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Application of Box–Behnken Design and Desirability Function in the Development and Optimization of Stealth Liposomes of Microtubule Inhibitor

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Authors' contributions

This work was carried out in collaboration among all authors. Author SPK designed the study, wrote the protocol and supervised the entire work. Author BLNR executed the study and carried out analysis of the study. Author CBR managed the literature searches and prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study was to develop and optimize a Stealth Liposomal Drug Delivery System of microtubule inhibitor using Box–Behnken Design and Desirability function.
Study Design: Development and Optimization of Stealth Liposomes.
Place and Duration of Study: The study was carried out in the Department of Pharmacy, Annamalai University, between September 2020 and May 2021.
Methodology: Stealth Liposomes were prepared by the thin-film hydration method (TFH). The formulation was optimized using Box – Behnken design to study the effect of independent variables, Amount of Egg Phosphatidylcholine (X1), Amount of Cholesterol (X2), and Amount of DSPE-PEG 2000(X3) on dependent variables Entrapment Efficiency (Y1) and *In-vitro* drug release (Y2).
Results: Entrapment efficiency of the Stealth Liposomes ranges from 56.35 to 84.25%and *in-vitro* release ranges from 62.38 to 94.26%. The optimized formulation was found using the desirability function to get maximum entrapment with maximum drug release. The optimized formulation showed entrapment efficiency of 80.46% and *in-vitro* release of 90.11%.

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Conclusion: Stealth Liposomal Drug Delivery System for microtubule inhibitor was successfully developed and optimized using desirability function in Design Expert software by a three-factor, three level Box – Behnken design.

Keywords: Stealth liposomal drug delivery; thin film hydration; box – behnken design; desirability function.

1. INTRODUCTION

A reasonable experimental design is very important, especially when complex formulations need to be developed, as it can save time, money and reduce experimental errors to obtain reliable experimental data. In particular, the multivariate strategy of experimental design allows simultaneous investigation of the effects of several variables, their actual significance on the considered response, and the possible interrelationship among them. This approach yields maximum information with a small number of experiments [1]. Response surface methodology (RSM) explores the relationships between several independent variables and one or more response variables. RSM includes central composite design (CCD), Box-Behnken design (BBD), and Doehlert design (DM). The present study used BBD since it has a high fitting correlation coefficient, good predictability, and high precision and has been considered to be a cost-effective technique compared to the other usual processes of formulation and optimization because it requires fewer experimental runs and therefore saves time [2].

Liposomes are spherical vesicles of different sizes consisting of a lipid bilayer and aqueous center compartment that is generated *in-vitro* [3]. These are popular in terms of biocompatibility, biodegradability, low toxicity, and can control the biodistribution of the drug by altering the size, composition of lipids, and hence the characteristics [4]. These are the carriers that are suitable for encapsulation of drugs with different lipophilicities, such as strongly lipophilic drugs, strongly hydrophilic drugs, and drugs with intermediate log P. Liposomes can protect the encapsulated drug or drugs and can target the organ or tissue passively [5]. But it was found that conventional liposomes suffer with 2 major drawbacks as sustained as well as targeted release system for drugs *in vivo*. First one is its attraction toward the reticuloendothelial system (RES), which will cause the removal of drug from the bloodstream as well as will result in adverse effects on the host defense system [6] and will decrease the availability of entrapped drug to the other tissues. The next is recognition of conventional liposomes by RES leads to nonlinear pharmacokinetics for the carrier, which makes calculating the amount of entrapped drug required to attain therapeutic drug dose difficult [7][8][9][10]. In addition, conventional liposome formulations containing saturated phospholipids and cholesterol are more prone to the influence of plasma proteins and other biologic fluids *in vivo*, which leads to rapid removal of drug contents [11][12]. To avoid the above mentioned difficulties, especially to avoid the RES uptake of the vesicles it is necessary to have previous administration of empty liposomes. Moreover, small unilamellar vesicles have the drawback of low aqueous entrapment volume; the use of charged liposomes could be toxic. Thus, mechanical or electrostatic stabilization cannot improve the long circulation of liposomes in biological systems. Further attempts to alter the biodistribution of liposomes resulted in the generation of new liposomal formulations called stealth liposomes (SLs), which have considerably reduced RES uptake, and remain in circulation for long period [13][14] with dose-independent pharmacokinetics and have reduced susceptibility to protein-induced leakage [15][16].

The rationale for the development of Eribulin mesylate in the form of stealth liposomes is to reduce drug toxicity, with maintaining the efficacy of the drug for a maximum period of time. Stealth liposomal drug delivery system increases the circulation time of the drug in the body by avoiding the reticuloendothelial system and achieves linear pharmacokinetics. This study aimed to optimize the formulation of Stealth containing Eribulin Mesylate prepared by the lipid film hydration method. An experimental design has been used to evaluate the influence of three formulation parameters, i.e. Amount Egg phosphatidylcholine (X1), Amount of Cholesterol (X2), and Amount of DSPE-PEG 2000 (X3), on encapsulation efficiency (Y1) and the cumulative drug release (Y2). Secondly, the experimental design was used to optimize the preparation formula to obtain maximum encapsulation efficiency and cumulative drug release.

2. MATERIALS AND METHODS

2.1 Materials

Eribulin Mesylate was a kind gift sample from Natco Pharma Limited, Hyderabad, whereas Egg Phosphatidylcholine from Vav Life Sciences, Mumbai. DSPE-PEG 2000 from Niram Chemicals, Mumbai, and Cholesterol from S.D. Fine Chemicals Limited, Mumbai. All other reagents were of analytical grade.

2.2 Compatibility Study

The compatibility study was carried out using Fourier transform infrared spectroscopy (FTIR). Drug alone and with the physical mixtures of Egg Phosphatidylcholine, Cholesterol, and DSPE-PEG 2000 was first solubilized in chloroform-methanol solvent mixture and dried to form a pellet. These pellets were kept for one month at room temperature that is 25°C±2°C and 60%±5% relative humidity for complete interaction between the drug and polymer. The drug and drug-polymer samples were dried in a hot air oven at 60°C for 30 min for the removal of moisture. These samples were scanned from 4000 to 400 cm^{-1} wavenumbers. Spectra obtained were compared with standard spectra of Eribulin mesylate, for changes in the peaks if any interaction transpires.

2.3 Preparation of Stealth Liposomes

Stealth Liposomes were prepared by the thin-film hydration method (TFH). The process variable parameters are used for the formulation according to the experimental design as shown in Table 1. The Egg phosphatidylcholine, Cholesterol, and DSPE-PEG 2000 were dissolved in a mixture of chloroform and methanol (ratio 2:1 v/v) in a 250 ml round bottom flask in different molar amounts. The solvent was evaporated in the rotary flask evaporator at 35°C under reduced pressure. The film was dried overnight in a vacuum desiccator, to remove any remaining solvent. The thin dry lipid film thus formed was hydrated using aqueous hydrating medium PBS pH 7.4 (Containing Eribulin mesylate) at 40°C. The formed liposomal dispersion was sonicated at 40°C in probe sonicator. Sonicated vesicles were stabilized and allowed to swell for one hour at ambient temperature and further were left to mature overnight to ensure full lipid hydration. Small unilamellar vesicles were formed by extruding this solution through a 0.2µm polycarbonate filter. Resultant Liposomes were subjected to centrifugation at 5,000 rpm, 8°C for 5 minutes using ultracentrifuge for separation of liposomes [17].

2.4 Box-Behnken Experimental Design

A Box-Behnken optimization design was applied using three independent variables to optimize the conditions and to analyze the sensitivity of responses to the changes made in the settings of experimental design [18][19]. A total of 15 experiments were performed out of which the center point was repeated three times. The center points improve the evaluation of the response surface curvature and simplify the estimation of the model error. The drawback of the conventional approach was to develop a formulation in which only one variable can be changed at a time which makes it hard to have an optimized formulation because the traditional method tells nothing about the interactions that may occur between the different variables. For this reason, a Quality by design approach is necessary. Therefore, a Box-Behnken design comprising of three factors was selected. A mathematical and statistical technique that enumerates the functional relationship between the measurable variables and many illustrative factors, to acquire an optimal response by using a sequence of tests is known as Response surface methodology. The foremost advantage of Response surface methodology is to reduce the experimental runs which are required as compared to the full factorial design, which needs a lot of trials and is already being extensively used in pharmaceutical studies. Using Design Expert software, experimental runs were generated and evaluated. The major response factors used to evaluate the liposome formulation, including Entrapment Efficiency (Y1), and Cumulative drug release (Y2), were determined. The selected factors with the actual and coded levels according to the design are represented in Table 1. The results obtained for each response were fitted to a quadratic polynomial model explained by a nonlinear equation as below;

$$\mathrm{Yo} = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

Where Yo is the dependent variable, corresponding to either Entrapment Efficiency (Y1) or Cumulative drug release (Y2), and A, B,

and C are the independent variables representing the amount of Egg phosphatidylcholine, Cholesterol, and DSPE-PEG 2000 respectively. b_0 is a constant; b_1, b_2, and b_3 are the coefficients translating the linear weight of A, B, and C, respectively; b_{12}, b_{13}, and b_{23} are the coefficients translating the interactions between the variables; and b_{11}, b_{22}, and b_{33} of the coefficients translating the quadratic influence of A, B, and C. Linear and second-order polynomials were fitted to the experimental data to obtain the regression equations, and their observed and predicted responses. By applying analysis of variance (ANOVA), lack of fit, and coefficient of determination (R^2) as a measure of goodness of fit of the fitted model, models were validated.

2.5 Evaluation of Stealth Liposomes

2.5.1 Drug entrapment efficiency

The liposomal suspension was centrifuged at 4000 rpm for 10 minutes at 4°C temperature by using cooling centrifuge. As the drug is water soluble so it will be in a molecular state and the free drug will not separate at lower rpm. Hence the supernatant containing liposomes in the suspended stage and the free drug was collected and again centrifuged at 10,000 rpm at 4°C temperature for 30 minutes for separation of liposomes. A clear solution of supernatant and sediment of liposomes was obtained. The liposomes free from the unentrapped drug were soaked in 30 ml of methanol and then sonicated for 10 min. The vesicles were broken to release the drug, which was then estimated for the drug content [20][21]. The percent drug entrapped (PDE) was then calculated using the following equation.

$$EntrapmentEfficiency\ (\%) = \frac{Amountofdrug\ entrapped}{Amountofdrug\ added} \times 100$$

2.5.2 *In-vitro* drug release studies

Studies of the drug release from the liposomal system are directed toward the approaches that are relevant to the *in-vivo* condition. The *in-vitro* release from the liposomal dispersion was studied through the dialysis membrane bag (14 KDa molecular weight, Himedia) in a glass beaker containing 200 ml of PBS (pH 7.4) with liposomal dispersion equivalent to 0.5 mg/ml of Eribulin mesylate at 37°C ± 0.5°C on a temperature controlled magnetic stirrer. The dialysis membrane tube previously soaked overnight in the ethanol was filled with 5ml of PBS containing liposomes. 2 ml volume was withdrawn at the interval of every 30 min and fresh PBS was added to make the volume. The samples were analyzed for drug content [22].

2.6 Optimizing using Desirability Function

To optimize manifold responses, they should be highly interconnected with each other. It is improbable that the values enviable to optimize the effect of one response will have the same effect on the second response, thus a variance can occur between them. Hence, the most favorable compromising zone must be required for each of the responses devoid of any bias. In the current study, all the responses were concurrently optimized by a desirability function that uses the numerical optimization method introduced by Derringer and Suich in the Design Expert software [23].

Table 1. Process parameters for Experimental design

Process Parameters	Levels		
Independent Variables	**(-1)**	**(0)**	**(+1)**
(A) Egg phosphatidyl choline (µM)	50	100	150
(B) Cholesterol (µM)	50	100	150
(C) DSPE-PEG 2000 (µM)	0.5	1	1.5
Dependent Variables	**Goal for dependent variables**		
(Y1) Entrapment Efficiency	Maximize		
(Y2) Cumulative drug release	Maximize		

In this approach, a specific goal was assigned to each response. A partial desirability function is associated with an individual response, where value 0 is assigned to an undesired/unacceptable response while for an acceptable response, the value lies between 0 and 1. The value between 0 and 1 indicates the closeness of the response to its target value (i.e., minimum to most desirable. Therefore, the desirability function helps in ascertaining the most favorable and appropriate point in the design space that accomplishes the set goals for dependent variables (response). In our study, Design Expert was utilized to conclude the maximum desirability value after assigning desired goals to the responses.

2.7 Stability Study of the Optimized Formulation

Stability studies were carried out on optimized formulations according to ICH guidelines. An accelerated stability study of the optimized liposomal formulation was performed to investigate the physical appearance and leak out of the drug from liposomes during storage. Optimized Liposomal suspension was sealed in glass vials and stored at refrigeration 5°C ± 3°C temperature and 25°C ± 2°C / 60% RH ± 5% RH temperature for 6 months. Samples were withdrawn at definite time intervals (0, 3, and 6 months) and observed for Visual appearances like colour, sedimentation, creaming, and extent of leakage. To calculate the extent of leakage, the percentage entrapment efficiency after storage was calculated at regular intervals and then correlated with the extent of leakage. The extent of leakage is estimated from the following formula;

$$Extent\ of\ leakage\ (\%) = \frac{Initial\ entrapment\ efficiency\ (\%)}{Amount of\ drud\ retained\ after\ storage\ (\%)} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Compatibility Study

The FTIR spectra of the drug alone and with the physical mixtures of Egg phosphatidylcholine, Cholesterol, and DSPE-PEG 2000 indicate no interaction between the drug and the excipients when compared with the infrared spectrum of the pure drug as all functional group frequencies are present. Figs. 1,2 shows the FTIR spectra of the drug alone and with the physical mixture of the drug with the excipients. Overlay spectra as in Fig. 3 shows that the peaks of pure Eribulin mesylate are identical with the peaks of Eribulin mesylate with the excipients.

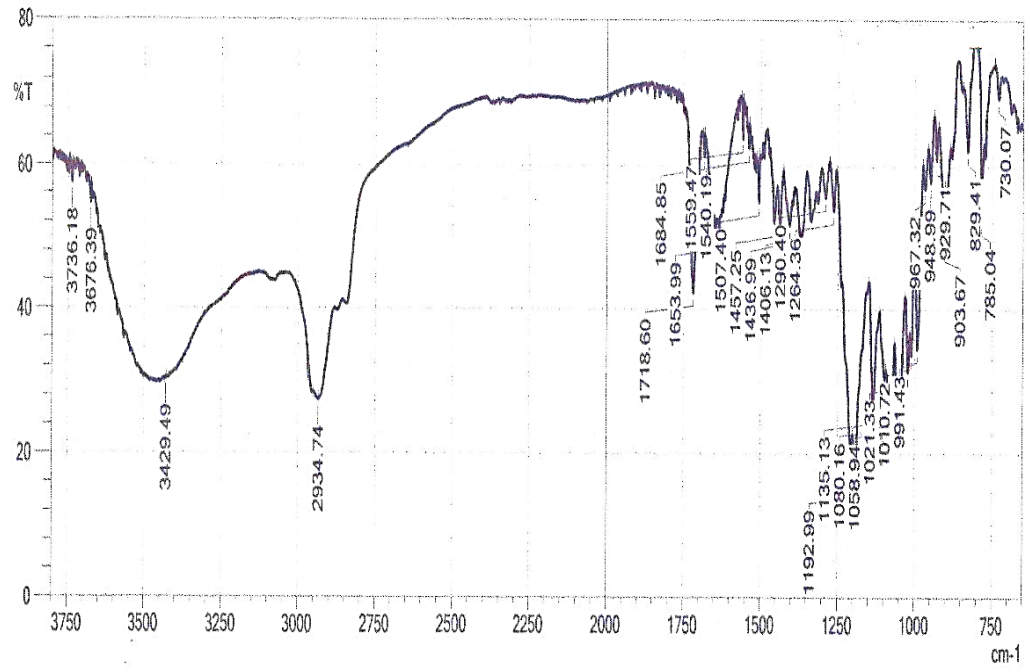

Fig. 1. FTIR Spectra of Eribulin mesylate

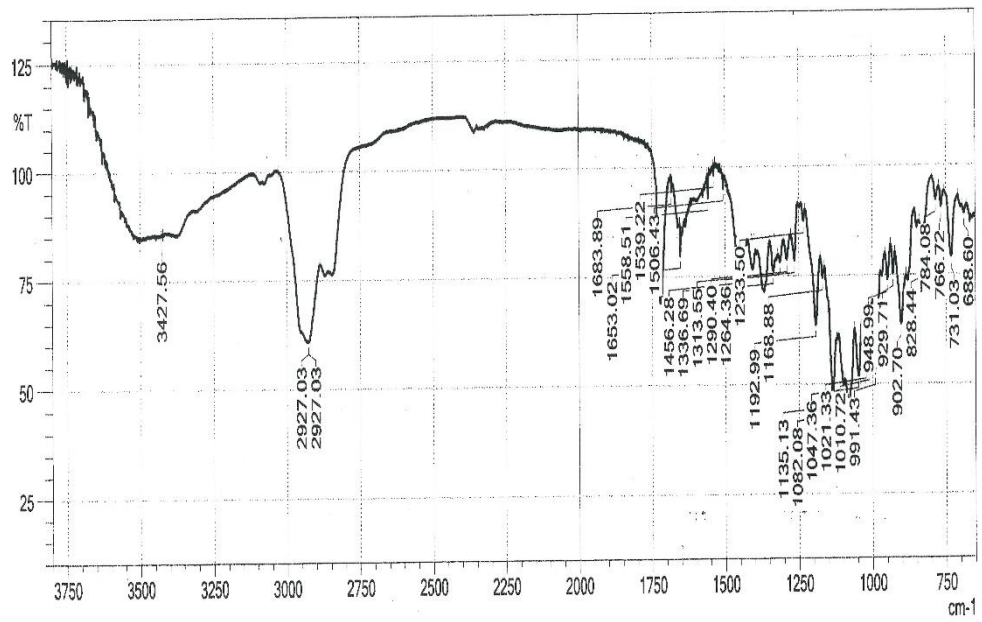

Fig. 2. FTIR Spectra of Eribulin mesylate with excipients

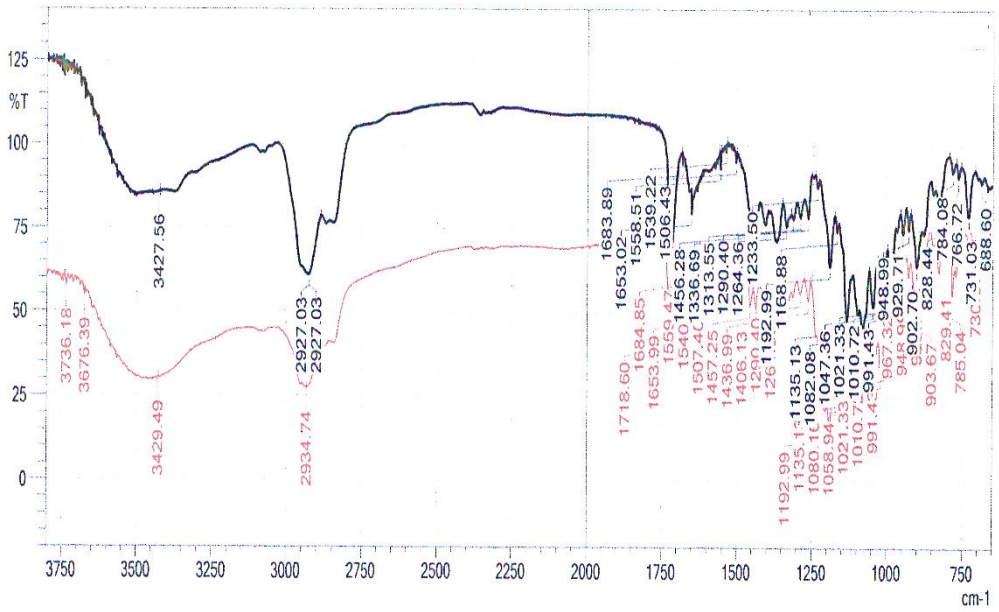

Fig. 3. Overlay spectra

3.2 Experimental Design

An experimental design of fifteen runs was generated for three factors at three levels to identify the optimum levels of different independent process parameters according to Box – Behnken design. Table 2 shows the observed responses along with the predicted values for designed formulations. The observed values for entrapment efficiency, and drug release range from 56.35 to 84.25%, and 62.38 to 94.26%, respectively. The responses were simultaneously fitted to linear, two-factor interaction (2FI), cubic and quadratic models

using Design Expert software. The values of R-squared, Adj -squared, Pred R-squared, SD, and % CV are shown in Table 3 along with the regression equation. Since the cubic model was aliased due to insufficient design points to estimate the coefficients, the quadratic model was chosen for its larger adjusted R-squared value. The ANOVA values for different responses are represented in Table 3, and all statistically significant ($p<0.05$) coefficients are included in the equations. As per the optimization design, a positive value shows favorable optimization, whereas a negative value shows an inverse relationship between the factor and the response. It is evident that all the three independent variables, namely the amount of Egg phosphatidylcholine (A), Cholesterol (B), DSPE-PEG 2000 (C), have interactive effects on the responses drug entrapment efficiency (Y1) and drug release (Y2).

3.3 Effect on Entrapment Efficiency (Y1)

The model proposes the following equation for Entrapment Efficiency;

$$EE = 77.9133 + 4.735A - 3.11875B - 0.91125C + 0.3625AB + 0.0975AC + 0.7BC - 14.5517A^2 + 1.00083B^2 + 0.625833C^2.$$

Where A is the amount of Egg phosphatidylcholine; B is the amount of Cholesterol, and C is the amount of DSPE-PEG 2000. The Model F-value of 866.61 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.47 implies the Lack of Fit is not significant. The Predicted R^2 of 0.9949 is in reasonable agreement with the Adjusted R^2 of 0.9982; i.e. the difference is less than 0.2. An adequate Precision value of 92.806 indicates an adequate signal. This model can be used to navigate the design space. Figures 4, 5, and 6 are the response surface plot showing the effect of different independent variables on the Entrapment Efficiency of liposomes.

From the equation, it can be qualitatively concluded that the amount of Egg phosphatidylcholine had a positive effect on the response of Y1, which indicated that as the amount of Egg phosphatidylcholine increases, entrapment efficiency increases. Oppositely the amount of Cholesterol and DSPE-PEG 2000 have a negative effect on entrapment efficiency as their values are negative. As the amount of Cholesterol and DSPE-PEG 2000 increases PDE decreases accordingly. All the independent variables have a significant impact on the dependent variable Y1.

Table 2. Observed and predicted values of responses of Box – Behnken design

Formulation Code	Run	Independent Variables			Dependent Variables			
					Actual		Predicted	
		(A)	(B)	(C)	(Y1)	(Y2)	(Y1)	(Y2)
F01	1	1	0	1	68.05	71.34	67.91	71.30
F02	2	0	0	0	77.46	84.18	77.91	83.89
F03	3	0	-1	-1	84.25	82.27	84.27	82.59
F04	4	1	-1	0	71.65	70.79	71.85	70.29
F05	5	-1	0	-1	60.12	91.05	60.26	91.09
F06	6	-1	-1	0	63.27	94.26	63.11	93.90
F07	7	1	0	-1	69.76	62.38	69.54	62.56
F08	8	0	0	0	78.36	83.26	77.91	83.89
F09	9	0	0	0	77.92	84.22	77.91	83.89
F10	10	0	-1	1	81.11	89.67	81.05	90.21
F11	11	1	1	0	66.18	62.39	66.34	62.75
F12	12	0	1	1	76.23	86.88	76.21	86.56
F13	13	0	1	-1	76.57	79.39	76.63	78.85
F14	14	-1	0	1	58.02	97.88	58.24	97.70
F15	15	-1	1	0	56.35	93.56	56.15	94.06

Table 3. Analysis of variance results of calculated model

ANOVA Results	Entrapment Efficiency	*In-vitro* Drug release
Regression		
Sum of Squares	1074.37	1724.58
Degrees of freedom	9	9
Mean Square	119.37	191.62
F-value	866.61	435.04
p-value	< 0.0001	< 0.0001
Residual		
Sum of Squares	0.6887	2.20
Degrees of freedom	5	5
Mean Square	0.1377	0.4405
Lack of Fit		
Sum of Squares	0.2837	1.61
Degrees of freedom	3	3
Mean Square	0.0946	0.5375
F-value	0.4669	1.82
p-value	0.7357	0.3735
Correlation Co-efficent (R^2)	0.9994	0.9987
Correlation of variation (%CV)	0.5226	0.8071

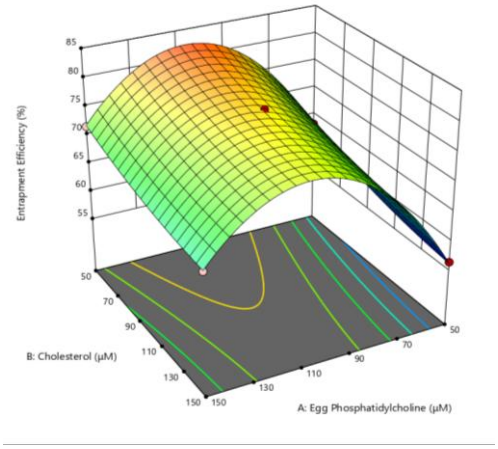

Fig. 4. Effect of Egg phosphatidylcholine and Cholesterol on Entrapment Efficiency

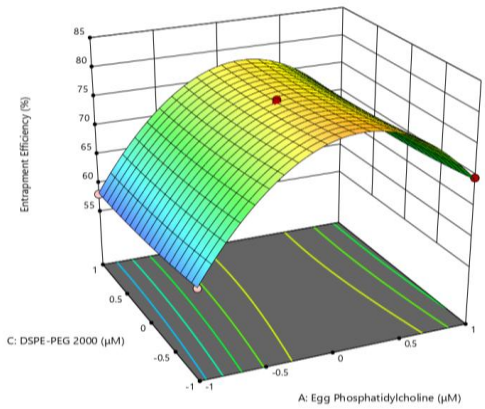

Fig. 5. Effect of Egg phosphatidylcholine and DSPE-PEG 2000 on Entrapment Efficiency

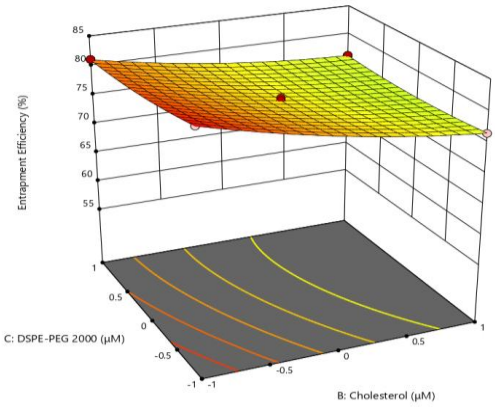

Fig. 6. Effect of DSPE-PEG 2000 and Cholesterol on Entrapment Efficiency

3.4 Effect on In-vitro Drug release (Y2)

The model proposes the following equation for In-vitro Drug release (Y2):

$$\begin{aligned}\mathbf{Drugrelease} = {} & 83.8867 - 13.7313A \\ & - 1.84625B + 3.835C \\ & - 1.925AB + 0.5325AC \\ & + 0.0225BC - 3.763335A^2 \\ & + 0.126667B^2 + 0.539167C^2.\end{aligned}$$

Where A is the amount of Egg phosphatidylcholine; B is the amount of Cholesterol, and C is the amount of DSPE-PEG 2000. The Model F-value of 435.04 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB, A^2 are significant model terms. The Lack of Fit F-value of 1.82 implies the Lack of Fit is not significant relative to the pure error. The Predicted R^2 of 0.9843 is in reasonable agreement with the Adjusted R^2 of 0.9964. An adequate Precision value of 64.833 indicates an adequate signal. This model can be used to navigate the design space. Figures 7, 8, and 9 are the response surface plot showing the effect of different independent variables on In-vitro Drug release. From the equation of the reduced model, it can be qualitatively concluded that Egg phosphatidylcholine and Cholesterol had a negative effect on the response of Drug release, which indicated that as the amount of Egg phosphatidylcholine and Cholesterol increases, Drug release decreases. Oppositely amount of DSPE-PEG 2000 has a positive effect as its value is positive. So, as the amount of DSPE-PEG 2000 increases, Drug release increases accordingly. All the independent variables have a significant impact on dependent variable Y2.

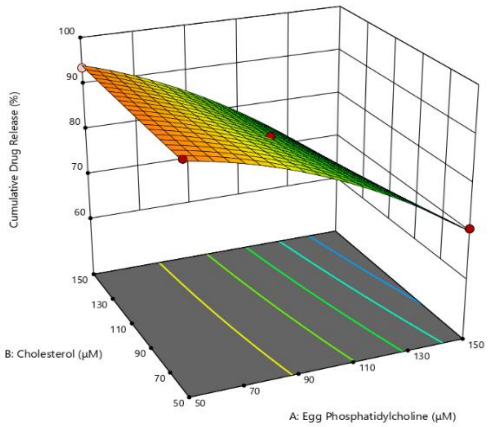

Fig. 7. Effect of Egg phosphatidylcholine and Cholesterol on *In-vitro* Drug release

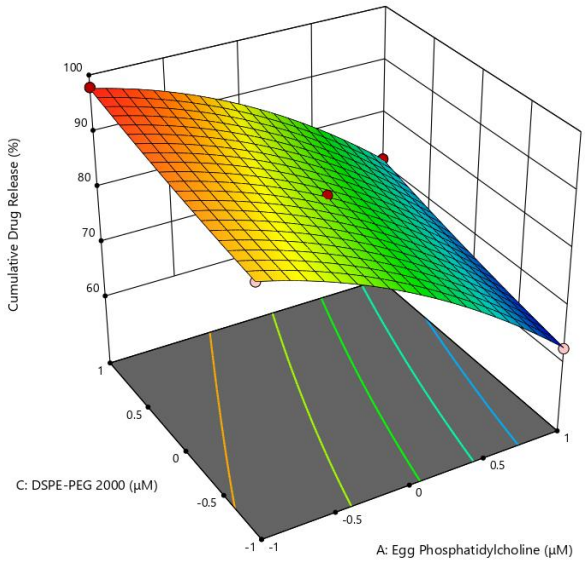

Fig. 8. Effect of Egg phosphatidylcholine and DSPE-PEG 2000 on *In-vitro* Drug release

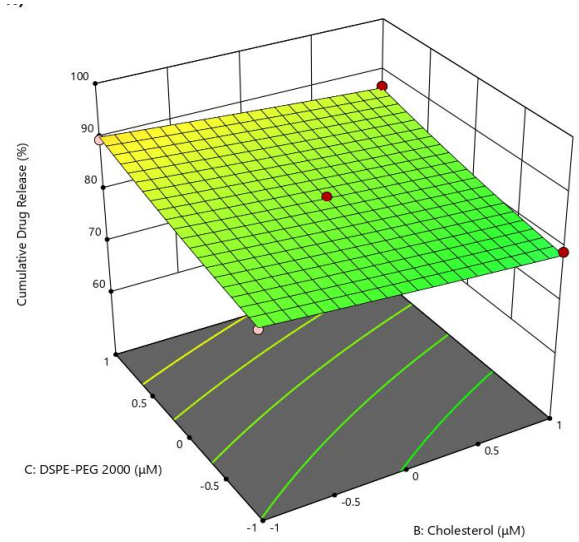

Fig. 9. Effect of DSPE-PEG 2000 and Cholesterol on *In-vitro* Drug release

3.5 Identification and Evaluation of Optimum Formulation using Desirability Function

The desirability function approach was applied in the present study using Design Expert. The constraints were set for all the responses. The independent variables (factors) were set in range as depicted in Table 1. The responses, Y1 and Y2were set to be maximized. Equal weight (1) and importance (+++) were given to all responses (weight and importance are the constraints of the software used where 3 pluses (+++) is a default setting that indicates equal importance of all responses). In the desirability function approach, the individual desirability function is calculated which is required for combining all the responses in one measurement. This will help in forecasting the optimum levels for the independent factors. Optimized formulation with the best desirability function, fulfilling the maximum requirement of response variables was selected. The selected optimized formulation contains X1= 93.34 µM, X2= 50 µM w/w, X3= 0.015 µM, and the overall desirability was found to be 0.839. The predicted

value of the optimized formulation for the response Y1 and Y2 was 80.19 and 91.64 % respectively. To confirm and validate the optimization, an optimized formulation was prepared in triplicate. All the responses were evaluated for each formulation as observed values. The comparison of the observed and predicted value is shown in Table 4. Figure 10 shows the counterplots for the desirability function between X1 and X2 (X3 at an actual amount of 0.015 µM). The percent biased range is between - 0.33 and + 1.66%.

Table 4. Composition of checkpoint formulation with predicted and observed values

Response Variables	Predicted Value	Observed Value	% Bias
Entrapment Efficiency (Y1)	80.19	80.46	- 0.33
In-vitro drug release (Y2)	91.64	90.11	+ 1.66

$$\textbf{Percent bias } (\%) = \frac{\textbf{Predicted value} - \textbf{Observed value}}{\textbf{Predicted value}} \times \textbf{100}$$

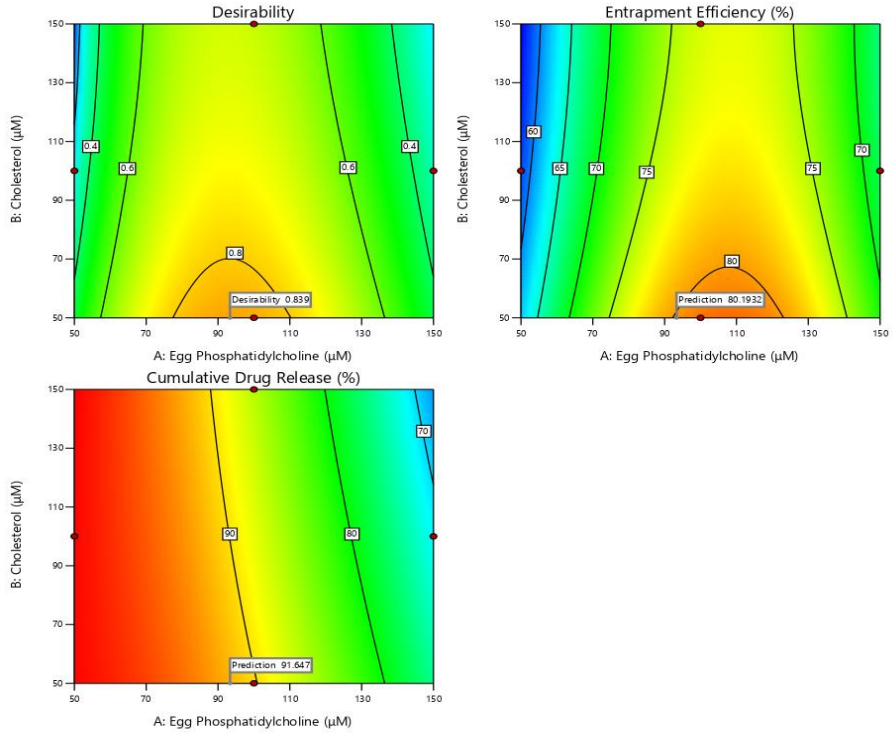

Fig.10. Contour plot of optimized formulation as a function of X1 and X2

Table 5. Stability study results of Optimized formulation

At Refrigeration Temperature (5°C ± 3°C)				
Time (Months)	**Colour**	**Sedimentation**	**Creaming**	**Extent of leakage (%)**
0	Off-White	No	No	0
3	Off-White	No	No	1.23
6	Off-White	No	No	2.85
At temperature (25±2°C) (60%± 5% RH)				
Time (Months)	**Colour**	**Sedimentation**	**Creaming**	**Extent of leakage (%)**
0	Off-White	No	No	0
3	Off-White with white sediment	Slight	Slight	69.54
6	Off-White with pale yellow sediment	Prominent	Intense	93.66

3.6 Stability study of the Optimized Formulation

The optimized batch was observed for colour, sedimentation, creaming, and extent of leakage during the stability study. The results were shown in Table 5. From the results, it was observed that liposomes remained more stable at refrigeration temperature.

4. CONCLUSION

The Stealth Liposomes of Eribulin mesylate were prepared using the thin-film hydration technique. The formulation was developed and optimized using Box-Behnken design by assessing the response of dependent variables, Entrapment efficiency, and in-vitro drug release using the independent variables, amount of Egg phosphatidylcholine, amount of Cholesterol, and the amount of DSPE-PEG 2000. Full model polynomial equation applied to determine the effect of independent variables on dependent variables was generated by integrating relative coefficients, and then it was reduced by removing insignificant values. Contour and 3D surface plot analysis was done and the applied method for optimization was evaluated statistically plotting predicted versus actual values of optimized formulation using desirability function. Percentage bias between the observed and predicted results of the quantitative responses of entrapment efficiency and in-vitro drug release of optimum formulation was found relatively less. Stability studies indicate that the liposomes are stable at refrigeration temperature. Therefore, it can be concluded that a Stealth Liposomal drug delivery system for Eribulin mesylate was developed using a three-factor, three-level Box – Behnken design and optimized by desirability function.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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