targeting purposes but without much good resolution. Subsequently, focus is being shifted towards integration of DoE concepts with the attributes. For example, size-dependent surface Plasmon resonance (SPR) of gold

nanoparticles face different degree of nanoparticles agglomeration so these are offering unique sense for specific analyte, i.e. a neurotransmitters [17]. Among nanomaterials; gold nanoparticles are being preferred because of their unique geometry, optical characteristics and surface (chemical) properties. These specific properties of metallic nanoparticles favor their biomedical applications in clinics as a part of a biosensor, in chemistry labs as a catalyst, in the field of immunology for immunological assay, in genomics for gene delivery, in chemotherapy as for photothermolysis, in microbiology for detection of pathogenic microorganism, in pharmaceutical domain for targeted delivery application and optical image-based monitoring of tracking biological macromolecules and cells [15]. So, In poorly controlled processes, if the size of starting bulky materials are being varied above the critical ranges, then such process would ultimately lead to a high degree of bias towards final performances of nanoparticles [16]. Alam et al. used the architects of DoE to simulate experimental conditions of hydrothermal based synthesis of colloidal gold through microwave-based process [15]. Experiment conditions were allowed to tune in microwave-based synthesis products in such a way that final product would able to match dimensions as of hydrothermal processed gold nanoparticles [15, 17]. It was quite interesting even microwave enabled colloidal gold samples are identical to size and surface of hydrothermal heating process. However, microwaved processed samples offer enhanced catalytic performances [15]. Such interesting comparisons of both systems would be possible only because of integrating DoE architects within the process of gold nanoparticle synthesis [15, 17]. Similarly, polymeric and lipid-based nanoparticles were also developed to encapsulate drugs in desired manners so that these nanoparticles would able to cross the biological bio-membranes owing to their low size [4, 64]. Different shapes of nanoparticles also affect penetrability, and potentials; along with rate of drug release, mechanism of drug release, bio-distribution index, loading efficiency, and stability profile [16, 35]. Javed et al. developed cuboidal shaped Limicubes using Monoolein with by their cuboidal geometry served purposes of high stability, sustained release, and better absorption performances in vivo [16].

9.7 Conclusion

As we move down scale of materials *i.e.,* from micron to nano-dimension; process of developing nanoparticles, nanomaterials, and nanocomposites type of systems face several quality-related challenges at the industrial stage. These regulatory hiccups lead into inconsistent products and poor performances; so federal agencies become forced to direct manufacturers to enable some quality based strict means and process to minimize the occurrence of variations within products. Hence among these tools, rationalized and judicious execution of DoE methodology are being adopted as indispensable tools to ensure building quality into the final products.

Briefly, low-resolution design architects are usually exploited by industrial scientists to screen out critical variables from non-critical variables. While high-resolution design architects enable them to elucidate roles and impacts of

independent process variables with respect to quality affecting parameters so that goal of quality performance within optimized products would be ensured.

Recently, different approaches such as Artificial Neural Network (ANN) and *Genetic Algorithm* (GA) are being coupled with DoE design to better control the process. And these are emerging trends for next genration

References

1. Nayak AK, Mazumder S, Ara TJ, Ansari MT, Hasnain MS (2019) Calcium fluoride-based dental nanocomposites. In: Applications of nanocomposite materials in dentistry. Elsevier, Cambridge, pp 27–45
2. Husen A, Iqbal M (2019) Nanomaterials and plant potential: an overview. In: Nanomaterials and plant potential. Springer, Cham, pp 3–29
3. Pottoo FH, Javed MDN, Rahman JU, Abu-Izneid T, Khan FA (2020) Targeted delivery of miRNA based therapeuticals in the clinical management of Glioblastoma Multiforme. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2020.04.001
4. Raman S, Mahmood S, Hilles AR, Javed MN, Azmana M, Al-Japairai KAS (2020) Polymeric nanoparticles for brain drug delivery - a review. Curr Drug Metab 21:649–660. https://doi.org/10.2174/1389200221666200508074348
5. Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS et al (2019) Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC Adv 9:20192–20206. https://doi.org/10.1039/C9RA03102B
6. Pottoo FH, Barkat MA, Harshita, Ansari MA, Javed MN, Sajid Jamal QM et al (2019) Nanotechnological based miRNA intervention in the therapeutic management of neuroblastoma. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2019.09.017
7. Harshita, Barkat MA, Rizwanullah M, Beg S, Pottoo FH, Siddiqui S et al (2019) Paclitaxel-loaded nanolipidic carriers with improved oral bioavailability and anticancer activity against human liver carcinoma. AAPS PharmSciTech 20:87. https://doi.org/10.1208/s12249-019-1304-4
8. Ahmad N, Ahmad R, Alam MA, Ahmad FJ, Amir M, Pottoo FH et al (2019) Daunorubicin oral bioavailability enhancement by surface coated natural biodegradable macromolecule chitosan based polymeric nanoparticles. Int J Biol Macromol 128:825–838. https://doi.org/10.1016/j.ijbiomac.2019.01.142
9. Chang J-H, An YU, Kim SJ, Im S (2003) Poly(butylene terephthalate)/organoclay nanocomposites prepared by in situ interlayer polymerization and its fiber (II). Polymer 44:5655–5661. https://doi.org/10.1016/S0032-3861(03)00613-X
10. Dhakal HN, Ismail SO (2019) Unsaturated polyester resins: blends, interpenetrating polymer networks, composites, and nanocomposites. In: Unsaturated polyester resins. Elsevier, Amsterdam, pp 181–198. https://doi.org/10.1016/B978-0-12-816129-6.00008-9
11. Sharma S, Javed MN, Pottoo FH, Rabbani SA, Barkat MA, Harshita et al (2019) Bioresponse inspired nanomaterials for targeted drug and gene delivery. Pharm Nanotechnol 7:220–233. https://doi.org/10.2174/2211738507666190429103814
12. Majeed S, Joel EL, Hasnain MS (2019) Novel green approach for synthesis of metallic nanoparticles and its biomedical application. Curr Nanomed 8:177–183. https://doi.org/10.2174/2468187308666180301142158
13. Mishra S, Sharma S, Javed MN, Pottoo FH, Barkat MA, Harshita et al (2019) Bioinspired nanocomposites: applications in disease diagnosis and treatment. Pharm Nanotechnol 7:206–219. https://doi.org/10.2174/2211738507666190425121509

14. Hasnain MS, Ahmad SA, Minhaj MA, Ara TJ, Nayak AK (2019) Nanocomposite materials for prosthetic devices. In: Applications of nanocomposite materials in orthopedics. Elsevier, Amsterdam, pp 127–144. https://doi.org/10.1016/B978-0-12-813740-6.00007-7
15. Alam MS, Javed MN, Pottoo FH, Waziri A, Almalki FA, Hasnain MS et al (2019) QbD approached comparison of reaction mechanism in microwave synthesized gold nanoparticles and their superior catalytic role against hazardous nirto-dye. Appl Organomet Chem. https://doi.org/10.1002/aoc.5071
16. Javed MN, Kohli K, Amin S (2018) Risk assessment integrated QbD approach for development of optimized bicontinuous mucoadhesive limicubes for oral delivery of rosuvastatin. AAPS PharmSciTech 19:1377–1391. https://doi.org/10.1208/s12249-018-0951-1
17. Alam MS, Garg A, Pottoo FH, Saifullah MK, Tareq AI, Manzoor O et al (2017) Gum ghatti mediated, one pot green synthesis of optimized gold nanoparticles: investigation of process-variables impact using Box-Behnken based statistical design. Int J Biol Macromol 104:758–767
18. Pandey M, Saleem S, Nautiyal H, Pottoo FH, Javed MN (2020) PINK1/Parkin in neurodegenerative disorders: crosstalk between mitochondrial stress and neurodegeneration. In: Quality control of cellular protein in neurodegenerative disorders. IGI Global, Hershey, pp 282–301. https://doi.org/10.4018/978-1-7998-1317-0.ch011
19. Nigar S, Pottoo F, Tabassum N, Verma S, Javed M (2016) Molecular insights into the role of inflammation and oxidative stress in epilepsy. J Adv Med Pharm Sci 10:1–9. https://doi.org/10.9734/JAMPS/2016/24441
20. Pottoo FH, Javed MN, Barkat MA, Alam MS, Nowshehri JA, Alshayban DM et al (2019) Estrogen and serotonin: complexity of interactions and implications for epileptic seizures and epileptogenesis. Curr Neuropharmacol 17:214–231. https://doi.org/10.2174/1570159X16666180628164432
21. Pottoo FH, Tabassum N, Javed MN, Nigar S, Rasheed R, Khan A et al (2019) The synergistic effect of raloxifene, fluoxetine, and bromocriptine protects against pilocarpine-induced status epilepticus and temporal lobe epilepsy. Mol Neurobiol 56:1233–1247. https://doi.org/10.1007/s12035-018-1121-x
22. Pottoo FH, Tabassum N, Javed MN, Nigar S, Sharma S, Barkat MA et al (2020) Raloxifene potentiates the effect of fluoxetine against maximal electroshock induced seizures in mice. Eur J Pharm Sci 146:105261. https://doi.org/10.1016/j.ejps.2020.105261
23. Beg S, Rahman M, Panda SS (2017) Pharmaceutical QbD: omnipresence in the product development lifecycle. Eur Pharm Rev 22:58–64
24. Beg S, Rahman M, Kohli K (2019) Quality-by-design approach as a systematic tool for the development of nanopharmaceutical products. Drug Discov Today 24:717–725. https://doi.org/10.1016/j.drudis.2018.12.002
25. Beg S, Swain S, Rahman M, Hasnain MS, Imam SS (2019) Application of design of experiments (DoE) in pharmaceutical product and process optimization. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 43–64. https://doi.org/10.1016/B978-0-12-815799-2.00003-4
26. Beg S, Hasnain MS, Rahman M, Swain S (2019) Introduction to quality by design (QbD): fundamentals, principles, and applications. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 1–17. https://doi.org/10.1016/B978-0-12-815799-2.00001-0
27. Beg S, Akhter S, Rahman M, Rahman Z (2017) Perspectives of quality by design approach in nanomedicines development. Curr Nanomed 7:191–197. https://doi.org/10.2174/2468187307666170519122653
28. Gilani SJ, Rizwanullah M, Imam SS, Pandit J, Aqil M, Alam M et al (2019) QbD considerations for topical and transdermal product development. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 131–150. https://doi.org/10.1016/B978-0-12-815799-2.00008-3
29. Nayak AK, Ahmed SA, Beg S, Tabish M, Hasnain MS (2019) Application of quality by design for the development of biopharmaceuticals. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 399–411. https://doi.org/10.1016/B978-0-12-815799-2.00019-8

30. Taleuzzaman, Chuhan S, Gilani SJ, SS Imam SB (2020) Systematic product and process development tools in life cycle management. In: Pharmaceutical drug product and process optimization. CRC Press, Boca Raton, FL, pp 33–52
31. Hasnain MS, Siddiqui S, Rao S, Mohanty P, Jahan Ara T, Beg S (2016) QbD-driven development and validation of a bioanalytical LC–MS method for quantification of fluoxetine in human plasma. J Chromatogr Sci 54:736–743. https://doi.org/10.1093/chromsci/bmv248
32. Alam T, Khan S, Gaba B, Haider MF, Baboota S, Ali J (2018) Adaptation of quality by design-based development of isradipine nanostructured–lipid carrier and its evaluation for in vitro gut permeation and in vivo solubilization fate. J Pharm Sci 107:2914–2926. https://doi.org/10.1016/j.xphs.2018.07.021
33. Gaba B, Khan T, Haider MF, Alam T, Baboota S, Parvez S et al (2019) Vitamin E loaded naringenin nanoemulsion via intranasal delivery for the management of oxidative stress in a 6-OHDA parkinson's disease model. Biomed Res Int 2019:1–20. https://doi.org/10.1155/2019/2382563
34. Kalam MA, Sultana Y, Ali A, Mohd A, Mishra AK, Aljuffali IA et al (2013) Part I: development and optimization of solid-lipid nanoparticles using Box-Behnken statistical design for ocular delivery of gatifloxacin. J Biomed Mater Res 101A:1813–1827. https://doi.org/10.1002/jbm.a.34453
35. Javed MN, Alam MS, Waziri A, Pottoo FH, Yadav AK, Hasnain MS et al (2019) QbD applications for the development of nanopharmaceutical products. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 229–253. https://doi.org/10.1016/B978-0-12-815799-2.00013-7
36. Moghddam SMM, Ahad A, Aqil M, Imam SS, Sultana Y (2017) Optimization of nanostructured lipid carriers for topical delivery of nimesulide using Box–Behnken design approach. Artif Cells Nanomed Biotechnol 45:617–624. https://doi.org/10.3109/21691401.2016.1167699
37. Imam SS, Aqil M, Akhtar M, Sultana Y, Ali A (2014) Optimization of mobile phase by 3^2-mixture design for the validation and quantification of risperidone in bulk and pharmaceutical formulations using RP-HPLC. Anal Methods 6:282–288. https://doi.org/10.1039/C3AY41562G
38. Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A et al (2007) Formulation development and optimization using nanoemulsion technique: a technical note. AAPS PharmSciTech 8:E12–E17. https://doi.org/10.1208/pt0802028
39. Hasnain MS, Javed MN, Alam MS, Rishishwar P, Rishishwar S, Ali S et al (2019) Purple heart plant leaves extract-mediated silver nanoparticle synthesis: optimization by Box-Behnken design. Mater Sci Eng C 99:1105–1114
40. Hasnain MS, Ansari SA, Rao S, Tabish M, Singh M, Abdullah MS et al (2017) QbD-driven development and validation of liquid chromatography tandem mass spectrometric method for the quantitation of sildenafil in human plasma. J Chromatogr Sci 55:587–594. https://doi.org/10.1093/chromsci/bmx010
41. Jazuli I, Annu, Nabi B, moolakkadath T, Alam T, Baboota S et al (2019) Optimization of nanostructured lipid carriers of lurasidone hydrochloride using Box-Behnken design for brain targeting: in vitro and in vivo studies. J Pharm Sci 108:3082–3090. https://doi.org/10.1016/j.xphs.2019.05.001
42. Barkat MA, Harshita, Pottoo FH, Singh SP, Ahmad FJ (2018) Therapeutic intervention of *Aloe* gel containing nano-sized and Micron-sized silver sulfadiazine gel on second-degree burn: a comparative study. Int J Low Extrem Wounds 17:176–183. https://doi.org/10.1177/1534734618791860
43. Alam T, Pandit J, Vohora D, Aqil M, Ali A, Sultana Y (2015) Optimization of nanostructured lipid carriers of lamotrigine for brain delivery: *in vitro* characterization and *in vivo* efficacy in epilepsy. Expert Opin Drug Deliv 12:181–194. https://doi.org/10.1517/17425247.2014.945416

44. Barkat MA, Harshita, Ahmad I, Ali R, Singh SP, Pottoo FH et al (2017) Nanosuspension-based Aloe vera gel of silver sulfadiazine with improved wound healing activity. AAPS PharmSciTech 18:3274–3285. https://doi.org/10.1208/s12249-017-0817-y
45. Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS et al (2019) A novel self-nanoemulsifying drug delivery system for curcumin used in the treatment of wound healing and inflammation. 3 Biotech 9:360. https://doi.org/10.1007/s13205-019-1885-3
46. Mishra SM, Rohera BD (2017) An integrated, quality by design (QbD) approach for design, development and optimization of orally disintegrating tablet formulation of carbamazepine. Pharm Dev Technol 22:889–903. https://doi.org/10.1080/10837450.2016.1199566
47. Jain A, Beg S, Saini S, Sharma T, Katare OP, Singh B (2019) Application of chemometric approach for QbD-enabled development and validation of an RP-HPLC method for estimation of methotrexate. J Liq Chromatogr Relat Technol 42:502–512. https://doi.org/10.1080/10826076.2019.1626742
48. Sandhu PS, Beg S, Katare OP, Singh B (2016) QbD-driven development and validation of a HPLC method for estimation of tamoxifen citrate with improved performance. J Chromatogr Sci 54:1373–1384. https://doi.org/10.1093/chromsci/bmw090
49. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M (2014) Design, formulation and optimization of valsartan transdermal gel containing iso-eucalyptol as novel permeation enhancer: preclinical assessment of pharmacokinetics in Wistar albino rats. Expert Opin Drug Deliv 11:1149–1162. https://doi.org/10.1517/17425247.2014.914027
50. Swain S, Jena BR, Madugula D, Beg S (2019) Application of quality by design paradigms for development of solid dosage forms. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 109–130. https://doi.org/10.1016/B978-0-12-815799-2.00007-1
51. Panda SS, Bera VVRK, Beg S, Sahu SK (2015) Ultrafast liquid chromatographic method development and its validation for quantification of telaprevir in pharmaceutical dosage form by using quality by design approach. J Chromatogr Sci 53:1193–1202. https://doi.org/10.1093/chromsci/bmu228
52. Fatima S, Beg S, Samim M, Ahmad FJ (2019) Application of chemometric approach for development and validation of high performance liquid chromatography method for estimation of ropinirole hydrochloride. J Sep Sci 42:3293–3301. https://doi.org/10.1002/jssc.201900458
53. Waghule T, Rapalli VK, Singhvi G, Manchanda P, Hans N, Dubey SK et al (2019) Voriconazole loaded nanostructured lipid carriers based topical delivery system: QbD based designing, characterization, in-vitro and ex-vivo evaluation. J Drug Deliv Sci Technol 52:303–315. https://doi.org/10.1016/j.jddst.2019.04.026
54. Bhatt P, Saquib Hasnain M, Nayak AK, Hassan B, Beg S (2018) Development and validation of QbD-driven bioanalytical LC-MS/MS method for the quantification of paracetamol and diclofenac in human plasma. Anal Chem Lett 8:677–691. https://doi.org/10.1080/22297928.2018.1429305
55. Hasnain MS, Ahmed SA, Beg S, Ansari MT, Nayak AK (2019) 'Quality by design' approach for development of multiparticulate drug delivery systems. In: Pharmaceutical quality by design. Elsevier, Amsterdam, pp 351–365. https://doi.org/10.1016/B978-0-12-815799-2.00016-2
56. Beg S, Chaudhary V, Sharma G, Garg B, Panda SS, Singh B (2016) QbD-oriented development and validation of a bioanalytical method for nevirapine with enhanced liquid-liquid extraction and chromatographic separation: QbD-based bioanalytical method for nevirapine. Biomed Chromatogr 30:818–828. https://doi.org/10.1002/bmc.3613
57. Nayak AK, Pal D, Pradhan J, Hasnain MS (2013) Fenugreek seed mucilage-alginate mucoadhesive beads of metformin HCl: design, optimization and evaluation. Int J Biol Macromol 54:144–154. https://doi.org/10.1016/j.ijbiomac.2012.12.008
58. Nayak AK, Kalia S, Hasnain MS (2013) Optimization of aceclofenac-loaded pectinate-poly (vinyl pyrrolidone) beads by response surface methodology. Int J Biol Macromol 62:194–202. https://doi.org/10.1016/j.ijbiomac.2013.08.043

59. Nayak AK, Hasnain MS, Malakar J (2013) Development and optimization of hydroxyapatite-ofloxacin implants for possible bone delivery in osteomyelitis treatment. Curr Drug Deliv 10:241–250. https://doi.org/10.2174/1567201811310020008
60. Sinha P, Ubaidulla U, Hasnain MS, Nayak AK, Rama B (2015) Alginate-okra gum blend beads of diclofenac sodium from aqueous template using ZnSO4 as a cross-linker. Int J Biol Macromol 79:555–563. https://doi.org/10.1016/j.ijbiomac.2015.04.067
61. Beg S, Kohli K, Swain S, Hasnain MS (2012) Development and validation of rp-hplc method for quantitation of amoxicillin trihydrate in bulk and pharmaceutical formulations using Box-Behnken experimental design. J Liq Chromatogr Relat Technol 35:393–406. https://doi.org/10.1080/10826076.2011.601493
62. Nayak A, Pal D, Hasnain M (2013) Development, optimization and in vitro-in vivo evaluation of pioglitazone- loaded jackfruit seed starch-alginate beads. Curr Drug Deliv 10:608–619. https://doi.org/10.2174/1567201811310050012
63. Saquib Hasnain M, Rishishwar P, Rishishwar S, Ali S, Nayak AK (2018) Isolation and characterization of Linum usitatissimum polysaccharide to prepare mucoadhesive beads of diclofenac sodium. Int J Biol Macromol 116:162–172. https://doi.org/10.1016/j.ijbiomac.2018.04.151
64. Pottoo FH, Sharma S, Javed MN, Barkat MA, Harshita AMS et al (2020) Lipid-based nanoformulations in the treatment of neurological disorders. Drug Metab Rev 52:185–204

Design of Experiments for the Development of Biotechnology Products

10

Suryakanta Swain, Bikash Ranjan Jena, and Sarwar Beg

Abstract

The development of biotechnology products containing large molecules such as nucleic acids, monoclonal antibodies, hormones, etc., requires critical understanding of the product-specific quality attributes and close monitoring of the manufacturing process for attaining quality consistency without batch-to-batch variability. The conventional product development strategy using one factor at one time approach only provides limited solution without any significant influence on the product quality for attaining the robust performance. However, the systematic approach of using design of experiments (DoE) is very useful for minimizing the variability and improving the product performance. A score of literature reports are available which vouch the applicability of experimental designs for efficient development of the biotechnology products. The present chapter, therefore, provides a holistic account on the implementation of DoE approach for manufacturing of the biotechnology, and also highlights the current challenges and opportunity associated with them.

Keywords

Quality · Variability · Product development · Experimental designs · Robustness

S. Swain (✉)
Department of Pharmaceutical Sciences, School of Health Sciences, The Assam Kaziranga University, Jorhat, Assam, India

B. R. Jena
School of Pharmacy and Life Sciences, Centurion University of Technology and Management (CUTM), Bhubaneswar, Odisha, India

S. Beg
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_10

10.1 Introduction

Statistical experimental planning, factorial design, and design of experiments (DoE) are synonymous concepts for investigating the mathematical relationships between input and output variables of a system. Although the fundamentals of the methodology have been known since the early 1900s, 1–3, it was not until recent years that it was widely applied in biotechnology. Methods such as factorial design, response surface methodology, and (DoE) provide robust and efficient ways to optimize cultivations and other unit operations and procedures using a reduced number of experiments. The multitude of interdependent parameters involved within a unit operation or between units in a bioprocess sequence may be substantially refined and improved by using such methods [1]. In an experiment, one or more process variables (or factors) are deliberately changed to observe the effect on one or more response variables. Statistical design of experiments is an efficient procedure for planning operations and analyzing the results such that objective conclusions can be drawn. Determining the objectives of analysis is the first step of experimental design. Selecting the process factors and responses to be analyzed is the second important step in optimizing the essential variables in a process [2].

In general, experimental design is the design of a system to obtain information using various innovative techniques. Experimental design can be used to evaluate physical objects, chemical formulations, structures, components, and materials. Bioengineering studies different production areas, including enzyme production, biological water treatment, tissue culture, nanoparticles, biofuel production, the isolation of microorganisms, and the industrial biological production of compounds such as proteins, lipids, and aromatic compounds. Every process is affected by different factors. Optimizing these parameters is an essential step in obtaining maximum production with minimum costs. Developing an experimental design is the most effective means of process optimization. This review summarizes the experimental design methods that are used to investigate the effects of various factors on various bioengineering processes, including full factorial design, fractional factorial design, Plackett–Burman (PB) design, Taguchi design, Box–Behnken design, and central composite design. Each design method is briefly introduced, the advantages and disadvantages of each are briefly discussed, and various processes are analyzed [3].

Other bioprocess-related applications include strain screening evaluation and cultivation media balancing. Because of the emerging regulatory demands on pharmaceutical manufacturing processes, exemplified by the process analytical technology (PAT) initiative of the United States Food and Drug Administration, the use of experimental design approaches improves process development for safer and more reproducible production is becoming increasingly important. Here, these options are highlighted and discussed with a few selected examples from antibiotic fermentation, expanded bed optimization, virus vector transfection of insect cell cultivation, feed profile adaptation, embryonic stem cell expansion protocols, and mammalian cell harvesting [4].

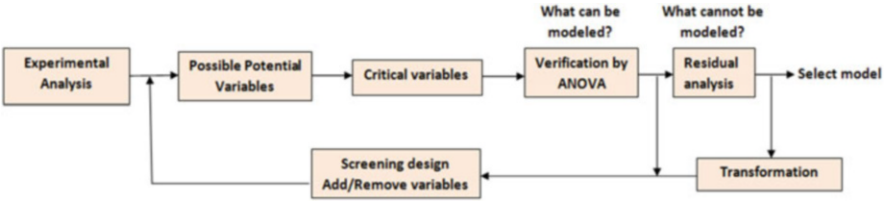

Fig. 10.1 Different critical steps for experimental design (Adapted from [5])

Biotechnology processes can be considered a transformation of nutrients and other medium components to biomass and molecular ones. The utilization of carbon and nitrogen sources is relatively quickly overviewed via total stoichiometric balances. To this, a degree of complexity is added by shifts in metabolism caused by activating or deactivating medium components such as growth, vitamins, and microbial stressors. Figure 10.1 illustrates the situation where all inputs exert their effects as an initial load of multiplicity effects onto the transforming bioprocess in a steady-state condition. However, in reality, the steady-state is unstable since the nutrient factors are degraded and depleted [6].

In most bioprocesses, products are recovered downstream. Additional factors, e.g., eluents, are sequentially supplied to purify the product. Here, too, input factors are distributed over time. The influence of upstream procedures follows the same pattern. Thus, any attempt to encompass all elements in a bioprocess ends up in an incomprehensible number of interacting and non-interacting factors that may, or may not, affect the specific outputs [7].

10.2 Efficient Strategy, Concept, and Parameters of Design of Experiments

Several simulated research problems have been programmed for the IBM 704 computer. Six different empirical research strategies have also been programmed for the computer. The "research problems" are "solved" by the computer using one of the six strategies to learn which is best. The computer uses each research strategy on each problem several times to get a statistical evaluation of its effectiveness [8].

Application of the design of experiments allows investigators to understand multiple method parameters and variables that tend to impact critical responses while unraveling the occurrence of (any) interactions and diminishing complexities ([9]). For the successful implementation of the experimental study design, the knowledge of response variables, critical method variables, their ranges, and suitable mathematical model(s) is mandatory. Response surface methodology (RSM) based various design of experiments like factorial design (FD), central composite design (CCD), Box–Behnken design (BBD), optimal design (OD), etc., are useful in the systematic development of analytical methods comprising significant variable-response relationship(s) [8]. The experimental designs assist in mapping the

responses based on the studied objective(s) and exploring the critical responses at high (coded as +1), medium (coded as 0), or low (coded as −1) levels of the variables. It tends to reveal the mechanistic understanding of the variables-responses relationship and their associated interactions via various pictorial/graphical tools. 3D and 2D-plots like response surface plots, contour plots, perturbation plots, linear correlation plots, outlier plots, and Box–Cox plot are beneficial in this regard ([9]). Once data acquired by the chosen design has been collected, the results can be analyzed using statistical methods like multiple linear regression (MLR) analysis so that objective conclusions can be drawn [10]. The flow layout of a typical design of the experiments-based regression model employed for method development and data analysis is illustrated below (Fig. 10.1).

10.3 Potential Benefits and Types of Experimental Design Used for the Development of Biotechnology Products

The design of experiments (DOE) based approach offers a solution to this problem and allows for efficient estimation of the main effects and the interactions with minimal operations. A systematic approach for evaluating the different DOE designs and choosing the optimal model (I-optimal and D-optimal), central composite, and Box–Behnken designs is mainly used for the development of biotechnology products. Other potential benefits and types of DoE-based software's for the development of biotechnology products are as follows [8, 10];

- Better understanding and control over critical variables
- Beyond traditional approach of method validation
- Flexibility in the analysis of the sample in various matrices
- Improved method robustness thereby reduction of variability
- Analytical attributes within the pharmacopeia restrictions, and away from Out-of-Specification (OOS) limits
- Smooth method transfers to the production level
- No obligation of revalidation within the design space.

10.4 Role of Central Composite Designs (I- and D-Optimal) and Box–Behnken Designs for Bioprocess Optimization

The schematic classifications of experimental design are shown in Fig. 10.2 and briefly described the central composite designs (I- and D-optimal) and Box–Behnken designs used for bioprocess optimization techniques as below.

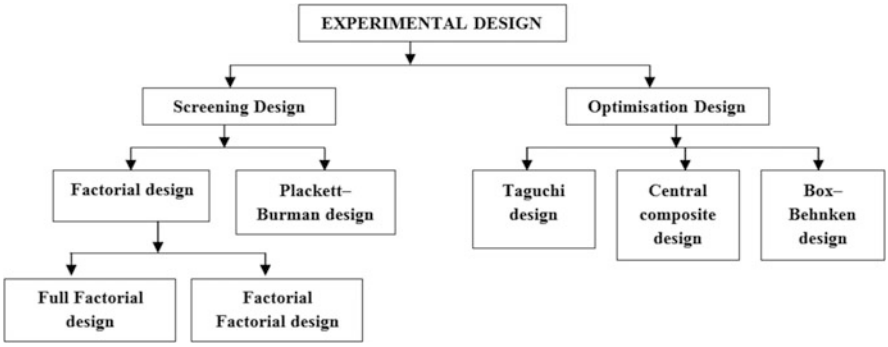

Fig. 10.2 Classification of experimental designs (Adapted from [5])

10.4.1 Central Composite Design

Box and Wilson developed a central composite design (CCD). Both linear and quadric models are allowed to be determined by this design. CCD seems to be a good alternative of a three-level full factorial design as it provides comparable results with a smaller number of experiments. CCD usually consists of a full factorial design or factorial design with two levels, additional axial or star points, and at least one central point of the experimental design. The axial design and the central design are almost the same for the two-level full factorial design except for one factor that may take on levels either above the high level or below the low level. CCD requires experiment numbers according to $N = k2 + 2k + \text{cp}$, here cp is replicate numbers of the center point, and k is factor numbers [1, 11–15].

10.4.2 Box–Behnken Design

Box and Behnken developed Box–Behnken design (BBD). This design consists of a factorial design with three levels and an incomplete block design in such a way to present as a rotatable or nearly rotatable design and to avoid the extreme vertices. BBD requires experiment numbers based on $N = 2k\ (k - 1) + \text{C0}$, here k is factor numbers, and C0 is central point numbers. BBD is useful in avoiding experiments that are in extreme conditions because the highest level and lowest level combinations for every factor cannot be included in BBD. Unsatisfactory results might be avoided in BBD [16–18]. ANOVA can examine the significant effects of BBD on the response, and the optimal response can be determined by the regression model with calculating the derivatives of the model. CCD has more factor levels than BBD. Thus BBD can be an economical alternative of CCD [19, 20].

10.5 DoE for Early Bioprocess Development or Modeling, Optimization, and Characterization

The universal implementation of DoE methodology to bioprocesses development is uncomplicated and unique outline of development. It investigates defined input factors to a converting bio system from which mostly common and well-defined output factors or responses are generated, such as product yield and productivity. The statistical design of experiments (DOE) is a collection of predetermined settings of the process variables of interest, which provides an efficient procedure for planning experiments. Experiments on biological processes typically produce long sequences of successive observations on each experimental unit (plant, animal, bioreactor, fermenter, or flask) in response to several treatments (combination of factors). Cell culture and other biotech-related experiments used to be performed by repeated-measures method of experimental design coupled with different levels of several process factors to investigate dynamic biological process. Data collected from this design can be analyzed by several kinds of general linear model (GLM) statistical methods such as multivariate analysis of variance (MANOVA), univariate ANOVA (time split-plot analysis with randomization restriction), and analysis of orthogonal polynomial contrasts of repeated factor (linear coefficient analysis). Statistical analysis of bioprocess with repeated measurements can help investigate environmental factors and effects affecting physiological and biochemical processes in analyzing and optimizing biotechnology based production. However, the strength of DoE is that it also reveals how interactions between the input factors influence the output responses. These interactions are often difficult to discover and interpret with other methods. The basic outline or layout containing different input factors, output responses, and stages involved in bioprocess and its upstream stages of experimental design model are depicted in Fig. 10.3 [21–23].

10.6 Statistical Analysis of Variance of Bioprocess for Analyzing and Optimizing Biotechnology Production

Cell culture and other biotech-related experiments used to be performed by repeated-measures technique of DoE coupled with diverse levels of several process factors to investigate dynamic biological process. Statistical analysis of bioprocess with repeated measurements can help investigate environmental factors and effects affecting physiological and bioprocesses in analyzing and optimizing biotechnology production. Data collected from this design can be analyzed by several kinds of general linear model (GLM) statistical methods such as multivariate analysis of variance (MANOVA), univariate ANOVA (time split-plot analysis with randomization restriction), and analysis of orthogonal polynomial contrasts of repeated factor (linear coefficient analysis). The statistical design of experiments (DOE) is a collection of predetermined settings of the process variables of interest, which provides an efficient procedure for planning experiments. Experiments on biological processes typically produce long sequences of successive observations on each experimental

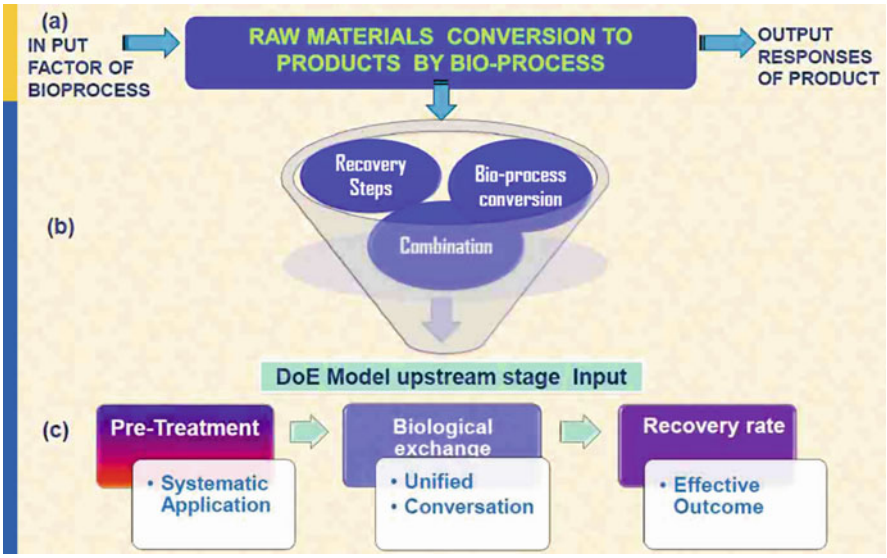

Fig. 10.3 Schematic diagram elucidating (**a**) Mechanism of input coded factors and output responses of a systematic bioprocess system as a basis for DoE, (**b**) Different steps involved in a typical bioprocess methodology: bioreactor and recovery (route of downstream processing), (**c**) Bioprocesses system upstream stage input factors be measured that add on further complication to the DoE models

unit (plant, animal, bioreactor, fermenter, or flask) in response to several treatments (combination of factors) [21, 24–26].

10.7 Application of DoE Methods Through the Use of Different Statistical Software's in Biotechnology

10.7.1 In Expanded Bed Optimization

In view of the emerging regulatory demands on pharmaceutical manufacturing processes, exemplified by the process analytical technology (PAT) initiative of the United States Food and Drug Administration, the use of experimental design approaches to improve process development for safer and more reproducible production is becoming increasingly important. Methods such as factorial design, response surface methodology, and (DoE) provide powerful and efficient ways to optimize cultivations and other unit operations and procedures using a reduced number of experiments. Design of experiments (DOE) based approach offers a solution to this conundrum and allows for an efficient estimation of the main effects and the interactions with minimal number of experiments. Statistical experimental planning, factorial design, and design of experiments (DoE) are more or less

synonymous concepts for investigating the mathematical relationships between input and output variables of a system [7].

10.7.2 In Antibiotic or Microbial Fermentation

Most notable applications of DoE have concerned optimization of the composition of growth and production culture media or microbial fermentation techniques. An elucidating example of this is from the production of the antibiotic clavulanic acid using the fungus *Streptomyces clavuligerus*. Wang et al. [27] optimized the medium composition by first screening a variety of media ingredients by a two-level fractional factorial design approach which subsequently was followed by optimizing their levels by response surface methodology [27]. Other successful examples of applying DoE to optimization of media composition for antibiotics production come from neomycin production by *S. marinensis* with solid-state fermentation [28] nisin by *Lactococcus lactis*, and meilingmycin by *S. nanchangensis*, [29–33] where fractional design methodology in combination with response surface was applied. Overviews of DoE methodology applicable for the optimization of microbial fermentation process and optimization of culture media of some vital antibiotics are enlisted in Table 10.1.

10.7.3 In Virus Vector Transfection of Insect Cell Cultivation

The cultivation of recombinant insect cell lines has been the subject of intense research since the 1980s and also allows the industrial production of recombinant proteins, vaccines, and insecticides. Gene therapy is a promising technology for the treatment of several acquired and inherited diseases. However, for gene therapy to be a commercial and clinical success, scalable cell culture processes must be in place to produce the required amount of viral vectors to meet market demand [34]. However, optimization of transformation conditions is carried out using OFAT approaches. Even though the optimization of transformation conditions for cloning experiments using DoE has not been reported, fractional factorial approaches have been successfully employed to identify the factors that significantly affect the transformation efficiency of bacteria for other purposes (e.g., drug development) [35]. evaluated the effect of five factors (Cell density, voltage, resistance, plasmid DNA concentration, and Mg^{2+} concentration) on the transformation efficiency of *Acinetobacter baumannii* using a three-level fractional factorial approach and the transformation efficiency was increased by four times. Thus, DoE approaches could be probably used as a tool to maximize the transformation efficiency of bacteria during cloning experiments. Each type of vector has its own distinct characteristics and consequently its own challenges for production [36].

Table 10.1 Applications DoE methodology for production of antibiotics fermentation and their responses outcome

Name of antibiotics	Rationale of methodology	Optimization process	Outcome and improvement	Reference
Neomycin (*S. marinensis*)	Medium composition maximizing neomycin yield by optimizing	Full FD and RSM	Yield of neomycin, optimum determined	[28]
Nisin (*Lactococcus lactis*)	Optimizing medium composition Maximizing nisin specific productivity by	statistical analysis Fractional factorial design and	productivity Nisin yield; specific. Increased activity yield	[31]
Clavulanic acid (*Streptomyces clavuligerus*)	Medium composition Maximizing clavulanic acid yield by optimizing	Optimizing by RSM Screening by fractional FD and	Yield of clavulanic acid with 50%	[27]
Meilingmycin	Medium composition maximizing meilingmycin yield by optimizing	Optimization by steepest ascent and RSM screening by fractional FD and	4.5-fold increase of Melingomycin yield	[33]
6-Aminopenicillanic acid (penicillin acylase)	Process parameters Maximizing 6-APA yield by optimizing	Full FD and modeling	Optimization has made by complete enzyme yield	[29], [32]
Antifungal antibiotic (*S. chattanoogensis*)	Process parameters Maximizing antibiotic yield by optimizing	Full FD and statistical analysis	Optimization has made by complete enzyme yield	[30]

RSM Response surface methodology, *FD* Factorial design

10.7.4 In Feed Profile Adaptation

Design of Experiment especially concerning the biological sciences, in particular for food microbiology, we can define DoE as a structured, systematic, and rigorous approach to problem solving that applies principles and techniques at the data collection stage, so as to ensure the generation of valid, defensible, and supportable conclusions. DoE can be successfully being implemented in feed profile adaptation. As the PAT initiative emphasizes, the use of DoE to exploit new optimization possibilities in biotechnology can be a very useful resource for bioprocess development. Especially the PAT is used to investigate in a more systematic manner the

factors that affect intrinsic responses in the cells/product protein of the bioprocess, such as glycosylation pattern and other modifications. Here, data mining techniques may provide a useful resource of analytical evaluation methods [37].

10.7.5 In Embryonic Stem Cell Expansion Protocols, and Mammalian Cell Harvesting

The development of new culture production systems for embryonic stem (ES) cells requires substantial work to find suitable production conditions. This is a particular concern in the pre-processing of time-consuming and labor-demanding in vitro differentiation procedures, where the complexities of factors that impact upon ES cell differentiation are profound. Chang and Zandstra [38] have developed and validated such a technology for ES cell differentiation analysis. They used a quantitative screening platform based on automated fluorescence microscopy, which enumerated the ES cells that had entered endodermal differentiation through expression of two biomarkers (Cytokeratin-8 and hepatocyte nuclear factor 3β). Chang and Zandstra [38] developed two-level fractional factorial design model based on 32 triplicate experiments, they screened important medium components for the differentiation process to endodermal cells (glucose, insulin, basic fibroblast growth factor, retinoic acid, and epidermal growth factor) with the biomarkers and cell numbers as responses. The model was further refined using a subsequent three-level factorial experiment for two of the factors. A statistical regression model was used to identify major and interactive effects on the endoderm formation. Retinoic acid was found to have an inhibitory effect on endoderm formation, while low glucose levels were beneficial. DoE proved to be a powerful tool for studying the factors impacting endoderm-specific ES cell differentiation; but it does require a relevant and sufficiently sensitive technique for the analysis of responses.

10.7.6 In Cancer Targeting and Gene Delivery

Implementation of a manufacturing process that assures a predefined quality of product is a critical requirement for the licensing and marketing of every cell and gene therapy (CGT) product. However, inadequate process knowledge and understanding constricts implementation of process changes as the impacts on product safety and efficacy are unknown. This often leads to the adoption of processes that, although compliant with established regulations, are not optimal for assuring broad availability to patients who depend on those therapies [39]. To improve the manufacturing of CGTs, Quality by Design (QbD) principles widely recognized as integration of scientific knowledge and risk assessment into process and build products can be adopted. Mostly the retro virus, Adeno virus, herpes viruses are used in gene therapy applications. QbD approach will involve development of an effective control strategy for it would ensure safe encapsulation of anticancer drugs for successful product development using polymeric nanoparticles. Chien et al. [40]

have developed a comparison technique where he has discussed about paclitaxel (PTX), a common chemotherapeutic agent, was loaded into poly-lactic-co-glycolic acid (PLGA) nanoparticles (NPs), and the coating process of chitosan (CS) onto PLGA NPs was focused for optimization by d-optimal designs and Artificial Neural Networks for targeting lungs and cervical cancer cells.

10.7.7 In Recombinant Protein Expression

Quality by Design (QbD) is a new approach to the development of recombinant therapeutic protein products that promotes a better understanding of the product and its manufacturing process [41]. Recombinant proteins are widely used in diverse fields in laboratory and industry, while many applications require sufficient amounts of high-quality proteins in terms of purity and activity. Viral transduction of eukaryotic cell lines is one possibility to efficiently generate recombinant proteins, and among all viral-based expression systems the baculovirus/insect cell expression system (BEVS) is certainly the most well-known and applied. In order to produce recombinant proteins, cost effectively, a satisfactory expression level has to be achieved in one of several species available for recombinant protein expression. Suitable hosts include bacteria (*Escherichia coli*), yeasts (such as *Pichia pastoris*), and cell lines of mammalian or insect origin [34]. These expression systems differ in terms of complexity, space-time-yield, and the ability to support protein folding and posttranslational modification. Several designs like full factorial, fractional factorial, Plackett–Burman, Taguchi orthogonal, RSM, and incomplete fractional are implemented in recombinant protein biotechnology. The design has so much advantages like both categorical and continuous variables can be simultaneously tested by full factorial design, results from the whole set of experiments are utilized. The Plackett–Burman method screens the design space to detect a large main effect. Taguchi orthogonal is a highly fractional orthogonal design allowing examining a selected subset of combinations of multiple factors at multiple levels with the fewest number of experiments. RSM can be useful for the only "real" optimization process and can be used to fine-tune the optimum conditions. Incomplete fractional is highly considered and can easily be set up using a freeware package (SAmBA)—Advance knowledge in statistics is not necessary—Factors can be examined in more than two levels. The other crucial steps include PRC amplification, Ligation, Purification, Functional assay, and Protein Crystallography, etc. [42].

10.8 DoE-Based Reported Bioprocess Case Studies

Mendes et al. [43] investigated that the daily relative growth rates of the red macroalgae *Gracilaria domingensis* in synthetic seawater. The effect of the combined influence of five factors [light (L), temperature (T), nitrate (N), phosphate (P), and molybdate (M) concentrations] was determined using a full factorial design. The ranges of the experimental cultivation conditions used were as follows: T, 18–26

C; L, 74–162 micromol photons m-2 s-1; N, 40–80 mmol/L; P, 8–16 mmol/L; and M, 1–5 nmol/L. The results were analyzed using the analysis of variance (ANOVA) test method. According to the ANOVA test, the factors N (p¼ 0.01327) and T (p¼ 0.00025) are highly significant at a¼ 0.05 level [43].

The production of poly-3-hydroxybutyrate (PHB) by *Methylocystis hirsuta* from natural gas was first studied in two different media by Rahnama et al. [44]. After selecting the medium, the effects of methane-to-air ratio (20/80–50/50–80/20) and nitrogen content (0–50–100 mg/L) on PHB production were determined in a bubble column using a 3^2 full factorial design. Both of these factors were significant, and a maximum accumulation of 42.5% w/w of dry cell weight was achieved [44].

Nalakath et al. [45] investigated the effect of four factors on the bioconversion of carbon monoxide to ethanol and acetic acid by *Clostridium autoethanogenum* using a 2^4 full factorial design. The four studied factors were initial pH (4.75–5.75), initial total pressure (0.8–1.6 bar), cysteine-HCl and H_2O concentration (0.5–1.2 g/L), and yeast extract (YE) concentration (0.6–1.6 g/L). The maximum ethanol production was enhanced by up to 200% at conditions pH 4.75 and a YE concentration of 1.6 g/L. All the main effects and the interaction effects were found to be statistically significant (p50.05). Main effect plots indicated that increasing the initial pH and using higher YE concentrations negatively affected ethanol production, whereas increasing the initial pressure and the cysteine-HCl and H_2O concentration had a positive effect. Ethanol production of 0.065 g/L was achieved using the following values of the factors: pH (4.75), pressure (1.6 bar), cysteine-HCl.H2O (1.2 g/L), and YE concentration (1.6 g/L; [45]).

The optimal conditions for microwave-assisted enzymatic biodiesel synthesis were investigated using a 22 full factorial design. The critical factors affecting biodiesel production were the ethanol-to-beef tallow ratio ($X1$, 6–12) and the reaction temperature ($X2$, 40–50° C). The transesterification yield (Y) was selected as the response. The reactions were catalyzed by a lipase from *Burkholderia cepacia*, which was immobilized on silica, and were performed using 8–15 W of microwave radiation. High conversions were achieved using lower molar ratios of ethanol-to-beef tallow, and the effect of temperature was observed to be not significant based on statistical analysis. Under optimized conditions (a 1:6 molar ratio of beef tallow to ethanol at 5° C), the fatty acids in the original beef tallow were almost completely converted into ethyl esters in 8 h of reaction time, and productivity of 92 mg ethyl esters/g.h was achieved (a six-fold increase; [46]).

Virological testing of bottled water is another bioengineering application. A study was conducted to choose the best tool for detecting viruses in bottled water. Different approaches were examined; for example, the recovery of viral RNA was measured following the in-situ lysis of virus particles in the aqueous phase. A second method detected the healing of viral RNA following the lysis of virus particles. The third detection method generated the lowest genome recovery, regardless of water and virus type. Two ways were compared because they were considered viruses. Using a 3^4 4^1 2^1 full factorial design, viral RNA recovery was determined. The independent variables used were three types of water with three different mineral compositions; four viruses (poliovirus, hepatitis A virus, Norovirus, and MS2

phage); three incubation times (1, 10, and 20 days), and two methods (A and B). Every factor except incubation time was found to be significant. Method A provided the best results, suggesting that this method should be used to detect hepatitis A [47].

A study of the metabolic responses of a new neuronal human cell line, AGE1HN, to various substrate values, showed that reduced substrate and pyruvate load improves metabolic efficiency leading to improved growth and a1 antitrypsin (A1AT) production. A 3^2 full factorial design was used to analyze metabolism's adaptation to various pyruvate and glutamate concentrations. The concentrations of pyruvate tested were 2, 5, and 9 mm, and the strengths of glutamate tested were 5, 7.5, and 10 mM. The most important result was that higher pyruvate concentrations in the medium decreased cell proliferation and reduced the efficiency of substrate use. However, the highest viable cell density and A1AT concentration (167% of the batch) could be achieved without adding pyruvate [48].

Most biotechnology unit operations are complex with numerous process variables, feed material attributes, and raw material attributes that can have significant impact on the performance of the process. Design of experiments (DOE) based approach offers a solution to the bioprocessing unit operations. I-optimal and D-optimal designs to the commonly used central composite and Box–Behnken designs for bioprocess applications [49]. Chang and Zandstra [38] have developed and validated such a technology for ES cell differentiation analysis [38]. They used a quantitative screening platform based on automated fluorescence microscopy, which enumerated the ES cells that had entered endodermal differentiation through expression of two biomarkers (cytokeratin-8 and hepatocyte nuclear factor 3β). Using a two-level fractional factorial design model based on 32 triplicate experiments, they screened important medium components for the differentiation process to endodermal cells (glucose, insulin, basic fibroblast growth factor, retinoic acid, and epidermal growth factor) with the biomarkers and cell numbers as responses.

The model was further refined using a subsequent three-level factorial experiment for two of the factors. A statistical regression model was used to identify major and interactive effects on the endoderm formation. Similarly, Yu Ji [50] discussed on Model based process design for bioprocess optimization: case studies on precipitation with its applications in antibody purification. The objective of this study was to design and optimize a precipitation based mAb purification process [50].

The process was selected from two precipitation systems with ammonium sulfate and PEG 6000 as precipitant, respectively. Then it was further evaluated as an alternative mAb capturing step in the general purification platform. The conditions of initial DoE and validation DoE used in model based algorithm for mAb precipitation by ammonium sulfate have carried out with that of the conditions of initial DoE and validation DoE used in model based algorithm for mAb precipitation by PEG. Final optimization was performed of ammonium sulfate using d-optimal design by taking total of 21 points.

Similarly, it has carried out for PEG precipitation also and evaluated. The model based process design approach includes bioprocess modeling, model based experimental design, and high-throughput microwell experimentation. The bioprocess design is based on experimental data and a computational framework with

optimization algorithm. Innovative model based experimental design is a core part in this approach. The method also employs Random design and Simplex to identify extra experiments to increase the accuracy, and will iteratively improve the process design solutions [50].

10.9 DoE-Based Media Formulation Optimization Technique

Design of Experiment (DoE) approaches allow the interaction effects of components to be explored. The response surface methodology (RSM) developed by Box and Wilson has been used to formulate media for bacteria, fungi, and mammalian cells. Several researchers have used the Central Composite design (CCC or CCF) with the RSM optimization technique for media optimization. Media Blending is a common technique used to optimize many media ingredients simultaneously. In this approach, mixtures are ranked based on the highest-performing blends by mixing with already available media. When blending media, their composition should be known and significantly different so that the resulting combinations are unique. However, since all components are changed simultaneously, concluding causal relationships is impossible. Ultimately, media blending reduces the time and cost of optimization compared to the traditional and DoE approaches. For example, Roullier et al. used media blending to increase the process titer by 40% using a high-throughput methodology that took 6 weeks. Sixteen base CDMs containing the same 47 components were combined to create 376 unique media formulations. The collected data was used to build statistical models that could identify critical elements in the media. It was found that ferric ammonium citrate, pantothenic acid, valine, methionine, arginine, biotin, and serine were the most significant components of titer. Effective media formulation requires accurate analytical methods to measure metabolites over the culture duration. Generally, metabolic analyzers (using combinations of biosensors and ion-selective electrodes) weigh around ten primary metabolites and ions. To measure other components, several different techniques are applied, for example, capillary electrophoresis (CE), HPLC, liquid chromatography-coupled with mass spectrometry (LCMS), gas chromatography (GC), and gas chromatography-coupled with mass spectrometry (GCMS). Genomic and transcriptomic studies have also been performed to increase production by altering the temperature of the cultivation, supplying butyric acid, and inducing osmotic shock. Griffin et al. used different levels of gene expression to identify metabolism changes. Millipore Sigma has developed a targeted approach to find the media components to which cells are expected to respond to, eliminating the need for random testing. Specifically, a high-throughput microarray analysis was employed to screen the cytokine receptors responsible for growth in a particular cell line and process. Media components that could then activate those receptors could be tested and used for a more targeted media optimization study. They found four ligands that could enable the receptors in the media. This information could be used to design a much smaller group of experiments around those four components that could optimize cell growth [51].

10.10 Current Challenges and Future Prospective

Modeling has always been an attractive prospect due to its potential to replace laboratory experiments or significantly reduce the amount of experimentation required. Models simply calculate an output as a function of given inputs. If the model inputs are media components, then the formulation can be optimized to produce the desired output. While no formal model based approach has been developed (although it could be argued the DoE approach is a model based approach), it is worth briefly introducing the types of models that have been developed for bio-manufacturing processes and how they might be adapted to media formulation applications. Customizing cell culture media according to individual cell line needs for each method is of paramount importance. Different cell lines and even cell clones require different formulations to optimize performance, and the process type, batch, fed-batch, and continuous require vastly different media. The intensification and concentration of fed-batch feed media have led to problems with precipitation and feed media without significant performance drops needed for perfusion culture to be more cost-competitive. Emerging high-throughput methodologies will accelerate the development and validation of such models. This then leads to a scenario in which on-line, in-line, or at-line PAT could provide inputs to models and controllers on the floor that can optimize feed media on the manufacturing floor, removing the need for months of process development and creating an incredibly robust process from a regulatory standpoint.

10.11 Concluding Remarks

The experimental design is a form of process analysis in which specific factors are selected to obtain the desired responses of interest. It may also be used to determine the effects of various independent factors on a dependent factor. The bioengineering discipline includes many different areas of scientific interest, and each study area is affected and governed by many different factors. Briefly analyzing the essential factors and selecting an experimental design for optimization are handy tools for the design of any bioprocess under question. This chapter summarizes innovative design methods that can be used to investigate various factors relating to bioengineering processes or biotechnology. The basic concepts and applications of the design of experiments (DoE) in recombinant protein biotechnology are also briefly discussed.

References

1. Montgomery DC (2005) Design and analysis of experiments, 6th edn. Wiley, New York
2. Karen LJ (2012) Experimental design in biotechnology. Technometrics 33(1):106–108
3. Perry DH (1991) Book Review. Dry Technol 9(3):817
4. Mandenius CF, Brundin A (2008) Beioprocess optimization using design-of-experiments methodology. Biotechnol Prog 24(6):1191–1203. https://doi.org/10.1002/btpr.67

5. Sahu PK, Rao RN, Cecchi T, Swain S, Patro CS, Panda J (2018) An overview of experimental designs in HPLC method development and validation. J Pharm Biomed Anal 147(5):590–611
6. Yates F (1937) Design and analysis of factorial experiments. Technical communication no. 35. Imperial Bureau of Soil Science, London
7. Pilat P, Votruba J, Dobersky P, Prokop A (1976) Application of mathematical optimization methods in microbiology. Folia Microbiol 21:391–405
8. Singh B, Beg S (2013) Quality by design in product development life cycle. Chron Pharmabiz 22:72–79
9. Singh B, Kumar R, Ahuja N (2004) Optimizing drug delivery systems using systematic "design of experiments." part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst 22(1):27–105
10. Singh B, Raza K, Beg S (2013) Developing "optimized" drug products employing "designed" experiments. Chem Ind Dig 12:1–7
11. Box GE, Wilson K (1951) On the experimental attainment of optimum conditions. J R Stat Soc Ser B (Methodological) 13(1):1–45
12. Bruns RE, Scarminio IS, Barros, N.B.d. (2006) Statistical design-chemometrics. Elsevier, Amsterdam
13. Ferreira SLC, Bruns RE, da Silva EGP, dos Santos WNL, Quintella CM, David JM, de Andrade JB, Breitkreitz MC, Jardim ICSF, Neto BB (2007a) Statistical designs and response surface techniques for the optimization of chromatographic systems. J Chromatogr A 1158(1):2–14
14. Hibbert DB (2012) Experimental design in chromatography: a tutorial review. J Chromatogr B 910:2–13
15. Tarley CRT, Silveira G, dos Santos WNL, Matos GD, da Silva EGP, Bezerra MA, Miro M, Ferreira SLC (2009) Chemometric tools in electroanalytical chemistry: methods for optimization based on factorial design and response surface methodology. Microchem J 92(1):58–67
16. Box GE, Behnken D (1960) Some new three level designs for the study of quantitative variables. Technometrics 2(4):455–475
17. Kuehl RO, Kuehl RO (2000) Design of Experiments: statistical principles of research design and analysis. Duxbury/Thomson Learning, Pacific Grove, CA
18. Otto M (1999) Chemometrics: statistics and computer application in analytical chemistry. Wiley-VCH, Weinheim, New York
19. Ferreira SLC, Bruns RE, Ferreira HS, Matos GD, David JM, Brandao GC, da Silva EGP, Portugal LA, dos Reis PS, Souza AS, dos Santos WNL (2007b) Box–Behnken design: an alternative for the optimization of analytical methods. Analytica Chim Acta 597(2):179–186
20. Hanrahan GZJ, Gibani S, Patil DG (2005) Chemometrics: experimental design. In: Worsfold PJTA, Poole CF (eds) Encyclopedia of analytical science. Elsevier, Oxford, pp 8–13
21. Haaland PD (1989) Experimental design in biotechnology. Marcel Dekker, New York
22. Kwang-Min L (2006) David Gilmore. Statistical experimental design for bioprocess modeling and optimization analysis: repeated-measures method for dynamic biotechnology process. Appl Biochem Biotechnol 135(2):101–116
23. Vijesh K, Akriti B, Anurag SR (2008) Design of experiments applications in bioprocessing: concepts and approach. Biotechnol Prog 24(6):1191–1203
24. Kwang-Min L, David FG (2006) Statistical experimental design for bioprocess modeling and optimization analysis: repeated-measures method for dynamic biotechnology process. Appl Biochem Biotechnol 135(2):101–116
25. Myers RH, Montgomery DC (2002) Response surface methodology: process and product optimization using designed experiments, 2nd edn. Wiley, New York
26. Wu CFJ, Hamada M (2000) Experiments: planning, analysis, and parameter design optimization. Wiley, New York
27. Wang YH, Yang B, Ren J, Dong ML, Liang D, Xu AL (2004) Optimization of medium composition for the production of clavulanic acid by Streptomyces clavuligerus. Process Biochem 40:1161–1166

28. Adinarayana K, Ellaiah P, Srinivasulu B, Bhavani DR, Adinarayana G (2003) Response surface methodological approach to optimize the nutritional parameters for neomycin production by Streptomyces marinensis under solid-state fermentation. Process Biochem 38:1565–1572
29. Cladera-Olivera F, Caron GR, Brandelli A (2004) Bacteriocin production by Bacillus licheniformis strain P40 in cheese whey using response surface methodology. Biochem Eng J 21:53–58
30. Gupte MD, Kulkarni PR (2002) A study of antifungal antibiotic production by Streptomyces chattanoogensis MTCC 3423 using full factorial design. Lett Appl Microbiol 35:22–26
31. Penna TCV, Moraes DA (2002) Optimization of nisin production by Lactococcus lactis. Appl Biochem Biotechnol 98:775–789
32. Torres BJ, Arroyo M, Torres-Guzman R, De La MI, Acebal C, Castillon MP (2005) Optimization of culture medium and conditions for penicillin acylase production by Streptomyces lavendulae ATCC 13664. Appl Biochem Biotechnol 126:119–131
33. Zhuang YP, Chen B, Chu J, Zhang S (2006) Medium optimization for meilingmycin production by Streptomyces nanchangensis using response surface methodology. Process Biochem 41:405–409
34. Zitzmann J, Sprick G, Weidner T, Schreiber C, Czermak P (2017) Process optimization for recombinant protein expression in insect cells. In: Gowder SJT (ed) New insights into cell culture technology. InTech Open, Rijeka, pp 43–98
35. Yildirim S, Thompson MG, Jacobs AC, Zurawski DV, Kirkup BC (2016) Evaluation of parameters for high efficiency transformation of Acinetobacter baumannii. Sci Rep 6:22110
36. Ikonomou L, Schneider Y-J, Agathos SN (2003) Insect cell culture for industrial production of recombinant proteins. Appl Microbiol Biotechnol 62:1–20
37. Ramirez J, Gutierrez H, Gschaedler A (2001) Optimization of astaxanthin production by Phaffia rhodozyma through factorial design and response surface methodology. J Biotechnol 88:259–268
38. Chang KH, Zandstra PW (2004) Quantitative screening of embryonic stem cell differentiation: endoderm formation as a model. Biotechnol Bioeng 88:287–298
39. Govind S, Ketaki K, Saritha S, Gupta MK, Yadav KS (2020) Quality by design (QbD) approach in processing polymeric nanoparticles loading anticancer drugs by high pressure homogenizer. Heliyon 6(4):e03846
40. Chien NB, Tran BT, Thao D, Thi NN (2018) D-optimal optimization and data-analysis comparison between a DoE software and artificial neural networks of a chitosan coating process onto PLGA nanoparticles for lung and cervical Cancer treatment. J Pharm Innov 14(4):1–15
41. Kozlowski S, Swann P (2009) Considerations for biotechnology product quality by design. In: Rathore AS, Mhatre R (eds) Quality by design for biopharmaceuticals. Wiley, New York, pp 9–30
42. Christos P (2019) Design of experiments as a tool for optimization in recombinant protein biotechnology: from constructs to crystals. Mol Biotechnol 61:873–891. https://doi.org/10.1007/s12033-019-00218-x
43. Mendes LF, Vale LA, Martins AP et al (2012) Influence of temperature, light and nutrients on the growth rates of the macroalga Gracilaria domingensis in synthetic seawater using experimental design. J Appl Phycol 24:1419–1426
44. Rahnama F, Vasheghani-Farahani E, Yazdian F, Shojaosadati SA (2012) PHB production by Methylocystis hirsuta from natural gas in a bubble column and a vertical loop bioreactor. Biochem Eng J 65:51–56
45. Nalakath AH, Veiga MC, Kennes C (2012) Biological conversion of carbon monoxide to ethanol: effect of pH, gas pressure, reducing agent and yeast extract. Bioresour Technol 114:518–522
46. Da Rós PC, de Castro HF, Carvalho AK et al (2012) Microwave assisted enzymatic synthesis of beef tallow biodiesel. J Ind Microbiol Biotechnol 39:529–536

47. Huguet L, Carteret C, Gantzer C (2012) A comparison of different concentration methods for the detection of viruses present in bottled waters and those adsorbed to water bottle surfaces. J Virol Methods 181:18–24
48. Niklas J, Priesnitz C, Rose T et al (2012) Primary metabolism in the new human cell line AGE1. HN at various substrate levels: increased metabolic efficiency and a1-antitrypsin production at reduced pyruvate load. Appl Microbiol Biotechnol 93:1637–1650
49. Kumar V, Bhalla A, Rathore AS (2014) Design of experiments applications in bioprocessing: concepts and approach. Biotechnol Prog 30(1):86–99
50. Ji Y (2012) Model based process design for bioprocess optimisation: case studies on precipitation with its applications in antibody purification. PhD thesis submitted to The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering University College, London
51. Shaun CG, Hemalatha B, Houlong L, Seongkyo Y (2018) Media formulation optimization: current and future opportunities. Curr Opin Chem Eng 22:42–47