Design of Experiment for the Development of Vesicular Drug Products

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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

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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

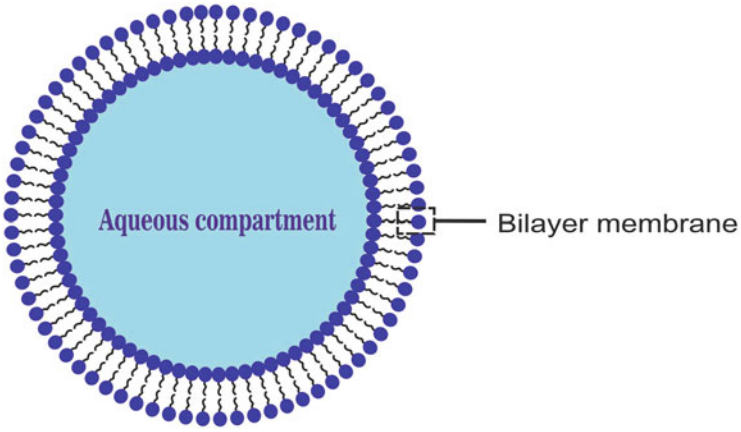

Fig. 8.1 General structure of vesicular systems

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

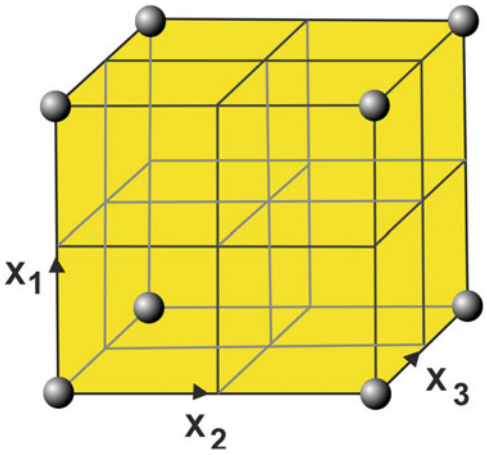

Fig. 8.2 Pictorial illustration of 2^3 full factorial design

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

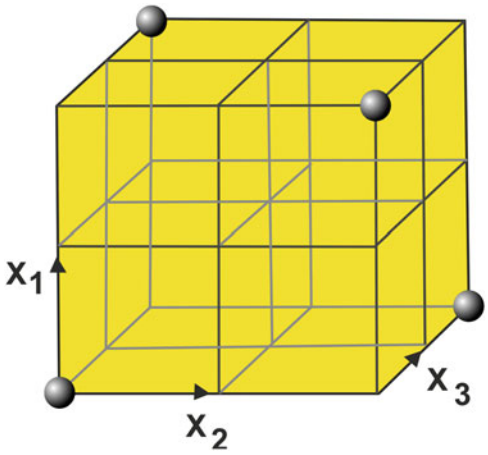

Fig. 8.3 Pictorial illustration of 2^{3-1} fractional factorial design

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

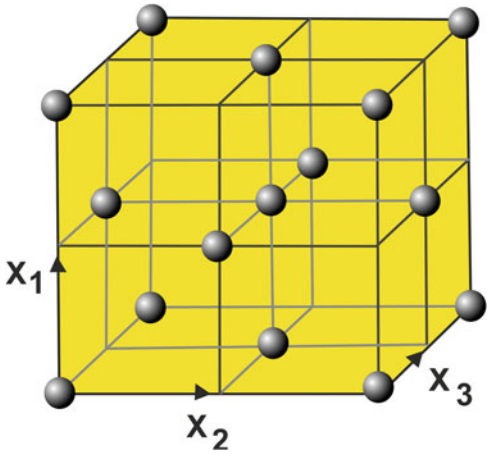

Fig. 8.4 Pictorial illustration of face-centered central composite design

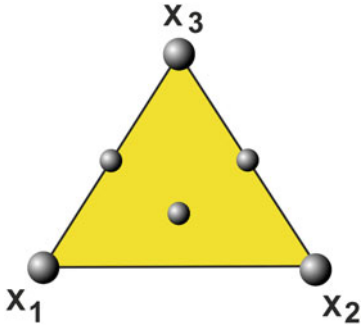

Fig. 8.5 Pictorial illustration of quadratic mixture design

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]

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