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Review

Development of liposomes using formulation by design: Basics to recent advances

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ABSTRACT

In couple of decennia, optimization tactics for drug delivery systems have been explored widely employing Design of Experiments (DoE) for desired outcomes to overcome drawbacks of "One Factor at a Time (OFAT)"conventional technique.. To pace with advances in computational approaches engaged in research domain, QbD-based tactic i.e. Formulation by Design (FbD) is under extensive investigation by budding scientists for better know-how of the product and process development for an unequivocal universal acceptation. Like other vesicular drug carriers, liposomes also demand robustness and reproducibility to scale up at industrial outset. Based on said outlook, this review focuses on the fundamentals and methodologies like Central Composite, Simplex Mixture, Box-Behnken, Factorial, Taguchi, Simplex Centroid, D-optimal, Placket Burman, and Orthogonal array with special reference to applications of FbD in the development of liposomes.

1. Introduction to DoE, QbD and FbD

Paul Ehlrich christened "the magic bullet concept" in 60′s to predict safe and efficacious drug delivery. With advances in nanotechnology, fabricated drug delivery approaches were introduced to establish this concept in true meaning (Svenson, 2004). Indeed, the nanometeric size drug delivery systems (DDS) have shown great promise for enhancing the efficacy of therapeutics and theranostics by many folds. Designing of DDS, essentially requires involvement of various excipients in optimum levels. Process and formula (product) optimization using one factor at a time (OFAT); which includes studying influence of one factor while keeping others constant to obtain a formulation with desired attributes (Singh et al., 2005a). In OFAT approach, the optimum level in terms of composition or process can never be assured owing to the presence of interactions among factors. The end product may be satisfactory but mostly sub-optimal, because there is still scope of a better formulation. Thus, the conventional OFAT approach of drug formulation development suffers from number of disadvantages of being tedious, uneconomical and in-apt to reveal interactions. "Cause and Effect" relationships cannot be established using OFAT method when variables affect each other disproportionately. The inconsistent results obtained in OFAT technique is attributed to lack of knowledge of factor – response relationship. Following the inadequacies posed by OFAT approach systemized approaches evolved. These rationalized methods consist of germane experimental technique which imbibes generation of mathematical equation coupled with graphical representation that typically depicts the response(s) (R) to factor(s) (F) variation where, R is a function of F (Singh et al., 2005a, b). DoE techniques are the most reliable as they can accomplish desired objectives of formulation as a result of fewer experimental runs. Moreover, the screening process in DoE helps in recognizing 'insignificant' and 'significant' input variables. It further saves time, efforts, and materials and is quite economic. Recently, a DoE-based doctrine of Quality by Design (QbD) is being continuously explored by scientists for systematic research. Fig. 1 represents schematic workflow of problem solving using QbD.

QbD is becoming need of the hour in the pharmaceutical field as regulatory agencies such as ICH and USFDA have incorporated it in their federal guidelines (Q8-11). Owing to obeying these federal guidelines, the pharmaceutical industries have started tailoring their strategies for better product and process outcomes. As DoE has much wider domain of application even beyond the pharma sector, therefore, on the heels of QbD paradigm, a brusque twaddle, viz. "Formulation by

Abbreviations: CQA, critical quality attributes; CPP, critical process parameters; CFA, critical formulation attributes; FD, factorial design; FFD, fractional factorial design; CCD, central composite design; BBD, Box-Behnken design; OD, orthogonal design; D-OD, D-optimal design; TgD, Taguchi design; PBD, Plackett-Burman design; SMD, simplex mixture design; ANN, artificial neural network

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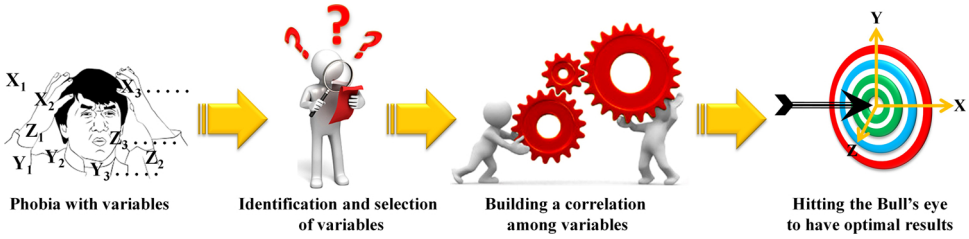

Fig. 1. Workflow of problem solving using QbD.

Design (FbD)", has recently been introduced for development of better pharmaceutical formulations (Singh et al., 2011a). FbD is very distinctive clairvoyant for formulation potential and for variable interaction or concurrence. Also, FbD is able to detect any flaw in formulation and consequently reveal the causes and probable solutions to resolve it. The FbD methodology explores a rational usage of DoE approach to formulate quality DDS in terms of effectiveness and economy. Owing to numerous advantages of FbD tactic, advanced DDS are being explored with systematic optimization process using FbD.

2. FbD tactics

FbD can be carried out using key elements as shown in Fig. 2. It requires meticulous drug product development, clever choice of experimental designs (EDs), identification of critical quality attributes (CQAs), critical formulation attributes (CFAs) and critical process parameters (CPPs), proper defining of design and control space and applying suitable computer-aided optimization. Objective of FbD optimization is comprised of five elements which are schematically represented in Fig. 3.

Meticulous FbD optimization of DDS includes careful "screening" of influential variables and subsequent Response Surface Method (RSM) analysis using EDs. Fig. 4 represents major FbD EDs which are used for optimization of different DDS. The factorial, composite and mixture designs are most explored designs for optimization of DDS. Formulation variables studied exert a significant influence ($p < 0.05$) on the response parameters. Numerical optimization using the desirability approach has been employed to develop an optimized formulation by setting constraints on the dependent and independent variables.

Table 1 summarizes a comparative overview of widely reported EDs used in optimization of DDS (Singh et al., 2011a; Meitz et al., 2014; Garg and Singhvi, 2015; Singh et al., 2017a).

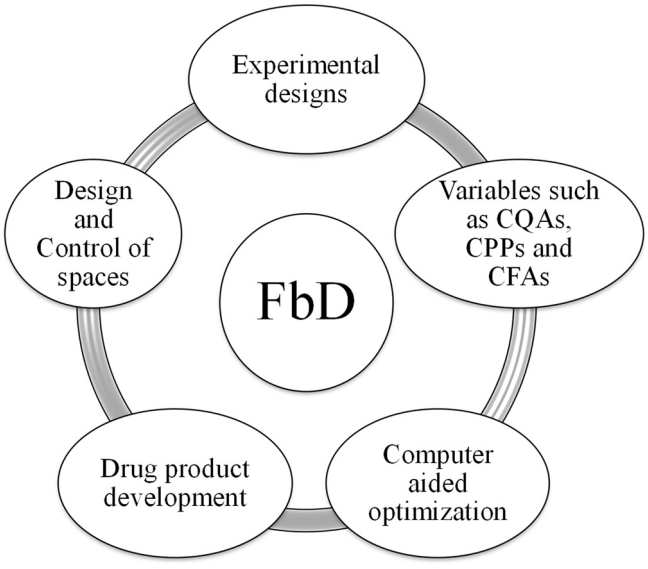

Fig. 2. Essentials to target FbD.

2.1. Selection of DoE

Selection of a suitable design out of existing alternatives depends upon the availability of resources and the degree of control for probable validity to make decisions over errors as a part of desired hypothesis (Box et al., 1978). A design with minimal runs allows verification of curvature in a 2-level screening design and avails reserved resources to rerun process so as to evade unfavorable consequences. FDs, PBDs or TgDs mostly are able to do convenient screening and support only linear responses. However, in order to detect non-linear responses or for more accurate response surface, complex designs are found suitable. Hence, response surface designs are used in order to estimate interactions leading to nonlinear response or even quadratic effects or to predict shape of response surface. It is important to screen out insignificant factors and identify significant ones using interaction effects. For understanding main effects and getting complete information about 2 factor interactions, FDs using color-coding or Min Run Res V designs are useful.

Generally, a product, process, or method is tested for ruggedness. A test method is verified whether it works for all operators or small variations in design don't adversely affect the product. The purpose is to achieve a minimal number of runs and test all variables at once. If no factors are supposed to be significant, then the changes that were made shouldn't affect the product, process, or method. If a factor is significant, then to predict which factor it is, a follow-up experimentation is required e.g. Factorial Design or a Min Run Res IV design. For characterizing the significant factors that affect the responses for optimization purposes, a Response Surface Design (RSD) can be used viz. central composite design (CCD) to study each factor at 5 levels, Box-Behnken design (BBD) at 3 levels or a D-optimal design (DOD) to create a custom design depending upon the type of polynomial. In case of mixture, or a formulation, such as food product, or drug formulation, or a chemical composition, then a Mixture Design should be used to set a total amount for the mixture. Each component range is constrained by the fact that as you increase the amount of one factor; the other factors must decrease so as to keep the total amount constant. Mixture D-optimal designs are most commonly used because they permit the flexibility in your component ranges. To select a suitable RSM design, a comparison must be made to understand the need of a design to carry out experimentation. In general, the major aspects to be considered while selecting an ED can be summarized in the following points:

- All designs can be applied for optimization of product characteristics, but SMDs should not be employed for process optimization.
- Any design out of 2^k FD, x^k FD, FFD, PBD, and D-OD or TgD can be employed for screening studies.
- For estimation of main effects, all 2-level designs except PBD can be employed. However, for higher number of factors (> 6), screening should first be carried out using FFD, and PBD or TgD.
- If there are only two factor levels, any design out of 2^k FD, FFD, and PBD or mixture design can be employed. However, in case of > 3 factor levels, CCD, BBD, Equiradial design, simplex and optimal designs are preferred.
- For quadratic models, x^k FD, CCD, and BBD or EqD are preferred.

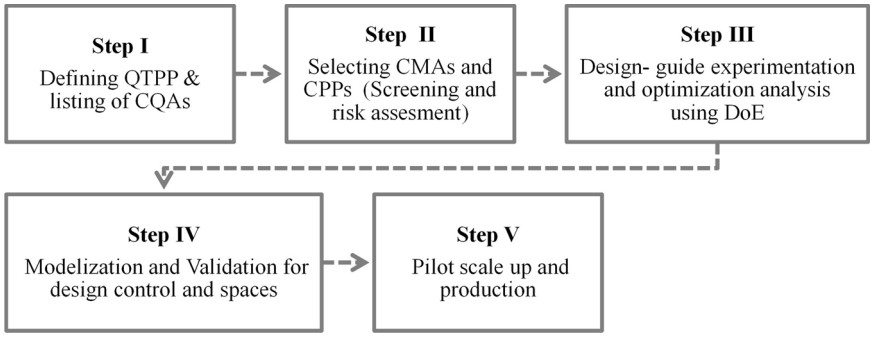

Fig. 3. Sequential flow of FbD opti-tactic.

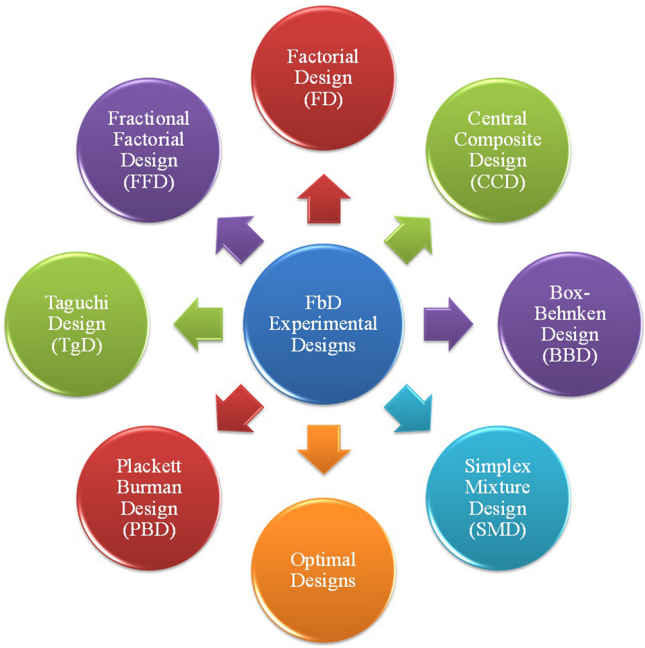

Fig. 4. EDs usually embarked upon during FbD optimization of drug delivery systems.

Table 2 provides a comparative overview of CCD, BBD, and DOD.

2.2. Development of tactic of FbD optimization

Since 1926, use of EDs has permeated in product or process optimization on industrial scale. The classic example includes 'FbD' which serves many objectives in pharmaceutical processing such as preformulation studies, characterization and optimization of drug delivery systems, stability kinetics, drug content assay and its validation etc. (Singh et al., 2005b; Plackett and Burman, 1946; Box and Wilson, 1951; Scheffe, 1958). Using FbD tactic, various DDS have widely been investigated such as nanoparticules, microparticules, macroparticules, vesicular and other innovative DDS. The FbD optimization approach has a crucial role in both product and process optimization of various carrier-based DDS using following factors:

CFAs: Average molecular weight, phospholipids, cholesterol, surfactant, and lipid charge **CPPs:**Temperature, hydration time, and sonication time

CQAs: Percent entrapment efficiency (EE%), vesicle size, dug payload, drug leakage, and percent drug content.

FbD involves mathematical modeling of data. A model is a mathematical or graphical expression intended to define the quantitative dependence of a response variable over the independent variables. Numeric models can either be empirical or theoretical. An empirical model provides a way to describe the factor-response relationship (Box et al., 1978). If a simple model is found to be inadequate for describing the phenomenon, the higher order models are followed. The coefficients for quantitative factors can be estimated using regression analysis. However, in case of the qualitative factors, as interpolation between discrete (i.e. categorical) factor values is meaningless, regression analysis is not employed. For more factors, interactions and higher order terms, multiple linear regression analysis (MLRA) is usually preferred. Multiple nonlinear regression analysis (MNLRA) should be preferred when the factor-response relationship is nonlinear. In multivariate studies, where there are large numbers of variables, the methods of partial least squares (PLS) or principal component analysis (PCA) can also be employed for regression (Box and Draper, 1987). PLS is an extension of MLRA and it is used when there are fewer observations than the number of predictor variables (MacGregor and Kourti, 1995). Model analysis is conducted considering statistical tools like ANOVA, MANOVA, Student's t test, predicted residual sum of squares (PRESS), and Pearson coefficient of determination (r^2). The basic steps involved in creating and analyzing a mathematical model include:

- The data are carefully examined for any deviations and/or apparent problems. Various graphs like response distributions, responses versus time order scatter plot, responses versus factor levels, main effects plots and normal or half-normal plots of the effects are plotted.
- The model assumptions are tested using residual graphs. If none of the model assumptions are violated, ANOVA is applied. The model is simplified further, if possible.
- If model assumptions are violated, suitable model transformation is proposed and a new model is generated. Table 3 summarizes crucial tools employed in FbD optimization (Yasinzai et al., 2011).

Key graphical tools used for validation of FbD models include (i) Normal probability plot of residuals: These plot graphically assesses the data for its distribution pattern, whether normal or not (Singh et al., 2005a), (ii) Response vs. predictions: Such plots divulge any interaction between the independent factors, (iii) Residual lag plots: Randomization of data can be estimated using residual lag plots, (iv) Residuals histogram: The purpose of residuals histogram is to graphically summarize the distribution of a univariate data set.

After selecting the models, optimization of one or multiple responses should be established graphically or numerically or with the help of artificial neural networks (ANN) (Schwartz et al., 1973). ANNs are the machine-based computational techniques that attempt to simulate some of the neurological processing abilities of the human brain. The ANNs offer unique advantages of nonlinear processing capacity and the ability to model poorly understood systems (Leonardi et al., 2009; Zhang et al., 2009; Gohel and Nagori, 2009; Barmpalexis et al., 2010; Miyazaki et al., 2008). Graphical optimization deals with selecting the best possible formulation out of a feasible factor space region (Lewis et al., 1999). It can be accomplished using Brute-force

Table 1
A comparative account of key EDs employed for optimization of DDS.

	FbD type	Cardinal remarks	Advantages/Disadvantages	Reference
RESPONSE SURFACE DESIGNS	**Factorial designs (FD)**	All levels (x) of a given factor (k) are combined with all levels of other factors in the experiment (total number of experiments = x^k).	It uses maximum data and efficiently estimate main effects and interactions but requires number of experimental runs.	(Box and Draper, 1978; Kobilinsky et al., 2017)
	Central composite designs (CCD) or Box-Wilson design	It is commonly used for non-linear responses, particularly in second-order models (Total number of factor combinations = $2^k + 2k + 1$).	It needs fewer experimental runs but problematic to deal with fractional values of α.	(Liu and Xiang, 2007)
	Box-Behnken designs (BBD)	It needs three levels (-1, 0 and +1) for each factor.	It is better alternative to CCD.	(Ferreira et al., 2007)
	Equi radial designs (EqD)	It represents first degree response surface design with N points on a circle around the center of interest in the form of a regular polygon.		(Singh et al., 2017b)
	Mixture designs	Simplex mixture designs (SMDs) are greatly suggested in case of multiple excipients, particularly when the characteristics of the final product depend on the proportions of each excipient.	It is suitable when combination of factor levels is involved but poses interpretive intricacy in polynomials along with failure in finding interactions and quadratic effects.	(Antony, 2014; Berger et al., 2018)
	Optimal designs	It is suitable for domain with irregular shape.	Design steps are somewhat complex.	(Mitchell, 1974; Dette et al., 2017)
SCREENING DESIGNS	**Fractional factorial designs (FFD)**	It represents a finite fraction ($1/x^r$) of a complete or full FD (r = the degree of fractionation and x^{k-r}= total number of experiments).	It can be used for large number of factors or factor levels but effects are puzzled with interaction terms.	(Gunst and Mason, 2009; Li et al., 2018)
	Plackett – Burman designs (PBD)	It is also called as Hadamard designs or symmetrically reduced 2^{k-r} FDs.	It requires minimum number of trials and can address very large number of factors but design structure is complex.	(Vanaja and Shobha Rani, 2007; Sahu and Jain, 2017)
	Taguchi designs (TgD)	It is also called as "off-line quality control" EDs. Depending upon the magnitude of signal-to-noise ratio, these are useful in factor screening for maximization or minimization of responses, or matching to a targeted value.	Variation is more interesting to study than the average. Experiments are run using controllable design factors and disturbing signal factors at two or three levels. It can estimate average effects and the variation at different design factor levels.	(Kumar et al., 2016)

search, Overlay plots, and Canonical analysis. However, mathematical or numerical optimization is suggested in case of multiple responses using desirability function (Shah et al., 2008).

Overall, FbD opti-tactic for the development of liposomal system can be summarized with crucial steps such as (Myers, 2003; Singh and Ahuja, 2004): (i) Problem definition: The FbD problem is clearly comprehended, QTPP (Quality Target Product Profile) vividly defined and possible responses (CQAs) ascertained. (ii) Selection of factors and factor levels: The independent factors (i.e., CMAs, CFAs and/or CPPs) are identified amongst the quantifiable and easily controllable variables. (iii) Design of experimental protocol: Based on the choice of independent factors and the response variables (i.e., CQAs), a suitable ED is selected and the number of experimental runs is determined. (iv) Formulation and evaluation of the dosage form: Various drug delivery formulations are prepared as per the chosen design and evaluated for the desired response(s). (v) Selection of design space and optimum formulation: The experimental data are used for generation of a mathematical model and an optimum formulation is located using suitable graphical and/or numeric methods. (vi)Validation of optimization: The predicted optimal formulation is prepared and the responses are evaluated. Results, if validated, are carried further to the production cycle via pilot plant operations and scale-up techniques. Computer software have been exploited at every stage of optimization process viz., selection of design, screening of factors, use of response surface designs, generation of the design matrices, plotting of 3-D response surfaces and 2-D contour plots (Singh et al., 2011b). Nowadays, problems associated with computers have largely been eliminated with availability of powerful and economical hardware and comprehensive FbD software, application of optimum search methods, partial interpretation of the results, and validation of the methodology (Singh, 1997). While selecting a FbD software package, it is pivotal to look for a statistical engine to obtain fast and accurate results. Widely accepted computer software for FbD optimization of liposomes has been summarized in Table 4.

3. FbD Opti-tactics for liposomes

Liposomes are vesicular carriers made of lipidic bilayer. They mimic biological membranes and are prepared using naturally-derived phospholipids with mixed lipid chains (e.g. egg PE) or other surfactants by sonication. These are being exploited to deliver drugs at the desired site for the treatment of plethora of diseases. To develop liposomes for in vivo use, it is important to design them with optimal in vivo kinetics, and high-throughput production conditions and specific liposome compositions. FbD can be promising approach for the identification of suitable liposome compositions via high-throughput screening methodologies, the design and optimization of vital elements (factors) for liposome production (Wong, 2010). Further, in case of new drug development based on "selectivity for the drug", there are two ways i.e. discovery of new compounds with high selectivity of drug or innovation of newer means of drug administration using novel formulation and/or method with high selectivity of drug by integration and harmonization of various techniques. Nagai (1997) nicely proposed "3H" formula for the new drug development by innovative drug administration i.e. 1. hybrid; 2. hi-quality; 3. husbandry, on which a total research activity (ROR, research on research) could be a milestone to play significant role in “Optimization technology in Pharmaceutical Fields". He also explained these aspects for the trials made on several topical mucosal adhesive dosage forms and parenteral administration of peptide drugs such as insulin and erythropoietin (Nagai, 1997). Gregoriadis in 1988 first time reported optimization studies for liposomes (Gregoriadis, 1988). Fig. 5 represents wagon wheel of possible opti-tactics for liposomes and Fig. 6 shows chronological reports on number of publications based on EDs applied on development of liposomes. Fig. 7 represents Ishikawa diagram showing critical factors (CQAs) affecting the development of liposomes.

Table 2
A comparative overview of CCD, BBD, and DOD.

CCD	BBD	D-OD
Created from a 2-level factorial design, augmented with center points and axial points.	Has specific positioning of design points	Position of design points is chosen mathematically according to the number of factors and the desired model. Therefore, the points are not at any specific positions – they are simply spread out in the design space to meet the d-optimality criteria.
Regular CCDs have 5 levels for each factor, although this can be modified by choosing α = 1.0, a face-centered CCD. The face-centered design has only three levels for each factor.	Always has 3 levels for each factor	For a quadratic model, factors may have either 3 or 4 levels
Created for estimating a quadratic model.	Created for estimating a quadratic model	Can be used to create a good design for fitting a linear, quadratic or cubic model. The user preferences can be changed to get up to a 6th order model.
Replicated center point provides excellent prediction capability near the center of the design space (where the presumed optimum exists)	Provides strong coefficient estimates near the center of the design space (where the presumed optimum exists), but weaker at the corners of the cube (where there weren't any design points)	The desired model can be edited by removing terms which aren't significant or cannot exist. This will turn in reduction of the required number of runs.
Insensitive to missing data	If runs are found to be missing at the end, the accuracy of the remaining runs becomes critical to the dependability of the model. BBD is not recommended if it gives a bad run or has missing data.	Constraints can be added to design space, for instance to exclude a particular area where responses are not obtained. D-optimality mathematically chooses points to minimize the integrated variation of the coefficients for the model to get the most precise coefficients.
The central composite designs have more runs initially and this makes them more robust to problems.		Generally, the D-OD has 1-2 more runs than BBD, so this provides a little more protection for the model coefficients in case of losing some data at the end.

For gene delivery, Zhong et al. (2007a,b) developed pro-cationic liposomes - protamine-DNA (PLPD) vectors using RSM design (3-factor/5-level/15-runs) with various independent variables such as protamine/DNA ratio (w/w), molar percent of cholest-5-en-3beta-yl[2-[[4-[(carboxymethyl)dithio]-1-iminobutyl]amino]ethyl] (CHETA), and CHETA/DNA ratio (w/w), and response variables such as PLPD size and

Table 3
Crucial tools used in FbD optimization.

Model Diagnostic Plots	Cardinal remarks
Half-normal plots	Used to select effects to be included in the model. The large effects (absolute values) appear in the upper-right section of the plot, while the lower-left portion of the plot contains effects caused by noise rather than a true effect. By rationally selecting the factor effects, the actual factors which are influencing the product and processes are screened out.
Pareto charts	Useful in factor screening exercise to find out the influential factors affecting the responses. The Pareto chart is an additional graphic used to display the t values of the effects. In Pareto analysis, the statistical significance of the effect of all the factors is analyzed. Pareto chart contains vertical bars and two horizontal lines i.e., t-value line and Bonferroni line. The factors above the t-limit and Bonferroni limit are selected to be most influential. However, the half normal plot of effects to choose the statistically significant effects is more recommended.
Actual *vs.* predicted	Graph plot between the observed and predicted response values, which helps in detecting a value or a group of values that are not easily predicted by the model. Ideally, such plots passing through origin exhibit linearity i.e., $r^2 \approx 1$.
Residuals versus predicted	"Residuals" (or error) is the numeric difference between the observed and the predicted response(s). Studentized residuals are the residuals converted to their standard deviation units. This plot of residuals versus the ascending predicted response values tests the assumption of constant variance. It should be a random scatter (constant range of residuals across the graph.) Expanding variance ("megaphone pattern < ") in this plot indicates the need for a transformation.
Residuals versus run	This is a plot of the residuals versus the order of the experimental runs. It checks for the "lurking variables" that may have influenced the response during the experiment. The plot should show a random scatter. Trends indicate a time-related variable lurking in the background.
Residuals versus factor	This is a plot of the residuals versus any selected factor. It checks whether the variance, not accounted for by the model, is different for different levels of a factor. If all is okay, the plot should exhibit a random scatter. Pronounced curvature may indicate a systematic contribution of the independent factor that is not accounted for by the model.
Normal probability plot	It investigates the normal probability distribution of residuals, as judged from the linear trend of the points, when plotted on a probit scale. It indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line. Definite patterns like an "S-shaped" curve indicate a transformation of the response may provide a better analysis.
Box–Cox plot	It helps in determining the most appropriate power transformation for application to response data. Most data transformations can be described by the power function, $\sigma = \text{fn}(\mu\alpha)$, where σ is the standard deviation, μ is the mean, and α is the power. If the standard deviation associated with an observation is proportional to the mean raised to α power, then transforming the observation by $(\lambda = 1 - \alpha)$ power yields a scale satisfying the equal variance requirement of the statistical model. This plot is not displayed when either the logit or the arcsine square root transformation has been applied.
Influence Plots	**Cardinal remarks**
Outlier T plot/R-Student	It is also called Externally Studentized Residual. It is the measure of how many standard deviations from the actual value deviates to the value predicted after deleting the point in question. Many times, it is referred as an externally studentized residual, and the outliers should be investigated to find out if a special cause can be assigned to them.
Cook's distance	This provides measures of the influence, or potential, of the individual runs. It is a measure of the effect that each point exerts on the model. A point that has a very high distance value relative to the other points may be an outlier.
Leverage plot	This is a measure of degree of influence of each point on the model fit. If a point has a leverage of 1, it controls the model and the model must go through that point. A point with leverage of near one should be reduced by adding or replicating points.
DFFITS	These measures the influence of each point has on the predicted value. This is a standardized value and can be interpreted as the number of standard deviation units. For small to medium size datasets, DFFITS should not exceed 1, while for large datasets it should not exceed $2\sqrt{\frac{p}{n}}$.
DFBETAS	These measure the influence of each point on each regression coefficient. Like DFFITS, it is a standardized value and follows the same guidelines.

Table 4
Widely accepted computer software for FbD optimization of liposomes.

Name of Software	Web address
Design Expert	www.statease.com
OPTIMA	www.optimasostware.co.uk
STATISTICA	www.statoftinc.com
Omega	www.winomega.com
COMPACT	www.fp.mcs.anl.gov
Isight	www.engenious.com
SOLVER	www.solver.com
JMP	www.jmp.com
GRG2	www.fp.mcs.anl.gov
DOE PRO XL & DOE KISS	www.sigmazone.com
SPSS	www.spss.com
ECHIP	www.echip.com
MINITAB	www.minitab.com
MATREX	www.rsd-associates.com/matrex.htm

PLPD transfectivity i.e. β-galactosidase (mU)/ total protein (mg), respectively. Optimized levels in the mathematical model showed optimal particle size (PS) and transfectivity as 228.9 ± 8.0 nm and of 24.26 ± 2.60 [β-galactosidase (mU)/total protein (mg)], respectively (Zhong et al., 2007a). Systematic characterization of encapsulation process can be carried out using DoE approach on the basis of the systematic screening of process parameters. Print 3 G is a peptidic agent which is reported to show anti-angiogenic activity against breast tumors. Ducat et al. (2010) encapsulated Print 3 G in PEGylated liposomes composed of SPC: CHOL: mPEG2000-DSPE (47:47:6, molar % ratio). Print 3 G adsorption on different materials such as glass beads, tubing, and connections for extrusion was used for liposome preparation. The freeze-thawing technique was used to enhance the amount of Print 3 G encapsulation into the liposomes. They employed DoE approach to get optimal EE with minimal experimental runs and influencing factors such as the lipid concentration and the number of freeze-thawing cycles. Results showed the optimal EE as 63% as represented in Fig. 8 (Ducat et al., 2010).

Zhao et al. (2012) optimized glycyrrhetinic acid liposomes (GAL) with RSM and studied for immunological activity. The optimal preparation conditions for GAL were found to be 9:1 of soybean phospholipid cholesterol (w/w) at 31 °C (bath temperature) which resulted in EE of 83.46 ± 0.55% (Zhao et al., 2012). Curic et al. (2013) characterized and optimized the encapsulation process of itraconazole (ITZ) into the PEGylated liposomes using thin film hydration technique combined with sonication. Optimization showed a confined design space to obtain the highest EE (%) and drug loading more than 90% and 0.3% (w/w), respectively, using 25 mg/ml of lipid, with robustness in results (Curic et al., 2013). After oral administration, local delivery to bowel tissue is a challenging task particularly in the treatment of

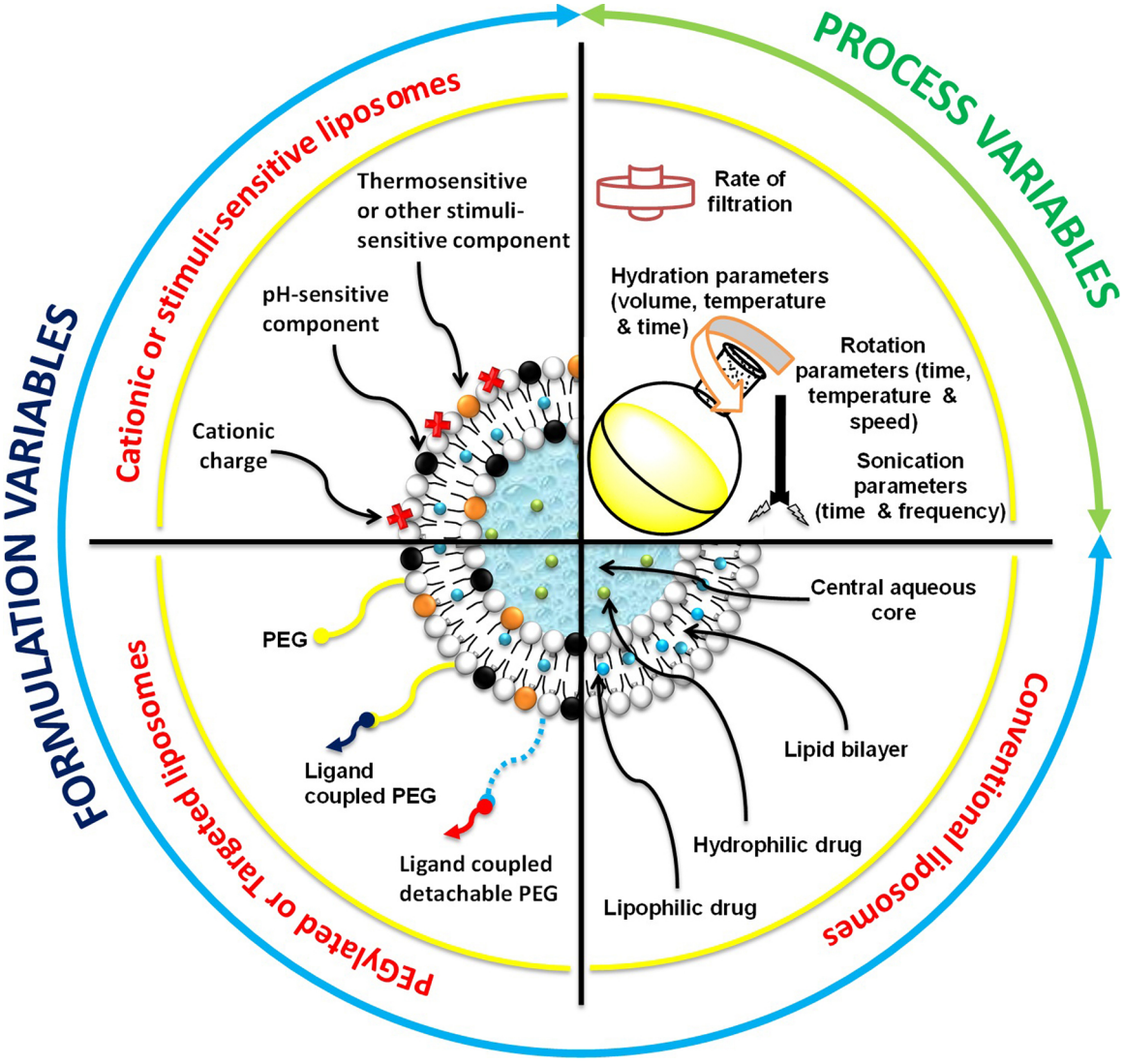

Fig. 5. Possible opti-tactics employed for development of liposomes.

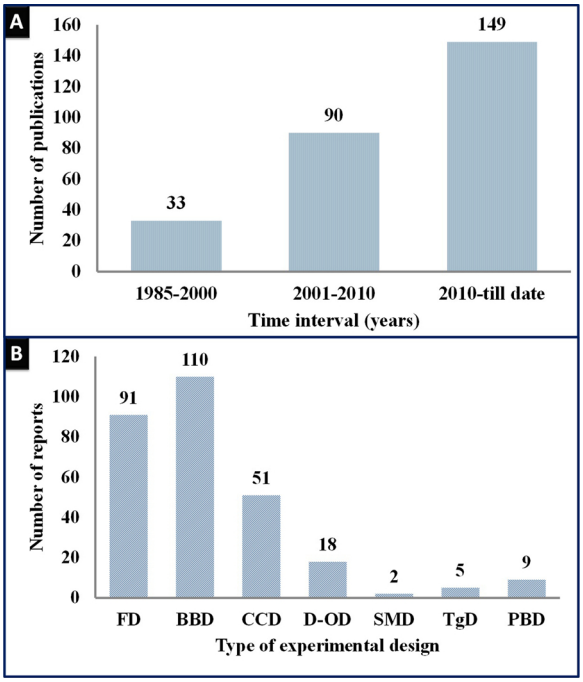

Fig. 6. Chronological reports on number of publications based on FbD applied on liposomes: (A) Total number and (B) Reports on Individual design till date. (Source: https://www.ncbi.nlm.nih.gov/pubmed, accessed on 6 May 2018)

inflammatory bowel disease (IBD). For this purpose, Gupta et al. (2013) developed colon specific liposomes using thin film hydration method with DoE approach to deliver the liposome spatially in the initial segment of colon. PS and EE were found to be 200–300 nm and 40–60%, respectively (Gupta et al., 2013). Wang et al. (2014) paid efforts to optimize process conditions for Madecassoside (MA) liposomes using RSM for maximum drug EE. MA liposomes were optimized for favorable uniformity and optimum PS (Wang et al., 2014).

A nonlinear RSM incorporating multivariate spline interpolation (RSM-S) is a useful technique for the optimization of pharmaceutical formulations. Duangjit et al. (2014) reported the bootstrap (BS) resampling technique to evaluate the direct reliability of the optimal liposome formulation predicted by RSM-S. They used the formulation characteristics such as vesicle size, size distribution, zeta potential, elasticity, drug content, EE, release rate, and the penetration enhancer factors as formulation factors, with its type and content were used as causal factors of the response surface analysis. The intended responses were high skin permeability in terms of flux and high stability of formulation in terms of drug leakage. Based on the dataset obtained, the simultaneous optimal solutions were estimated using RSM-S. Leave-one-out-cross-validation (LOOCV) showed satisfying reliability of the optimal solution (as shown in Fig. 10). The correlation coefficients of the estimated and experimental values for the skin permeability (Y1) and the stability of formulation (Y2) were extremely high (RLOOCV = 0.9653 and 0.9984, respectively). These results suggested that RSM-S successfully predicted the relationship between the causal factors (formulation characteristic and formulation factors) and pharmaceutical response variables. These results indicated that an original optimal solution with acceptable characteristics (e.g., high skin permeability and good stability formulation) could be estimated with RSM-S (Duangjit et al., 2014). Using RSM, Luo et al. (2014) optimized liposomes bearing epigallocatechin-3-gallate (EGCG) for prevention of carcinogenesis. The optimal preparation conditions were as follows: phosphatidylcholine-to-cholesterol ratio of 4.00, EGCG concentration of 4.88 mg/mL, Tween 80 concentration of 1.08 mg/mL, and rotary evaporation temperature of 34.51 °C. Under these conditions, the experimental EE and size of EGCG nano-liposomes were 85.79% ± 1.65% and 180 nm ± 4 nm, respectively, which were close to the predicted value. The malondialdehyde value and the in vitro release study revealed increased stability of developed EGCG nanoliposomes. Moreover, encapsulation of EGCG in nanoliposomes enhanced its inhibitory effect on tumor cell viability at higher concentrations in comparison to free EGCG (Luo et al., 2014). Yu et al. (2014) optimized gypenosides liposomes (GPSL) using RSM. Optimal conditions of GPSL preparation were found to be the lipid to drug (8:1, w/w), soybean phospholipid to cholesterol (6:1, w/w), the temperature of incubation of drug in water bath of 55 °C, and the time of incubation of drug of 11 min. With these conditions, the EE of GPSL was found to be 90.17 ± 0.38% (Yu et al., 2014). Pandey et al. (2014) employed QbD tactic for the development of chitosan-coated nanoliposomes (CH-NLPs) bearing a hydrophilic drug. Screening of factors like the concentrations of lipid, cholesterol, drug and chitosan; stirring speed, sonication time; organic: aqueous phase ratio; and temperature was conducted. For optimized CH-NLPs, various responses were observed i.e. 111.3 nm, 33.4% and 35.2% for PS, %EE and coating efficiency (%CE), respectively, which were in

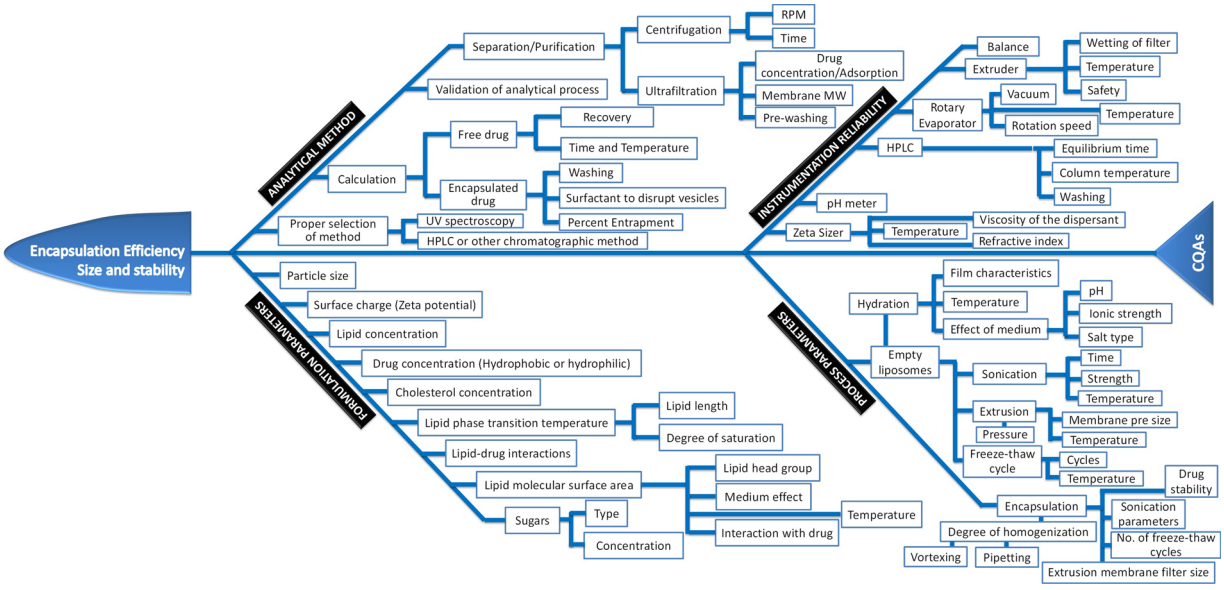

Fig. 7. Ishikawa diagram showing critical factors (CQAs) affecting the development of liposomes.

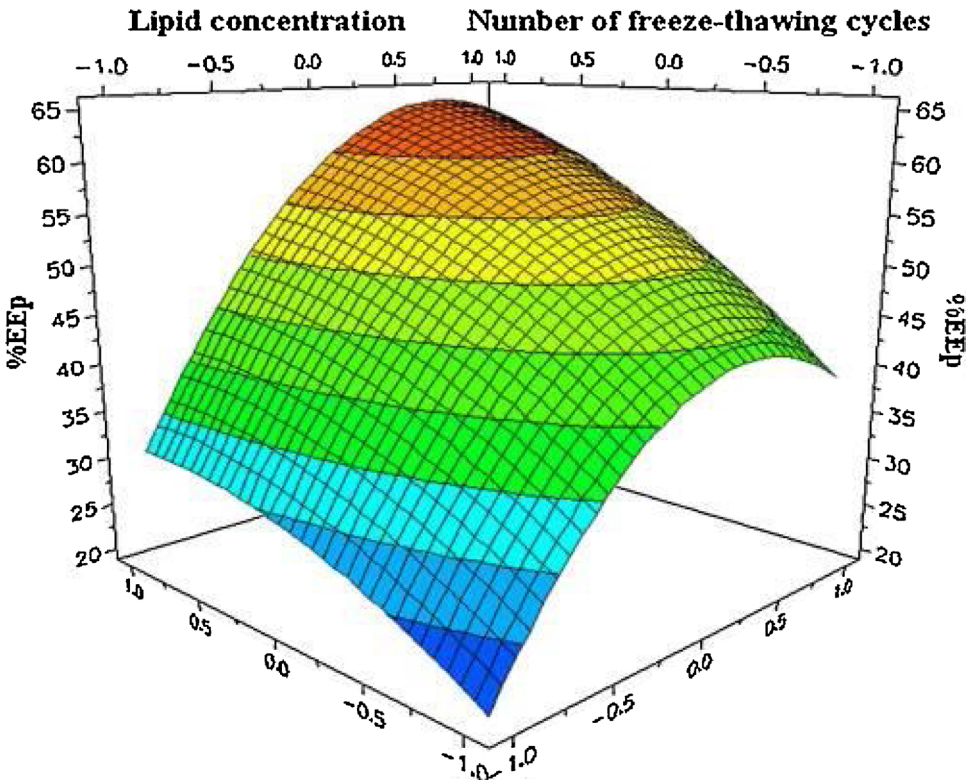

Fig. 8. The upper darker area represents the optimal condition for the peptide encapsulation.

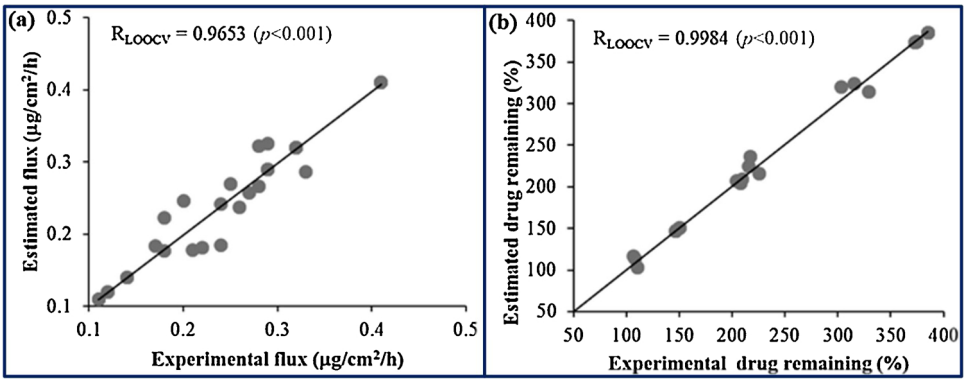

Fig. 9. The LOOCV estimated accuracy and reliability of the response surface variables: (a) Skin permeability (flux) and (b) stability of formulation (drug remaining at 25 °C for 30 day).

accord to the predicted values, that assured the suitability and robustness of the design space minimizing both intra- and inter-batch variations (Pandey et al., 2014) (Fig. 9).

4. Central composite design (CCD)

The most popular RSM design is the CCD. A CCD has three groups of design points: (a) two-level factorial or fractional factorial design points (b) axial points (sometimes called "star" points) (c) center points. CCDs are designed to estimate the coefficients of a quadratic model. All point descriptions will be in terms of coded values of the factors. RSM based on a CCD has been successfully employed to model and optimize pharmaceutical processes (Baş and Boyacı, 2007; Anderson and Whitcomb, 2016).

Lipid Protamine DNA vectors are promising non-viral gene delivery cargoes. Sun and Zhang (2004) developed anionic LPD vectors having protamine-DNA complexes and pH sensitive (DOPE/CHEMS) liposomes. Optimization was performed using CCD (three factor, five-level) with protamine/DNA (w/w), CHEMS/DNA (w/w) and CHEMS/DOPE (molar ratio) as the independent variables and LPD size (Y1) and LPD protection efficiency against nuclease as the response variables. The model fitting showed an optimized LPD formulation with PS of 185.3 nm and protection efficiency of 80.22% (Sun and Zhang, 2004). In similar study, procationic-liposome-protamine-DNA (PLPD) vector and transferrin (Tf) coupled PLPD (Tf-PLPD) have been prepared and modeled (CCD) using PC, CHOL, and cholest-5-en-3-ol(3beta)-[2-[[4-[(carboxymethyl)dithio]-1-iminobutyl] amino] ethyl] carbamate) (CHETA) with the help of film dispersion-filtration technique. PLPD and Tf-PLPD showed stability in serum. PLPD showed lesser cytotoxicity to HepG2, SMMC7721, and Chang's normal hepatocyte as compared to LPD. This modeled approach increased transfection efficiency of PLPD in terms of effective condensing capacity of protamine and the targeting ability of Tf (Zhong et al., 2007b). Nobiliside A (Nob) causes hemolysis and toxicity. Xiong et al. (2009) developed Nob-liposomes using central composite rotatable design (CCRD). These liposomes were optimized for concentration of PC, CHOL, and lipids/drug ratio to get maximum

EE and minimum hemolytic rate (HR). It was found that EE was significantly affected by CH proportion and lipids/drug ratio while HR was affected by PC/CH ratio. The optimal values of PC, CHOL, and lipids/drug ratio were found to be 2% (w/v), 0.9% (w/v), 40 (w/w), respectively, with EE more than 95% and less than 1% HR at 80 μg /mL (Xiong et al., 2009). Guan et al. (2013) optimized saikoside (SS) liposomes for various preparation methods such as thin film hydration, ether injection, reverse phase evaporation and pH gradients and analyzed for critical factors affecting EE. They observed optimum EE of 60% at size of 110 nm by single factor analysis and CCD (Guan et al., 2013). Ingvarsson et al. (2013) prepared an inhalable powder formulation of cationic liposomes [dimethyldioctadecylammonium (DDA) bromide/ trehalose-6,6′-dibehenate (TDB)] by optimizing key spray drying parameters (CPPs and CQAs) with the use of risk assessment and DoE for getting an optimal operating space (OOS). A CCD (face-centered) was applied with the subsequent employment of MLRA. They demonstrated four CQAs: the mass median aerodynamic diameter (MMAD), in process liposome stability (size), the moisture content and the yield, and five CPPs: drying airflow, feed flow rate, feedstock concentration, atomizing airflow and outlet temperature. The OOS reflected that optimal moisture content should be below 3% with modeling of the liposome size stability (size ratio after and before spray drying) (Ingvarsson et al., 2013). Ma et al. (2014) developed β-lactoglobulin (β-Lg) liposomes to prevent allergic reaction using RSM − CCD technique. EE was taken as a dependent variable which was optimized using various independent variables *viz*. PC and CHOL, β-Lg, sonication time (5–25, min) and temperature (25–40 °C). Desirability criteria suggested PC to CHOL ratio, β-Lg conc., sonication time and temperature as 8.05, 9.09 mg/mL^{-1}, 17.71 min, and 30 °C, respectively. Optimized conditions resulted in 189 nm size liposomes with 61.55% EE (Ma et al., 2014). Dithranol (anthralin) poses potential formulation hurdles such as photolability and high lipophilicity. Dithranol-loaded emulsomes have been developed using thin film casting method with FbD approach for enhancing permeation and skin retention. Compritol 888 ATO and Phospholipon 90 G were optimized critically. As compared to marketed product of dithranol, the optimized emulsomes were comparatively non-irritant, stable and biocompatible (Raza et al., 2013).

5. Simplex mixture design (SMD)

Sinomenine liposomes (SLs) were developed using ether injection and optimized with SMD (applied single-factor test)) for phospholipids (PL), cholesterol (CHOL) and Vitamin E (Vit. E) to obtain maximum PDE. The optimal proportion (mass ratio) of sinomenine: PL: CHOL: Vit. E was found to be 8.92: 60.35: 28.81: 1.91, respectively, for developed SLs at 50 °C and ultrasonic time of 20 min (Wang et al., 2009).

6. Box-Behnken Design (BBD)

BBD are response surface designs, specially made to require only 3 levels, coded as -1, 0, and +1. Box-Behnken designs are available for 3–10 factors. They are formed by combining two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial. Because there are only three levels, the quadratic model is appropriate. Blocking options are also offered for most of these designs. Categorical factors can also be added to this design. Lin et al. (2014) systematically optimized and characterized the co-encapsulation process of Salvianolic acid B (Sal B), Tanshinone II A (TSN) and Glycyrrhetinic acid (GA) into liposomes. The liposomes (GTS-lip) were prepared using film hydration method combined with probe sonication to encapsulate two hydrophobic components (TSN and GA), and using pH gradient method to load hydrophilic component Sal B. Systematic optimization of encapsulation process was performed using single factor test, orthogonal test in combination with BBD. Optimum conditions are as follows: ratio of GA to lipid (w/w) = 0.08, ratio of Sal B to lipid (w/w), 0.12, and pH of buffer, 3.3, respectively. Based on the conditions mentioned above, EE of Sal B, TSN and GA reached target levels: 96.03 ± 0.28%, 80.63 ± 0.91% and 88.56 ± 0.17%, respectively. The GTS-lip had a unimodal size-distribution and a mean diameter of 191.3 ± 6.31 nm. The accumulative release percentage of TSN, GA and Sal B were 10% in 36 h, 4% in 36 h and 77% in 24 h, respectively (Lin et al., 2014). Rane and Prabhakar (2013) investigated the combined influence of 3 independent variables in the preparation of paclitaxel containing pH-sensitive liposomes. A 3 factor, 3 levels BBD was used to derive a second order polynomial equation and construct contour plots to predict responses. Selected independent variables were PC: DOPE (X1, molar ratio), CHEMS (X2, molar concentration) and amount of drug (X3). Fifteen batches were prepared by thin film hydration method and evaluated for percent drug entrapment, vesicle size, and pH sensitivity. The transformed values of the independent variables and the percent drug entrapment were subjected to multiple regression to establish full model second order polynomial equation. F was calculated to confirm the omission of insignificant terms from the full model equation to derive a reduced model polynomial equation to predict the dependent variables. Contour plots were constructed to show the effects of X1–X3 on the percent drug entrapment (Fig. 10). A model was validated for accurate prediction of the percent drug entrapment by performing checkpoint analysis. The computer optimization process and contour plots predicted the levels of independent variables X1–X3 (0.99, -0.06, 0, respectively), for maximized response of percent drug entrapment with constraints on vesicle size and pH sensitivity (Rane and Prabhakar, 2013).

Shi et al. (2012) reported optimization of paeonol (PAE) loaded liposomes gel for transdermal delivery using a BBD (three-factor, three-level) with a second-order polynomial equation and 3-D contour plots. Various independent variables studied such as the DC − CHOL concentration (X1), lipid/drug (X2, molar ratio), and the polymer concentration (X3), at low, medium, and high levels were studied. %EE (Y1), flux (Y2), and viscosity of the gels (Y3) were taken as the dependent variables. There was proportionate increase in %EE of PAE with increase in lipid/drug ratio while opposite results were found with increasing polymer concentration. However, the flux was found to be increased with increasing concentration of polymer and DC − CHOL. In case of viscosity of gels, the polymer concentration directly affected the viscosity (Shi et al., 2012). Yang et al. (2012) developed plasmid DNA-loaded cationic liposomes (DC − CHOL/DOPE) using different methods i.e. dry-film, reverse phase evaporation and ethanol injection with BBD. Various parameters such as DC − CHOL/DOPE, total lipid content, cationic lipid to pDNA charge ratio were optimized. Interestingly, the results reflected the best quality and stability of liposomes prepared using dry-film method with high desirability (Yang et al., 2012).

7. Factorial design (FD)

A factor is an independent variable that is controlled and varied during the course of an experiment. Bioadhesive liposomes modified with pectin and chitosan for peroral administration of bovine lactoferrin (bLf) were prepared using a 2^4 -FD to identify the key formulation variables influencing PS and drug EE (Yao et al., 2014). In another study, coating process of lidocaine hydrochloride (LID) liposomes by a biodegradable polymer (chitosan, CH) was optimized using fractional factorial ($2^{(5-1)}$) screening matrix on the basis of the concentration of CH coating solution, the dripping rate, the stirring rate to maximize % EE and coating efficiency (%CE). FFD helped in quantifying the interactions between the critical factors. It was found that amount of LID was the predominant factor affecting EE directly while the concentration of the CH solution and the stirring rate affected the CE (%) (Gonzalez-Rodriguez et al., 2007). Fusion proteins and the pVIII major coat protein are being investigated for targeting tumors using various

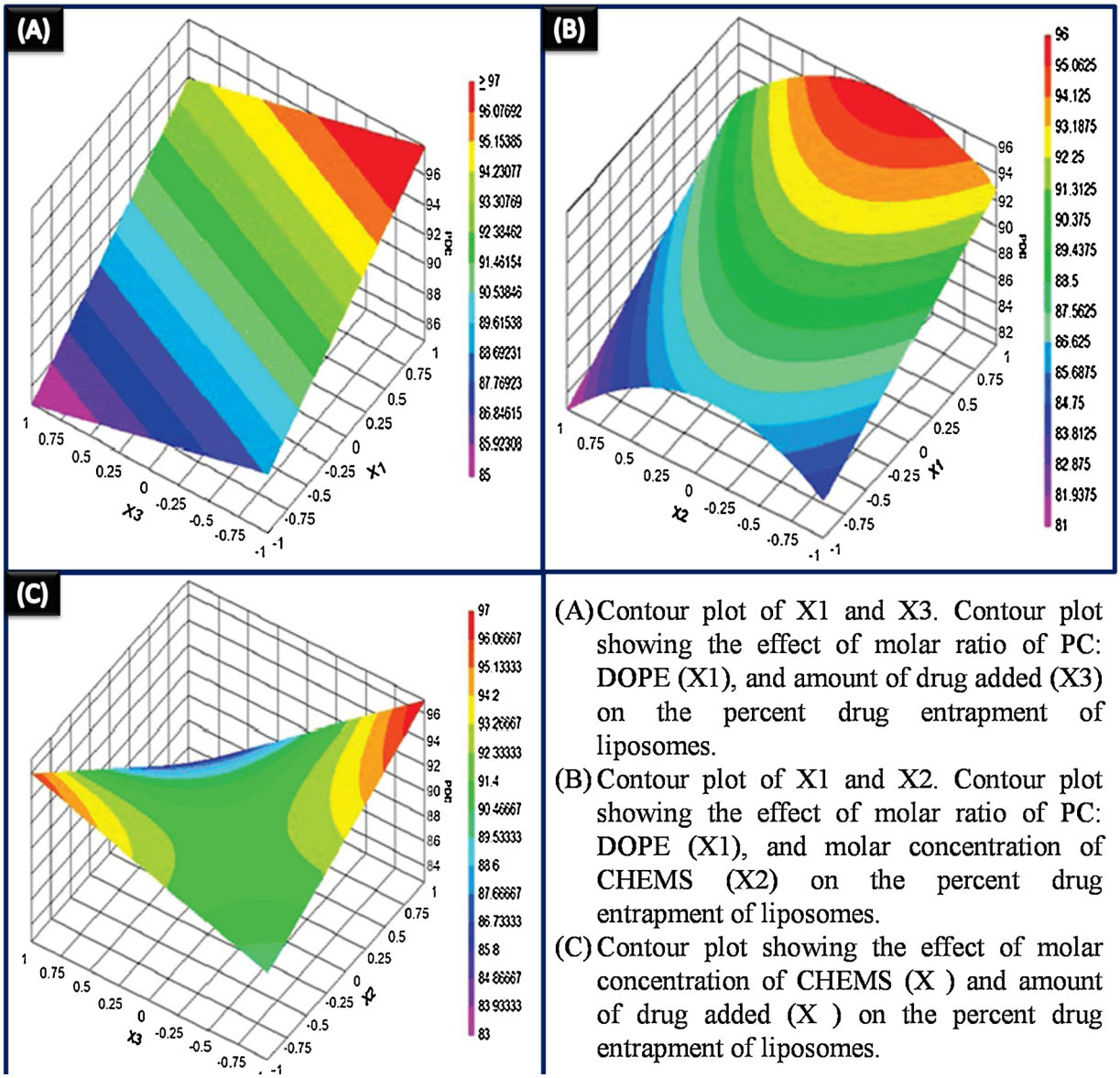

Fig. 10. Contour plots showing the effects of X1, X2, and X3 on the percent drug entrapment.

nanocarriers including liposomes. Such phage protein-fabricated liposomes demand optimization for lipid composition and phage protein content so as to improve the targeting efficiency. Using co-culture targeting assay and a FD, various parameters were optimized including size, zeta potential and morphology for increasing stability and homogeneity of these liposomes (Wang et al., 2011). Table 5 discusses application potential of FD in development of liposomes.

8. Taguchi design (TgD)

Taguchi design is a conventional methodology. It consists of orthogonal arrays (OA) which are two-level, three-level, and mixed-level fractional factorial designs. The use of signal and noise factors, inner and outer arrays, and signal-to-noise ratios are a unique aspect of this design (Taguchi, 1986). The objective of TgD is to screen the dominant or controlling factors which impact the response variables to generate acceptable values of responses irrespective of natural, environmental and process variability. In each experiment, TgD approach employs two designs called the inner and outer array. The Taguchi experiment is the cross product of these two arrays. The control factors form the inner array, while the noise factors, associated with process or environmental variability, form the outer array. Taguchi's signal-to-noise ratios are functions of the observed responses over an outer array. We choose from inner and outer array designs, which use the traditional Taguchi orthogonal arrays, such as L4, L8, and L16. Dividing system variables according to their signal and noise factors is a key ingredient in robust engineering. Signal factors are system control inputs. Noise factors are variables that are typically difficult or expensive to control. A signal-to-noise ratio is a statistic calculated over an entire outer array. Its formula depends on whether the experimental goal is to maximize, minimize or match a target value of the quality characteristic of interest. A Taguchi experiment repeats the outer array design for each run of the inner array. The response variable in the data analysis is not the raw response or quality characteristic; it is the signal-to-noise ratio (Taguchi et al., 2005; Montgomery, 2017). Xia et al. (2006) studied coenzyme Q10 (CoQ10) nanoliposomes and their feasibility of production using ethanol injection and sonication techniques. These liposomes were optimized using OAD. The optimal liposomes were found to have PL/CoQ10/CHOL/ Tween 80 in the weight ratio of 2.5:1.2:0.4:1.8, size of 68 nm, %EE of 95% with more than 90% retention ratio. Interestingly, incorporation of CoQ10 increased the microviscosity of liposomal formulation and inhibited the peroxidation of PL (Xia et al., 2006). Saroja

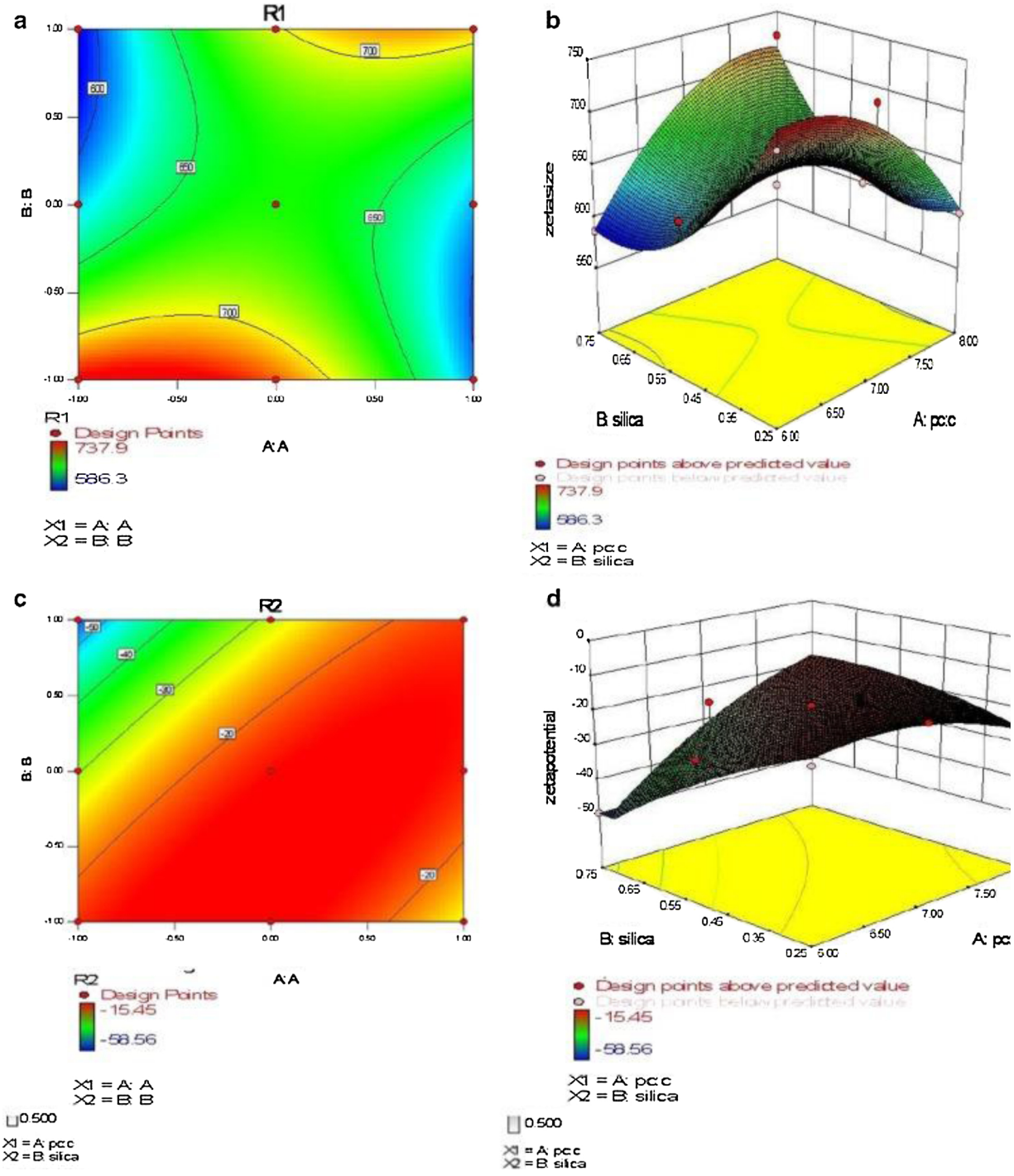

Fig. 11. Graphical evaluation of the design a one factor; b interaction with the silica (second factor); c contour graph representing the standard error of the design; d 3D graph of both factors representing the standard error of the design.

and Lakshmi (2013) developed fenofibrate lipospheres using the melt dispersion method. Various important parameters effecting PS and EE were optimized by employing the L9 TgD. With olive oil up to 30%, maximum EE was found to be 87% in the optimized formulation (Saroja and Lakshmi, 2013). The adjuvant activity of epimedium polysaccharide (EPS) was improved by incorporation into the liposomes (EPSL) using an orthogonal L9 (3^4) TgD. 14-day-old chickens (350, 7 groups) were vaccinated with Newcastle disease (ND) vaccine and EPSL, EPS and blank liposomes were administered simultaneously. The optimal EPSL was found at 1:30 drug to lipid ratio, 4:1 soya PC to CHOL ratio, 10 min ultrasonic time, and 40 °C temperature of water bath. EPSL showed enhancement of the immune response of ND vaccine and cytokines secretion thereby improving the adjuvant activity of EPS (Gao et al., 2012).

9. Simplex-centroid design (SCD)

SCD is a type of mixture design used to analyze the relationship involved in a process that contains several variables. It is constructed to form a triangle with data points located at each corner, the three midpoints on each side, as well as the center and is highly effective at demonstrating the significance related to the three primary

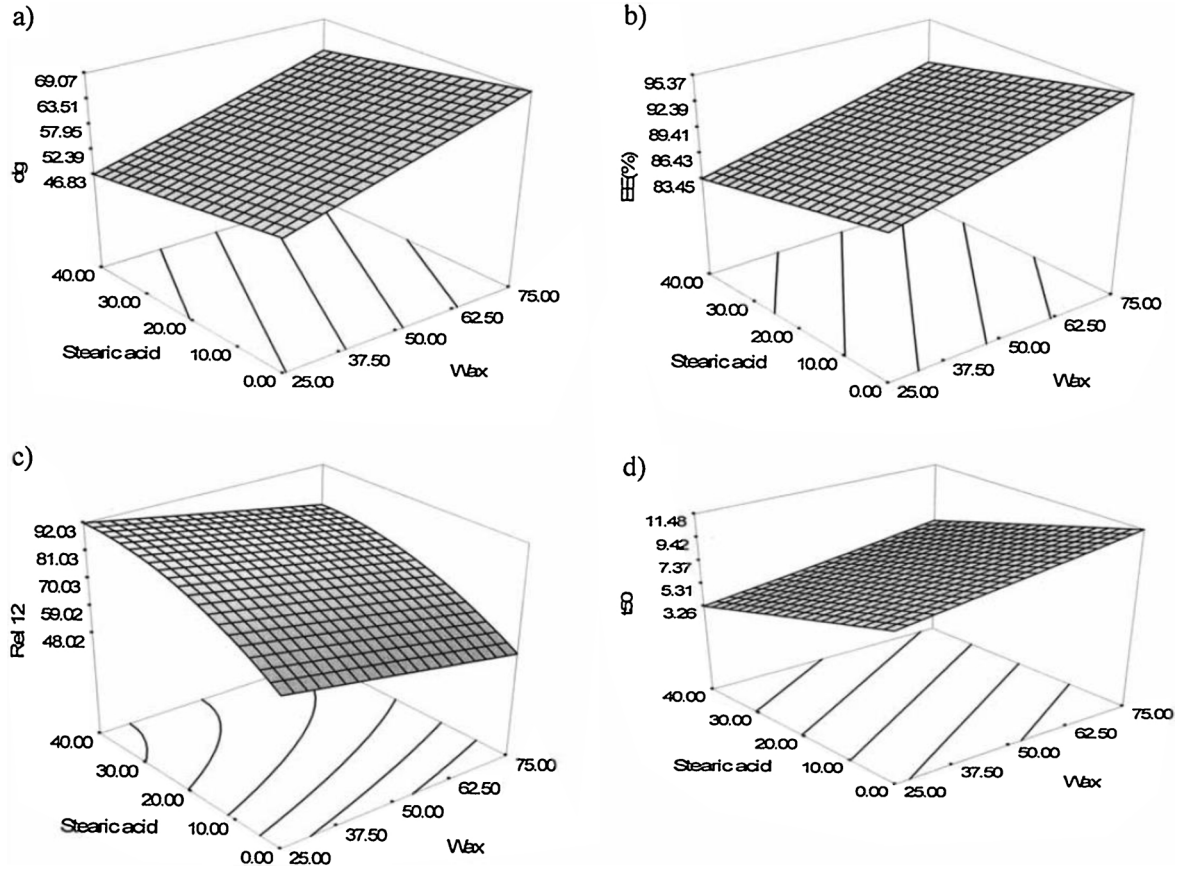

Fig. 12. Response surface plot showing the effect of wax loads (X1) and stearic acid levels (X2) on: (a) mean diameter (Y1), (b) encapsulation efficiency (Y2), (c) release at the end of 12 h (Y3), and (d) time taken for 50% drug release (Y4).

components. It is an ED used to determine the relationship between a variable that impacts the process and resulting response, with each variable altered in a systematic approach. It is one of the type of mixture design. In the q-component simplex-centroid design, the number of distinct points is 2q - 1. These points correspond to q permutations of (1, 0, 0, …, 0) or q single component blends, the (q/2) permutations of (.5, .5, 0, …, 0) or all binary mixtures, the (q/3) permutations of (1/3, 1/3, 1/3, 0, …, 0), …, and so on, with finally the overall centroid point (1/q, 1/q, …, 1/q) or q-nary mixture. Maherani et al. (2012) explored the application potential of the mixture design (SCD) approach to develop liposomes composed of varying percentage of DOPC, POPC and DPPC using the extrusion method for better EE% (calcein), size, and ζ-potential. The optimal liposomal formulations showed incorporation percentage of DOPC, POPC and DPPC to be 46%, 12%, and 42%, respectively. Moreover, other parameters included an average size: 127.5 nm, a phase-transition temperature: 11.43 °C, a ζ-potential: -7.24 mV, fluidity value: 2.87 and %EE: 20.24% (Maherani et al., 2012).

10. D-optimal design (D-OD)

The D-optimal criterion was developed to select design points in a way that minimizes the variance associated with the estimates of specified model coefficients. Mura et al. (2008) optimized benzocaine-

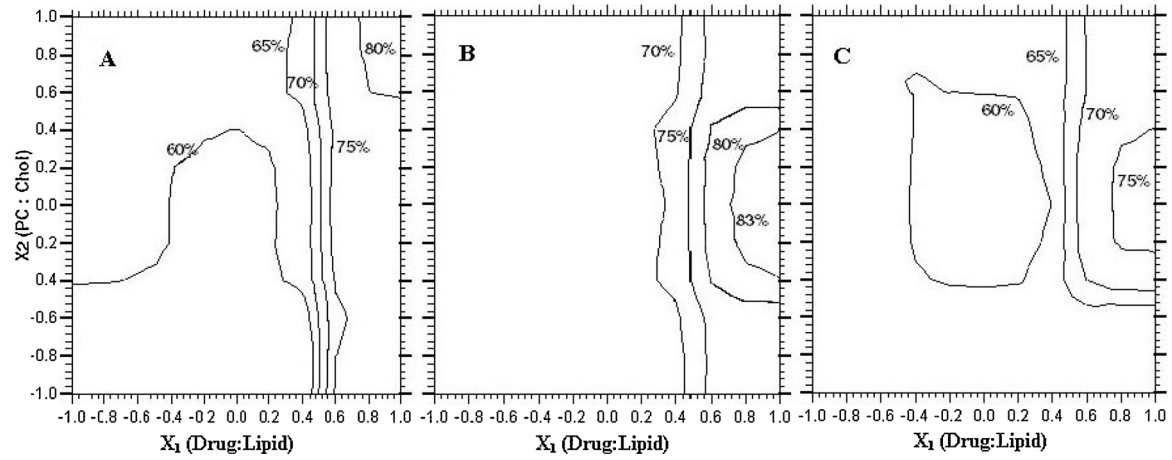

Fig. 13. Contour plots (A) at –1 level of variable X3, (B) at 0 level of variable X3, (C) at 1 level of variable X3.

Table 5
Application potential of FD in development of liposomes.

FD type	Agent loaded	Method of preparation	Variables selected		Critical remarks	Ref.
			Independent	**Dependent**		
3^2FD	Sirolimus	Thin film hydration method	DPPC/CHOL and DOPE/DPPC (molar ratios)	PS and EE %	DPPC/Chol molar ratio was the major contributing variable in PS and EE%. In the prediction of PS and EE % values, the average percent errors are found to be as 3.59 and 4.09%.	(Ghanbarzadeh et al., 2013)
3^2FD	Silica particles	–	PC to CHOL molar ratio and concentration of silica nanoparticles	Size and zeta potential	Protocell (total 9 formulations) with 7:3 ratio of PC to CHOL and 0.5 mg/ml of silica nanoparticles demonstrated better colloidal behaviors (Fig. 11).	(Kaur et al., 2012)
FFD	Gadolinium (Gd) chelate	–	PL type and amount of CHOL, drug/lipid ratio (loading) and nature of the Gd-chelate	Incorporation efficacy and MR contrast efficacy [longitudinal (T1) relaxivity]	Liposomes with CHOL and phospholipids with short acyl chain lengths i.e. DMPC and DMPG provided high T1-relaxivity with high loading of CHOL and Gd-chelate. Nuclear magnetic resonance dispersion (NMRD) analysis showed the presence of Gd-chelates with a long tau(R). This statistical modeling helped in surface modification with PEG without compromising the T1-relaxivity.	(Glogard et al., 2002)
2^5 full FD	Ciprofloxacin (CPF) HCl	Reverse phase evaporation	Five independent variables including CHOL	EE, size, and percentage of drug released after 1 and 10 h	The results obtained pointed out that the molar concentration of cholesterol was the predominant factor that increased the EE% of the drug and the PS responses.	(Mehanna et al., 2009)
FD	Topotecan	hydration method followed by extrusion	lipid, CHOL (%), PGs (%), PEG-lipids (%), and drug to lipid in molar ratio	entrapment and retention, size and zeta-potential	The optimized liposomes prepared from DSPC/CHOL/DSPG/DSPE-PEG_{2000} with molar ratio of 7:7:3:1.28 and conventional DSPC/CHOL/DSPG with molar ratio of 7:7:3 revealed low PDIs i.e. 0.15 and 0.10, an average diameter of 103.0 ± 13.1 and 95.2 ± 11.10 nm, respectively. However, the drug loading was only 11.44 and 6.21% with zeta-potential of -10 ± 2.3 and -22 ± 2.8 mV for PEGylated and conventional liposomes, respectively.	(Vali et al., 2008)
3^2 FD	Glipizide	Emulsification phase separation technique	Amount of paraffin wax and proportion of stearic acid in the wax	geometric mean diameter (dg), %EE, release at the end of 12 h (rel_{12}) and time taken for 50% of drug release (t_{50})	The experimental values of dg, % EE, rel_{12} and t_{50} values for the optimized formulation were found to be 57.54 ± 1.38 μm, 86.28 ± 1.32%, 77.23 ± 2.78% and 5.60 ± 0.32 h, respectively, which were in close agreement with those predicted by the mathematical models.	(Shivakumar et al., 2007)
3^2 FD	Nimesulide	Thin film hydration technique	Concentration of PLs and CHOL	%EE, size distribution, drug diffusion, and drug leakage	The optimized batch of liposomes was subjected to drug permeation and drug retention studies employing rat skin and human cadaver skin. In comparison to methanolic solution of pure nimesulide, liposomal formulations were found to retain higher amounts of nimesulide in the skin.	(Singh et al., 2005c)
3^3 FD with ANN and MLR	Leuprolide acetate	Reverse phase evaporation method	Volume of aqueous phase, HSPC/DSPG, and HSPC/CHOL	percent drug entrapment (PDE)	ANN showed less error compared to MLR. The normalized error (NE) value observed with the optimal ANN model was 0.0211 while it was 0.0658 for the full model. Applied designs helped in maximizing PDE for leuprolide acetate into the liposomes (Fig. 12).	(Arulsudar et al., 2005)
3^3 FD (26-term logit model)	Flutamide	–	Volume of organic phase, the volume of aqueous phase, and the drug: PC: CHOL in molar ratio	%EE	In turn, the regression equation is used to develop contour plots that show the %EE is maximized at the level of 1:15:2 of the drug: PC: Chol molar ratio with the volume of organic phase (chloroform:	(Murthy and Umrethia, 2004)

(*continued on next page*)

Table 5 (*continued*)

FD type	Agent loaded	Method of preparation	Variables selected		Critical remarks	Ref.
			Independent	**Dependent**		
					methanol) (1:2) at 5 ml and the volume of distilled water at 1.5 mL.	
3^3 FD	Idoxuridine (IDU)	Reverse phase evaporation (REV) method	Volume of organic phase, volume of aqueous phase, and drug/PC/CHOL in molar ratio	PDE, size, and size distribution	Liposomal gels in 2% w/w or 5% w/w HPMC K4M gel bases to get 1% w/w IDU i.e. liposomal IDU gel – 1 (LIG-1) and LIG-2, respectively. Maximum percentage of drug retention (PDR) was obtained with the optimized formulation at 2-8 °C as compared to storage at 25 ± 2 °C, and 37 °C, and about 2.2- and 2.5-times higher skin drug retention was found in LIG-1 and LIG-2, respectively. This improved PDR resulted in increase in healing of the lesions in patients suffering from HSV-1 and HSV-2 diseases. DoE with the help the derived equations and contour plots predicted the critical values of independent variables required to prepare IDU liposomes using the REV method.	(Seth et al., 2004)
3^3 FD with ANN and MLR	Cytarabine	–	Drug/PC and CHOL molar ratio, PC/CHOL in percentage ratio of total lipids, and the volume of hydration medium	PDE	A second-order full-model polynomial equation and a reduced model were established by subjecting the transformed values of independent variables to multiple regression analysis, and contour plots were drawn using the equation. ANN showed less error compared with multiple regression analysis (Fig. 13).	(Subramanian et al., 2004)
2^4 FD	–	–	CHOL/lipids ratio and polyelectrolyte concentration	polymer molecular weight (mwt), surface affinity (S), number of adsorption shots (Sh), temperature (T), and the combinations mwt x S, mwt x Sh	Positive or high surface affinity liposomes (DSPC:CHOL:DMTAP, 5:4:1), and neutral ([N]) or low surface affinity liposomes (DSPC:CHOL, 1:1) were used as adsorption surfaces for the hydrophilic anionic polyelectrolytes i.e. Carboxymethylchitin (CMC) and Carboxymethyl/Glycolchitin (CO) at physiological ionic strength (I). There was reduced polymer-induced particle aggregation upon doubling of the uncoated liposomes size i.e. from 0.22 to 0.45 μ.	(Mobed and Chang, 1998)
$2^{(k-p)}$ FD	Riboflavin (vitamin B2), Oil Red O, deoxybenzone, oxybenzone, β-carotene and sulisobenzone	–	The presence or absence of these five different light absorbers in multilamellar liposomes containing the vitamin free or complexed with gamma-cyclodextrin comprised the six factors of the system	The stabilization ratio of the vitamin and its percentage entrapment in liposomes	The response variables were predicted by multiple regression equations comprising combinations of the six formulation factors. Higher entrapment and higher protection for the drug should characterize the optimum formulation.	(Loukas, 1997)
$2^{(k-p)}$ FFD	sodium ascorbate, oil red O and/or oxybenzone, and sulisobenzone	–	light absorbers and drug (free or complexed with alpha-cyclodextrin)	stabilization ratio and %EE	The response variables were predicted by multiple regression equations comprising combinations of the four formulation factors. Both the higher entrapment and the higher protection for the drug should characterize the optimum formulation.	(Loukas, 1998)

*ANN = artificial neural networks, MLR: multiple linear regression.

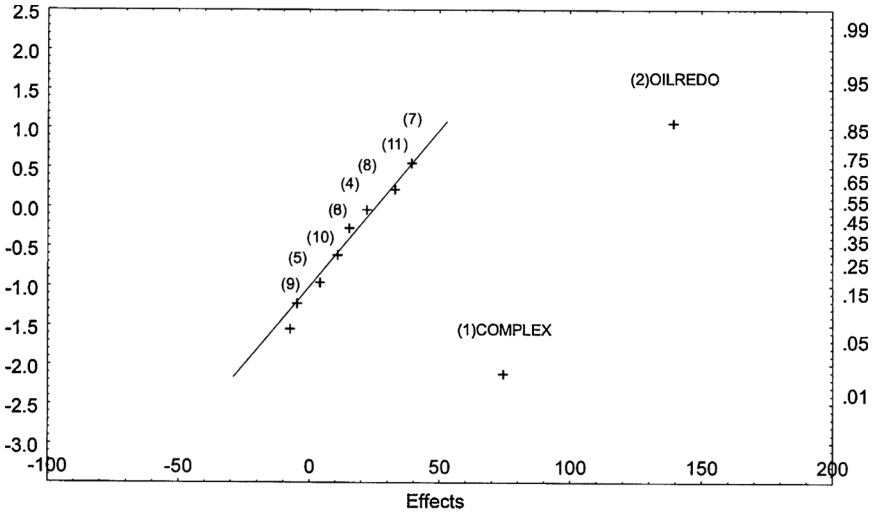

Fig. 14. Normal probability plot of factors main effects on the stabilization ratio. (The right y axis denotes the percentage cumulative frequency, which equals the cumulative frequency divided by (n + 1), where cumulative frequency for a measurement denotes the measurements less than or equal to that measurement, and n is the total number of measurements).

liposomes for topical delivery. Optimization using DOD approach included various variables such as potassium glycyrrhizinate (KG) as an alternative to CHOL and stearylamine (SA, cationic) or dicethylphosphate (DCP, anionic), and the percentage of ethanol and the total volume of the hydration medium so as to obtain maximum %EE and drug permeation (%P after 3 h).Graphical analysis revealed that KG and SA increased %EE, and CHOL and DCP increased %P. The Doehlert design was used for investigating response-surface analysis and it showed a negative interaction between ethanol (%) and volume of the hydration medium (Mura et al., 2008).

11. Plackett-Burman Design (PBD)

Plackett-Burman design (PBD) is the most frequently used screening design because of its ability to estimate all main effects with the same precision (Antony, 2014). It is a fractional factorial design with the advantage of minimizing the experimental runs from large number of variables to smaller most significant factors (El-Refai et al., 2010; Fang et al., 2010). Using this design, N factors can be screened with N + 1 experimental runs (Medina-Morales et al., 2011). Being an orthogonal design, the main effects are independent, while the interactions are not of interest at this screening stage (Antony, 2014; Yingling et al., 2011). The PBD based on the first order model with no interaction can be represented by this equation (Rajendiran et al., 2011).

$$Y = \beta_0 + \sum \beta_i X_i$$

Where, "Y" represents the response, "β_0" is the model intercept, "i" represents the variable number, "β_i" is the variable estimate and X_i corresponds to the independent variables.

Loukas (2001) developed the multicomponent protective liposomal formulations bearing riboflavin (free or complexed form with γ-cyclodextrin) along with various light absorbers and antioxidants. Various factors related to the composition and method of preparation was studied using PBD to get maximum stabilization ratio of the vitamin in presence or absence of light absorber and antioxidants as shown in Fig. 14 (Loukas, 2001).

12. Conclusion and future panorama

A formulation scientist can derive unique benefits of FbD in rational development of various drug delivery systems and pharmaceutical processes. As a rule, when finding the correct solution is not simple, a pharmaceutical scientist should mandatorily consider the use of FbD for developing novel and nanostructured drug delivery systems, wherein the variability and vulnerability of the systems make them ultrasensitive to diverse formulation factors and processes these systems tend to undergo. FbD is a quality-centric approach, which provides enormous benefits to meet the unmet needs of patients as well as pharmaceutical manufacturers for development of efficacious, cost-effective, safe and robust drug products. As with any other coherent scientific methodology, FbD also requires a thorough envisioning of the formulation development exercise as a whole, from the transition of laboratory scale development to pilot plant, and to scale-up into a robust and stable drug product. The 'process understanding' is the keystone of FbD initiatives. Better understanding of the system can help the formulator to define and monitor it with higher precision. The difficulties in optimizing a DDS using FbD are due to the difficulties in understanding the real cause and effect relationship. Execution of FbD techniques, therefore, allows gaining the requisite conception of CFAs and CPPs which tend to impact CQAs, and eventually, the holistic product performance during laboratory scale, scale-up and production of exhibit batches. Defining such relationships between these formulation or process variables and quality traits of the formulation is almost an impossible task without apt application of an FbD model. On industrial fronts, it can lead to the systematic development of generic drug products in an optimized manner. FbD EDs have been applied to almost on all the kinds of DDS for optimizing not only the drug formulations, but also the processes.. It has proved to be useful even if the primary aim is not the selection of the optimum formulation, as it tends to divulge the degree of improvement in the product characteristics as a function of the change in (any) excipient or process parameter(s). Today, the federal agencies in terms of QbD need "inbuilt product quality" rather than testing the quality of the finished product. However, notwithstanding the immense vitality of this QbD-based philosophy in industrial milieu, its importance is enormous in diverse fields beyond the formulation development such as in analytical method development, pharmaceutical manufacturing, and stability studies too. Comprehending the formulation or process variables rationally using FbD not only would help in federal compliance but also in attaining the desired goals of product/process excellence with phenomenal ease at low cost. Albeit the practice of systematic development of drug delivery systems has undoubtedly spiced up over the past few decades, yet it is far from being adopted as a standard practice. Several more initiatives are needed to underscore the growing utility of FbD before this can happen perceptibly. The current review highlights the FbD applications, methodology and potential cautions for liposome scientists.

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