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Original Article

Design and Optimization of Capecitabine Proniosomes

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ABSTRACT

The present research study is to investigate the combined influence of 4 independent variables in the preparation of niosomes derived from capecitabine proniosomes. A 4-factor, 3-level Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. The independent variables selected were span 60, cholesterol, hydration volume and sonication time; dependent variables percentage entrapment efficiency (PEE) mean vesicle size (MVS). Based on the Box-Behnken design 29 trial runs were studied and optimized for PEE and MVS. The transformed values of the independent variables and the dependent variables were subjected to multiple regressions to establish a full-model second-order polynomial equation. Further 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 PEE and MVS of niosomes derived from proniosomes. 3D plots and contour plot were constructed to show the influence of independent variables on dependent variables. The model showing highest value of R^2 was considered as best model for release mechanism. It was found that the best fit model for optimized capecitabine niosomes F24 was Korsmeyer-peppas model and for capecitabine niosome was Higuchi matrix model, The Box-Behnken design demonstrated the role of the derived equation, 3D plot and contour plots in predicting the values of dependent variables for the preparation and optimization of capecitabine proniosomes.

KEYWORDS: Capecitabine, Niosomes derived from proniosomes, Box-Behnken design, Design Expert 11, optimization.

1. INTRODUCTION

Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Although capecitabine has a strong therapeutic effect, it is associated with several side effects such as gastrointestinal irritation, edema, dizziness and peptic ulceration when taken orally for a prolonged period[1]. One of the major obstacles in designing the formulation of

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novel drugs is their limited aqueous solubility. This problem can be overcome by entrapping the drug in a vesicular structure. Encapsulation of a drug in vesicular structures like niosomes and liposomes can be expected to prolong the existence of the drug in the systemic circulation, enhance penetration into target tissue and reduce toxicity, if selective uptake can be achieved. Chemically capecitabine is a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR) figure 1, which is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue[2,3].

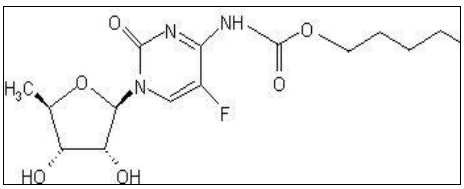

Fig 1: Structure of capecitabine

Niosomes are unilamellar or multilamellar vesicles that are made up of nonionic surfactant and can entrap amphiphilic and hydrophobic solutes [4, 5]. Niosomes have shown advantages as drug carriers, such as being economic and chemically stable alternatives to liposomes[6], but they are associated with problems related to physical stability, such as fusion, aggregation, sedimentation and leakage on storage. The proniosome approach[7-9] minimizes these problems by using dry, free-flowing product, which is more stable during sterilization and storage. Ease of transfer, distribution, measuring and storage make proniosomes a versatile delivery system. Proniosomes are water-soluble carrier particles that are coated with surfactant and can be hydrated to form niosome dispersion immediately before use on brief agitation in hot aqueous media. The resulting niosomes are very similar to conventional niosomes and more uniform in size. Reported methods for preparation of proniosomes are the spraying of surfactant on water-soluble carrier particles and the slurry method.

In the present investigation conventional slurry method was adapted to formulate niosomes derived from capecitabine proniosomes. To check the influence of formulation variables on response optimization technique was studied. A Box-Behnken design was created using Design Expert 11 (Trial Version 11, Stat-Ease Inc., Minneapolis, MN) to interpret the results.

2. MATERIALS AND METHODS

2.1. Materials: Capecitabine gift sample was obtained from Shilpa antibiotic Pvt Ltd, Raichur. Maltodextrin was procured from Himedia, Hosur, Cholesterol, Span 60, were purchased from Loba chem Pvt Ltd, Mumbai. All other ingredients and reagents used were of analytical grade.

2.2. Methods

Preparation of proniosomes: The proniosomes were prepared by the slurry method[10]. 250µmol stock solution of span 60 and cholesterol was prepared in chloroform:methanol (2:1). The accurately measured volumes of span 60 and cholesterol stock solutions and capecitabine (50mg) dissolved in chloroform: methanol (2:1) solutions were added into a 250ml round bottom flask containing previously 2g of maltodextrin powder used as carrier. Additional chloroform: methanol (2:1) solution added to form slurry. Further the flask was attached to a rotary flash evaporator rotated at 60 to 70 rpm. The solvent is allow to evaporate at temperature of 45±2ºC in a reduced pressure of 600mm/Hg until the mass in the flask had become a dry, free flowing product. The obtained proniosome powder was further dried overnight in desiccators under vacuum at room temperature. The obtained dry proniosome powders were stored in air tight amber coloured vials kept in a refrigerator for further evaluation.

Preparation of niosomes derived from proniosomes: Proniosomes were transformed to niosomes by hydrating with phosphate buffer saline (PBS) with a pH of 7.4 at 80°C using vortex mixer for specified time. The niosome were sonicated using a 250-W probe-type sonicator (MAGNA-PAK-250, Libra Ultrasonic, India). Niosome were characterized for PEE and MVS.

2.3. Experimental design

The developed formulations were optimized using 4-factor 3- level Box-Behnken statistical design (Design Expert 11). The rationale behind this Box Behnken design based on the salient principles of design of experiments (DoE) and quality by design (QbD) approach. It provides understanding of the plausible interactions among the different levels of variables and helps in selecting "the best" formulation with minimal expenditure of time, effort and developmental cost vis-a'-vis the traditional one factor at a time (OFAT) approach[11]. The QbD methodology involves defining the critical process parameters using screening and risk assessment, optimization data analysis and optimum search through response surface methodology to embark upon the design space and postulation of control strategy for continuous improvement[12,13]. This property prevents a potential loss of data in those cases. The design matrix generated the nonlinear quadratic equation for the response as shown below,

$$\mathbf{Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \ldots\ldots\ldots (Eq.1)}$$

Where Y is the response related with each factor level combination; b_0 is constant; b_1, b_2, b_3 are linear coefficients, b_{12}, b_{13}, b_{23} are interaction coefficients while b_{11}, b_{22}, b_{33} are quadratic coefficients generated from the observed experimental values of response from experimental runs, while A, B and C are the coded intensity of independent variables. The terms A^2, B^2 and C^2 (i-1, 2 or 3) represent the interaction and quadratic terms respectively[14]. The concentration range of independent variables along with their low, medium and high levels were showed in table 1. The selected independent variables for the experimental design were span 60 concentration (A/X_1), cholesterol

concentration (B/X_2), hydration volume (C/X_3) and sonication time (D/X_4) and their effect were observed on PEE (Y_1), MVS (Y_2) with five centre point were shown in table 2.

Table 1: Various independent and dependent variables used by Box-Behnken design

Factors	Levels used, actual (coded)		
Independent variable	**Low (-1)**	**Medium (0)**	**High(+1)**
A/X_1 -Span 60 (mM) %	40	60	90
B/X_2-Cholesterol(mM) %	10	30	60
C/X_3-Hydration volume(ml)	5	10	15
D/X_4-Sonication time (min)	5	10	15
Dependent variable			
Y_1-Percent entrapment efficiency (PEE)			
Y_2-Mean vesicle size (MVS)			

The resulting experimental values of the responses were compared with predicted value and linear regression plot between actual and predicted value of the responses was plotted. The model was evaluated in terms of statistically significant coefficient and R^2 values and finally one optimum formulation was selected from point prediction method.

2.4. Evaluation

Drug content: Extract equivalent to 10mg of capecitabine from noisome derived from proniosome with 100ml of methanol, further it was appropriately diluted and assayed by UV spectroscopic method at 303 nm. The analysis was performed in triplicate and the content of capecitabine in each sample was determined in terms of percentage of drug content.

In-vitro drug release and release kinetics: The release study was performed for the optimized niosomes formulation (F24) by the paddle method using PBS (pH 7.4). The release evaluated to check the goodness of fit for zero-order model, first-order model, Higuchi's matrix model and Korsmeyer– peppas model. The correlation coefficient (R^2) for each model was calculated.

Table 2: Observed actual and predicted experimental values of Y_1 and Y_2 for Box-Behnken design.

Runs	Independent variable				Dependent variable			
					Y_1(Mean ±SD)		Y_2(Mean ±SD)	
	A/X_1 (%)	B/X_2 (%)	C/X_3 (ml)	D/X_4 (min)	Actual value	Predicted value	Actual value	Predicted value
1	0	0	0	0	70.21±2.35	78.37±1.71	600.00±3.25	608.60±2.75
2	0	0	0	0	72.21±5.42	78.37±1.28	602.00±1.25	608.60±2.85
3	0	0	-1	1	74.23±4.52	80.15±2.06	604.00±2.55	614.04±3.11
4	0	-1	0	-1	76.54±3.52	79.26±1.17	625.00±3.22	626.88±2.55
5	0	1	-1	0	78.21±5.21	78.50±2.16	625.00±2.22	625.92±3.22
6	1	-1	0	0	80.10±6.21	81.11±1.98	627.00±2.33	636.38±1.22
7	-1	0	0	-1	55.21±2.11	59.80±1.69	606.00±1.85	614.75±2.11
8	1	1	0	0	80.98±3.54	85.18±2.54	630.00±2.45	644.38±3.22
9	0	-1	1	0	80.21±2.51	80.79±2.67	630.00±3.11	627.58±1.22
10	0	0	0	0	82.01±3.49	78.37±1.78	607.00±2.11	608.60±4.21
11	-1	1	0	0	58.21±3.65	58.49±1.59	630.00±3.22	628.88±3.22
12	0	0	-1	-1	82.00±4.08	82.06±1.78	608.00±2.22	613.04±4.11
13	-1	0	1	0	60.21±4.13	59.87±1.45	610.00±1.22	609.71±3.22
14	-1	0	0	1	62.32±2.72	63.34±1.25	612.00±2.22	616.25±1.22
15	0	1	1	0	81.21±1.78	83.90±1.11	635.00±1.22	637.58±2.22
16	0	0	0	0	82.23±2.11	78.37±2.53	614.00±3.22	608.60±3.22
17	0	0	1	1	83.20±2.89	84.43±1.53	616.00±3.75	619.21±1.85
18	0	0	1	-1	84.21±4.21	79.58±2.36	618.00±1.75	616.21±1.95
19	0	1	0	1	84.21±2.59	79.33±1.89	640.00±1.85	631.38±2.12
20	0	0	0	0	85.21±2.35	78.37±1.96	620.00±4.11	608.60±3.12
21	1	0	-1	0	82.01±3.85	80.19±1.85	622.00±3.55	615.54±2.25
22	0	-1	0	1	85.21±4.27	85.63±2.35	632.00±4.21	633.38±3.45
23	0	1	0	-1	85.36±1.83	82.78±2.35	642.00±3.25	633.88±3.65
24	0	-1	-1	0	86.21±2.38	84.39±1.25	635.00±4.01	630.92±2.85
25	-1	0	-1	0	66.21±2.11	63.57±3.26	625.00±2.22	619.54±2.95
26	1	0	0	-1	83.25±2.72	83.10±3.25	630.00±2.35	624.25±2.65
27	1	0	1	0	85.21±5.01	85.69±4.21	635.00±3.25	633.71±1.25
28	-1	-1	0	0	68.25±4.58	65.34±4.22	638.00±1.12	631.88±2.25
29	1	0	0	1	86.21±3.56	82.49±1.22	637.00±2.33	626.75±3.21

A/X_1 = Span 60 (mM) %; B/X_2 = Cholesterol (mM) %; C/X_3 = Hydration volume (ml); D/X_4 = Sonication time (min); Y_1 -PEE; Y_2 - MVS.

Niosome size and size distribution The vesicles size and size distribution were determined by dynamic light scattering (DLS) method, using a computerized inspection system (Zetasizer, HAS 3000; Malvern Instruments, Malvern, United Kingdom). For vesicle size measurement, the vesicular suspension was further diluted with the phosphate buffer saline to avoid multiscattering events and the measurements were conducted in triplicate [15].

Entrapment efficiency: The entrapment efficiency (PEE) of capecitabine niosome from proniosome formulations was determined by the centrifugal method. The formulations were centrifuged at 7000 rpm for 20 min and supernatant was taken and diluted with PBS (pH 7.4). The drug concentration in the resulting solution was assayed by UV spectroscopic method at 303 nm. This process was repeated to ensure that free drug was completely removed[16]. The PEE of niosomes formulations was calculated by the following equation.

PEE = (Total drug - Drug in supernatant)/(Total drug)

3. RESULTS AND DISCUSSION

3.1. Formulation and optimization of capecitabine niosome: A 4-factor, 3-level Box–Behnken statistical design was used to prepare niosomes (table 2) using Design Expert 11 (Trial Version 11, Stat-Ease Inc., Minneapolis, MN). All individual and interactive effects of independent variables were investigated and all the responses of these runs fitted to first order, second order and quadratic models and found that best fit model was quadratic ($p < 0.0001$). The Summary of results of regression analysis for responses $Y_1$and Y_2 for fitting to quadratic model and the polynomial equation of each response and each model were shown in table 3. Three-dimensional plots showed the interaction effects of the independent variables on the responses as well as their usefulness in studying the effects of two factors on one response at a time as shown in figures 2-3.

Table 3: Summary of regression analysis for responses Y_1 (PEE), Y_2 (MVS), for fitting to different models.

Model	R^2	Adjusted R^2	Predicted R^2	SD	%CV
Response (Y_1)					
Linear	0.5755	0.5048	0.3735	6.48	-
2FI	0.6223	0.4125	-0.0782	7.06	-

Quadratic	**0.8483**	**0.6966**	**0.4434**	**5.07**	**6.56**
Response (Y_2)					
Linear	0.0878	-0.0642	-0.2868	12.87	-
2FI	0.1576	-0.3105	-1.1485	14.28	-
Quadratic	**0.7169**	**0.4338**	**-0.3619**	**9.39**	**1.51**

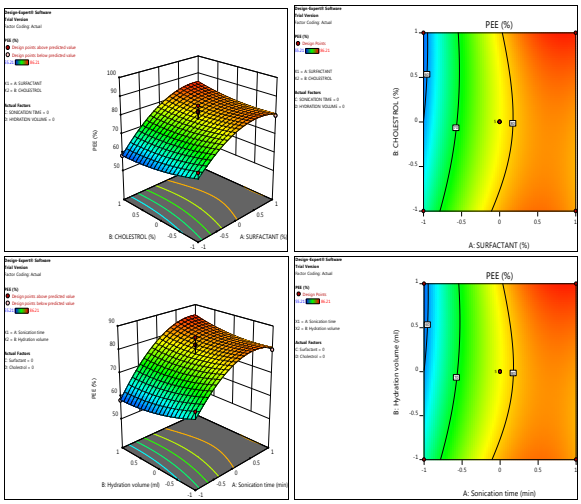

Fig 2: Three-dimensional (A-B) and contour response surface plot (C-D) image showing influence of independent variables (A/X_1-span 60 ; B/X_2 -cholesterol; C/X_3 -hydration volume; D/X_4-sonication time) on Y_1 response.

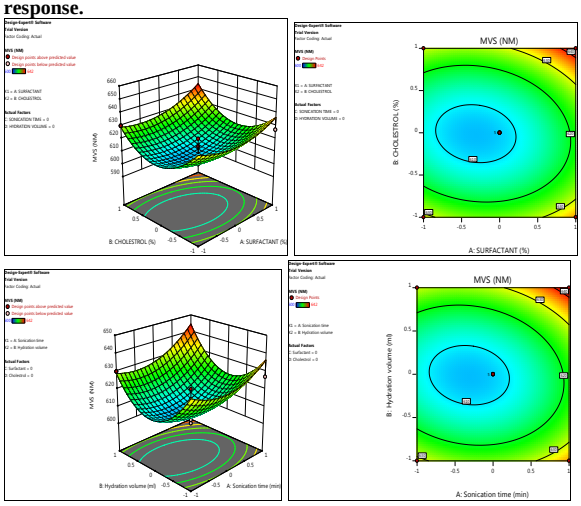

Fig 3: Three-dimensional (A-B) and contour response surface plot (C-D) image showing influence of independent variables (A/X_1-span 60; B/X_2 -cholesterol; C/X_3 -hydration volume; D/X_4 -sonication time) on Y_2 response.

3.1.1. Fitting of data to the model: The results of regression analysis of different responses are given in table 4. Larger values of the standard error for coefficients shows that the quadratic nature of the relationship. From table 2, it is evident that the one independent variables viz. the concentration of the span 60 has positive effects on the response Y_1(PEE), whereas the response Y_2 (MVS) has an inverse relationship with span 60. The concentration of cholesterol has the positive effect on response Y_2 (MVS) whereas the concentration of cholesterol has inverse effect on response Y_1 (PEE). Hydration volume and sonication time have an inverse effect on response Y_1 (PEE).

Table 4: In vitro release kinetics data to different mathematical models for capecitabine optimized niosome.

Model Fitting (Average)		**R**	**k**
Zero order	mo - m = kt	0.8275	3.1727
T-test		6.252	(Passes)
1st order	ln m = kt	0.9760	-0.0627
T-test		19.026	(Passes)
Matrix	mo - m = kt1/2	0.9867	15.8429
T-test		25.778	(Passes)
Best fit model-	**Matrix**		
Peppas	log (mo-m) = log k + n logt	0.9849	11.4653
T-test		24.149	(Passes)
n	0.6335		
Hix.Crow.	(% unreleased)$^{1/3}$=kt	0.9429	-0.0162
T-test		12.007	(Passes)

3.1.2. Effect of independent variables on PEE: PEE is the percentage fraction of the total drug entrapped into the vesicles. The maximum and minimum entrapment efficiency obtained were 86.21% for F24 (table 2). It is observed from the experimental design that entrapment efficiency has a direct positive relationship with the concentration of span 60 as revealed by the following equation.

Y_1 (PEE) = 78.374 + 10.6125A - 0.695B + 0.448333C + 0.7341670D + 2.73AB + 2.3AC -1.0375AD + 2.25BC - 2.455BD + 1.96CD -7.70617A^2 + 1.86008B^2 + 1.66258C^2 + 1.51633D^2

The Model F-value of 5.59 implies the model is significant. There is only a 0.14% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.05 indicated that the model terms are significant. In this case, A, B, AB, A^2, B^2, C^2, D^2 are significant model terms. Values greater than 0.1 indicated that the model terms are not significant. The "Lack of Fit F-value" of 0.4027 implies the "Lack of Fit" is not significant which is relative to the pure error. There is 88.90% chance that this large "Lack of Fit F-value" could occur due to noise. This model can be used to navigate the design space and result of this calculated model for entrapment efficiency represented by 3-dimentional plots and contour plot as shown in figure 2. As the concentration of span 60 increases, % PEE also increases. According to[17] the entrapment of drug occurred in both, bilayer and aqueous compartment of the vesicles. When the lipid compartment and aqueous phase becomes saturated with the drug, the vesicles provided limited entrapment capacity. The entrapment of drug occurs in both the baitlayers and the aqueous compartment of the vesicles[18] . When the lipid compartment and aqueous phase became saturated with the drug, the vesicles provided limited entrapment capacity[18]. Hence, niosome could entrap capecitabine only to an optimum extent, after which any further increase in hydration volume would lead to leakage of capecitabine from vesicles.

3.1.3. Effect of independent variables MVS: Small vesicular size is most important criteria for the effective drug delivery of niosome. The size of the vesicles was found to vary between 600.0 to 642.00 nm (table 2). Initially, average

vesicle size increased with increase in the concentration of span 60 from 1 to 2 mM. However, an additional increase in the concentration of span 60 from 2 to 3 mM leads to decrease in the average vesicle size. This is due to the development of a micellar structure instead of the vesicles, which are comparatively smaller in size. This relationship is presented by the following equation.

$$\mathbf{Y_2\ (MVS) = 608.6 + 5A + 1.25B + 2.08333C + 1D + 2.75AB + 7AC + 0.25AD + 3.75BC\text{-}2.25BD + 0.5CD + 7.95A^2 + 18.825B^2 + 3.075C^2 + 3.95D^2}$$

The Model F-value of 2.53 implies the model is significant. There is only a 4.66% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.05 indicated that the model terms are significant. In this case, A, B, C, D, AB, AC, BC, BD, CD, A^2, C^2, D^2 are significant model terms. Values greater than 0.1 indicates the models are not significant. The "Lack of Fit F-value" of 1.37 implies the "Lack of Fit" is not significant which is relative to the pure error. There is only 40.89% chance that this large "Lack of Fit F-value" could occur due to noise. There existed a direct relationship between the span 60 concentration and cholesterol on the MVS, entrapment efficiency of the vesicles of vesicles with capecitabine. This model can be used to navigate the design space and result of this calculated model for MVS represented by 3-dimentional plots and contour plot as shown in figure 3. The plot showed the effect of two formulation factors on particle size at one time.

3.1.4. Optimization: The optimized capecitabine niosomal formulation was selected based on the criteria of attaining the maximum value of entrapment efficiency whereas minimizing the vesicle size by applying point prediction method of the Design Expert 11 software[19]. Upon 'trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with span 60 (2mM), cholesterol (1mM), hydration volume (10ml) and sonication time (10min) was found to fulfill requisites of an optimum formulation i.e**.** F24. The optimized formulation has the MVS of 635.00±4.01nm with PEE 86.21±2.38mg/cm^2/h, respectively. Figure 4 showed the quantitatively linear relationship between resultant experimental values of the responses with that of the predicted value of all dependents variables. Optimized capecitabine niosome formulation (F 24) was converted into formulation.

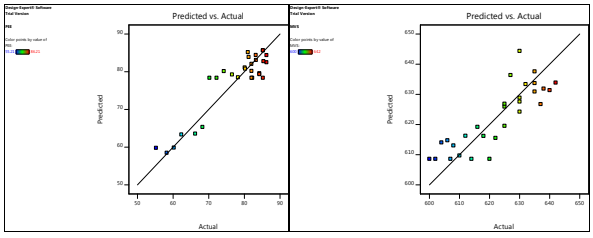

Fig 4: Linear correlation plots between actual and predicted values and the corresponding residual plots for all responses.

3.1.5. *In vitro* drug release and release kinetics: The obtained *in vitro* release data for both optimized capecitabine niosome and data was fitted to various release kinetic models. The correlation coefficients (R^2) values for different models for both formulations are presented in table 4. The model showing highest value of R^2 was considered as best model for release mechanism. It was found that the best fit model for optimized capecitabine niosomes F24 was Korsmeyer-peppas model and for capecitabine niosome was Higuchi matrix model, respectively. The highest R^2 obtained for optimized capecitabine niosome formulation F24 and optimized capecitabine niosome formulation were 0.9429 and 0.9849 respectively.

4. CONCLUSION

The present study conclusively demonstrates the use of Box-Behnken design in formulation and optimization of capecitabine niosome derived from proniosome formulations to avoid its systemic toxicity. This study indicated that niosome can be optimized to achieve desired properties using span 60 concentrations, cholesterol concentration, sonication time, and hydration volume. The optimized niosome formulation demonstrated enhanced entrapment efficiency and mean vesicular size.

5. ACKNOWLEDGEMENT

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