Design of Experiments for the Development of Ophthalmic Products

7

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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

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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]

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