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FORMULATION AND OPTIMIZATION OF CURCUMIN-LOADED LIPOSOMES

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ABSTRACT

The study aimed to formulate and optimize curcumin loaded liposomes. In the present study Box Behnken design is used for optimization of curcumin liposomes. The responses obtained from the design matrix i.e. particle size and percentage entrapment efficiency were statistically evaluated and an optimum formula was suggested by the design expert software. The optimised formula was prepared and evaluated. Seventeen runs formulations were prepared according to Box-Behnken design. The formulations were F1 to F17. Formulation F13, F14, F15, F16, and F17 were fixed as the central formulation.

Key Words: Curcumin, Optimisation, Box Behnken Design, Liposomes, Entrapment Efficiency.

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INTRODUCTION

Turmeric has been used in Asia for thousands of years in food, preservation of food, and as traditional medicine. It is the yellow spice derived from the roots, rhizome, of the plant Curcuma longa. Curcumin is a herbal drug isolated from Curcuma longa family zingiberaceae, is used in the treatment of Cancer, Alzheimers and other inflammatory conditions. It is soluble in oil and insoluble in water at acidic and neutral P^H4 But the solubility increases when the medium becomes alkaline. The bioavailability of the drug is very poor and it is in very low level in plasma and tissues. Hence there is a demand to increase the bioavailability of curcumin before administering to the patients. Lot of ideal ways and drug delivery systems are available for increasing the bioavailability. Based on the drug character, liposomal approach seems to be good when comparing to other approaches.

Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a lipid bilayer mainly composed of natural or synthetic phospholipids. Liposomes were discovered in the early 1960́ s by Bangham and Colleagues and subsequently became the most extensively explode drug delivery system. The word liposome derives from two Greek words: lipo ("fat") and soma ("body"); it is so named because its composition is primarily of phospholipid.

A liposome is defined as a self forming structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartment. Phospholipids are the backbone of these structures. Phosphatidylcholine (PC), also called lecithin, is a biocompatible phospholipid that exists in plants and animals and used frequently in liposomal preparation. Moreover, there are other molecules widely used in combination with phospholipids, such as cholesterol. The exact location of a drug in liposomes will depend upon its physicochemical characteristics and the composition of the lipids. However, as a general rule, the hydrophilic drug

molecules can be encapsulated in the aqueous space whereas the hydrophobic and amphiphilic molecules can be incorporated into the lipid bilayer.

Liposomes are thought to shield all or most of the drug molecules resulting in decreasing the direct contact of drug with biological environment, thus the pharmacokinetic profile of the drug will be determined by the physiochemical properties of liposomes, rather than the drug itself. Incorporating the drug into a vehicle capable of delivering it intact would overcome many of the disadvantages of the free drug administration. Improving the pharmacokinetics of the drug by this method could lead to beneficial effects such as reduced dosages, increased cellular permeability and delayed drug elimination. It is worth mentioning that liposomes are also non-toxic, biodegradable and can be manufactured on large scales (1-3).

MATERIALS AND METHODS

Materials

Curcumin was purchased from High Purity Laboratory Chemicals, Mumbai. Cholesterol, Lecithin and Carbopol 934 were purchased from Yarrowchem Products, Mumbai and all the other excipients were purchased from Nice chemicals, Cochin.

Preparation of Curcumin Liposomes (4)

Liposomes were prepared by conventional rotary evaporation method. The mixture of phospholipids and cholesterol was dissolved in volatile organic solvent chloroform along with 4 mg of drug. The organic solvent was then evaporated in rotary vacuum evaporator. The rpm was fixed at 100 and the temperature of the water bath at 60^0C. The deposited lipid films were hydrated with 5 ml phosphate buffer PH 6.5 with same speed and temperature. The resulting vesicles were swollen for 2 hr at room temperature. The 17 prepared liposome formulations were optimized based on drug content, entrapment efficiency and particle size.

Optimization of Curcumin Liposomes (5)

Liposomes with different drug: polymer ratio was prepared and optimized by Box-Behnken design.

Optical Microscopy

The particle size of all the liposomes were evaluated by using optical microscope fitted with a calibrated eyepiece micrometer. The particle diameters of about 50 liposomes were measured randomly. The average particle size was determined by using the Edmondson's equation,

$$D \text{ mean} = \Sigma nd / \Sigma n$$

Where n = number or frequency of microspheres observed and

d = mean size range

Drug Content and Entrapment Efficiency (6)

Dissolved 1ml liposomal suspension in suitable organic solvent and made up to 10 ml with phosphate buffer pH 5.8. From this 5 ml was made up to 10 ml. Again 1ml was made up to 10 ml phosphate buffer. The absorbance was measured at 425 nm by UV Spectroscopy.

Optimization of Liosome Preparation by Box Behnken Design (7-10)

Response surface methodology using Box – Behnken was chosen for the optimization of liposome formulation because it allows the determination of influence of the factors with a minimum number of experiments. The independent factors were Cholesterol concentration (X1), lecithin (X2) and chloroform (X3). The levels were fixed based on the preliminary evaluation. The response variables were Particle size (μm) (Y1) and Entrapment Efficiency (%) (Y2). Seventeen formulations were prepared according to Box - Behnken design. ie F1 to F17. Formulation F13, F14, F15, F16, and F17 were fixed as the central formulation. The responses obtained from the design matrix were statistically evaluated using Design expert 9 statistical software trial package, Stat – Ease (Table-1).

Table-1 Factors and levels used for formulation

Factors	Levels used		
	1	0	1
X1= Cholesterol(mg)	5	10	15
X2=lecithin (mg)	60	80	100
X3=chloroform (ml)	5	10	15
Responses	Constraints		
Y1=Particle size	6.3-11.1(μm)		
Y2=Entrapment Efficiency	67.5-92.4(%)		

Development of the Optimum Batch

Based on the statistical evaluations the software suggested one optimum batch which experted to give the response within the range.

Evaluation of Optimized Batch

The developed optimized batch was evaluated for particle size and entrapment efficiency.

RESULTS AND DISCUSSION

Preparation of Liposomes

Seventeen formulations of liposomal suspension of Curcumin were prepared by conventional rotary evaporation method. Liposomes were prepared with cholesterol, soya bean lecithin, and chloroform in different ratios.

Optimization of the Batches by Box- Behnken Design

A three factor three level, Box-Behnken design was used for the optimization procedure. The design consists of a replicated centre point and a set of point lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The non linear computer generated quadratic model is given as

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_{12} + b_{22}X_{22} + b_{33}X_{32} + E$$

Where Y = the measured response associated with each factor level combination.

b_0 = intercept b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y. X_1, X_2 and X_3 = coded levels of independent variables E = error term

In the preliminary studies, it was found out that the three most important factors affecting entrapment efficiency are amount of lecithin, cholesterol and chloroform. Hence these three variables were selected as the independent variables. The levels of these parameters were determined from the preliminary studies. All other factors were kept constant throughout the present study. To elucidate the influences of the decision variables on the response variables, a three-factor, three-level seventeen run 'Box-Behnken' design was employed. Design Expert software 9.0.3.1 (Statease, Minneapolis USA) trial package was used for the generation and evaluation of the statistical experimental design. The response variables were percentage entrapment and particle size of the liposome. Consideration of three levels of the independent variables instead of the usual two level studies was necessary to check the possible non-linearity of these influences. 17 formulations were prepared according to Box-Behnken design. The formulations were F1 to F17. Formulation F13, F14, F15, F16, and F17 were fixed as the central formulation (Table-2).

Table-2 shows the factors and responses used for Box Behnken design

Formulation Code	Factors			Responses	
	X1 Cholestrol (mg)	X2 Lecithin (mg)	X3 Chloroform (ml)	Particle size (µg)	Entrapment Efficiency (%)
F1	10	100	15	7.42	81.44
F2	10	60	5	7.89	85.28
F3	10	100	5	6.98	81.83
F4	10	60	15	6.88	80.79
F5	15	80	15	9.42	77.41
F6	5	80	5	8.74	78.52
F7	15	80	5	9.73	76.41
F8	5	80	15	9.15	79.17
F9	15	100	10	10.42	68.40
F10	5	60	10	10.76	69.27
F11	15	60	10	10.98	67.50
F12	5	100	10	11.18	68.73
F13	10	80	10	7.25	89.84
F14	10	80	10	6.81	90.28
F15	10	80	10	6.36	91.45
F16	10	80	10	6.53	89.74
F17	10	80	10	7.35	92.42

A suitable polynomial equation involving the individual main effects and interaction factors were obtained for each individual responses based on the statistical parameters such as the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of squares (PRESS). The summary of the model analysis, lack of fit and R-square analysis for the responses were depicted in the tables below:

The Model F-value of 28.64 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_2X_3, X_3^2 significant model terms. The "Lack of Fit F-value" of 0.81 implies the Lack of Fit is not significant. There is a 5.05% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. Mathematical relationship in the form of polynomial equation for the measured responses was obtained with the statistical software. The equation was as follows

$$\mathbf{Y1 = +8.41+0.11X_1+0.029X_2-0.014X_3+0.16X_1X_2+0.12X_1X_3-0.36X_2X_3+2.500E\text{-}003X_2^2-0.24X_3^2+0.94X_3^2}$$

The Model F-value of 72.72 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_1, X_2^2 are significant model terms. The "Lack of Fit F-value" of 1.69 implies the Lack of Fit is not significant relative to the pure error. There is a 0.59% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good Mathematical relationship in the form of polynomial equation for the measured responses was obtained with the statistical software.

The equation was as follows:

$$\mathbf{Y2 = +3.94250 +1.166600\ X_1 +0.43283\ X_2+2.327\ X_3+4.0050E\text{-}003\ X_1\ X_2+0.0621\ X_1\ X_3+1.6E\text{-}003\ X_2\ X_3 - 0.067825X_1^2 -1.30225E\text{-}003\ X_2^2 -0.1433\ X_3^2}$$

The equations represent the quantitative effect of variable (X_1, X_2, and X3) and their interactions on the responses. Coefficients with more than one factor term and those with higher order term represent interaction terms and quadratic relationships respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect.

Contour plots are two dimensional representations of the responses for the selected factors. Three dimensional surface plots for the obtained responses were drawn based on the model polynomial functions to assess the change of response surface. The contour plot and the response surface plots of the significant interaction terms of the factors were given in Fig-1 and 2

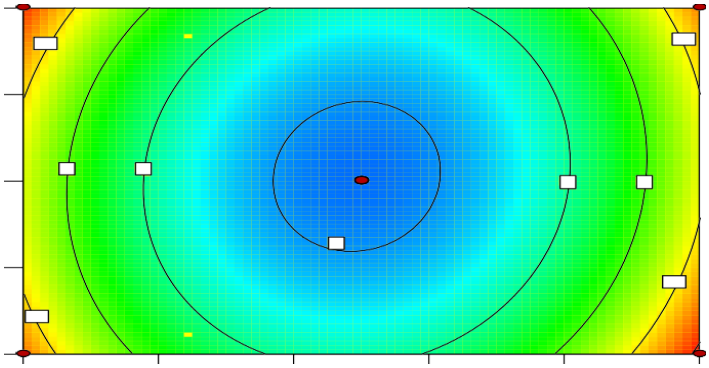

Fig-1 contour plot for the effect of cholesterol, lecithin, and chloroform on particle Size.

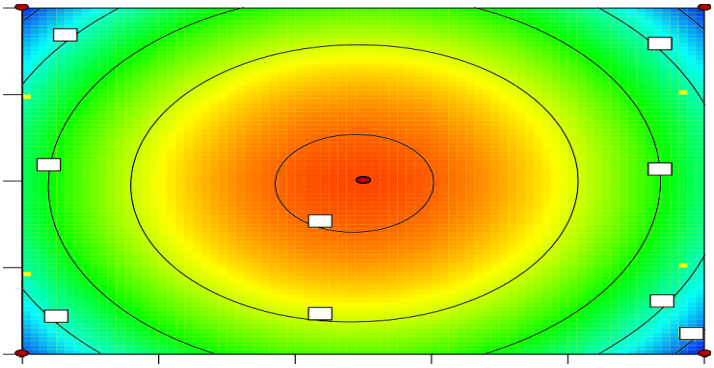

Fig-2 Contour plot for the effect of cholestol, lecithin, and chloroform Concentration on entrapment efficiency

After generating the polynomial equation relating to the dependent and independent variables, the formulation was optimized for the responses. The desirable ranges of the responses were restricted to maximize the entrapment efficiency in the range of 80- 95%. The optimum values of the variables were obtained by the numerical analysis based on the criterion of desirability. Therefore a new batch of liposome with the predicted levels of formulation factors was prepared to confirm the validity of the optimization procedure.

Development of the Optimum Batch

Based on the statistical evaluations the software selected one optimum batch. The formula for the optimum batch is given in table-3.

Table-3 Formula for optimum batch

Sl no:	Cholesterol (mg)	Lecithin (mg)	Chloroform (ml)	Particlesize (µm)	EntrapmentEfficiency (%)
1	9.82	79.60	5	6.3	91.63

Evaluation of Optimized Liposome

The liposomes were evaluated for average particle size, Drug content and Percentage drug entrapment. The evaluation is depicted in table-4.

Table-4 Evaluation of optimized formulation of liposome

Parameters	Results
Particle size (µm)	6.1±0.06
Drug content (mg)	3.47±0.08
Percentage drug entrapment (%)	90.21±0.04

CONCLUSION

The drug was identified through preformulation studies and also ensured the compatibility of Curcumin with cholesterol and soya bean lecithin. Curcumin loaeded liposomes were formulated using rotary evaporator by film hydration method and characterized. The formulation was optimized using Box – Behnken design (Design Expert software 9.0.3.1. Statease) keeping cholesterol, lecithin and chloroform levels as independent variables. The responses obtained from the design matrix i.e. particle size and percentage entrapment efficiency were statistically evaluated and an optimum formula was suggested by the design expert software. The optimised formula was prepared and evaluated.

REFERENCES

1. Shang, Y.J.; Jin, X.L.; Shang, X.L.; Tang, J.J.; Liu, G.Y.; Dai, F.; Qian, Y.P.; Fan, G.J.; Liu, Q.; Zhou, B. Antioxidant capacity of curcumin-directed analogues: Structure-activity relationship and influence of microenvironment. *Food Chem.* 2010, 119, 1435–1442.
2. Lee, K.H.; Aziz, F.H.A.; Syahida, A.; Abas, F.; Shaari, K.; Israf, D.A.; Lajis, N.H. Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities. *Eur. J. Med. Chem.* 2009, 44, 3195–3200.
3. Tang, H.D.; Murphy, C.J.; Zhang, B.; Shen, Y.Q.; van Kirk, E.A.; Murdoch, W.J.; Radosz, M. Curcumin polymers as anticancer conjugates. *Biomaterials* 2010, 31, 7139–7149.
4. Hua, W.F.; Fu, Y.S.; Liao, Y.J.; Xia, W.J.; Chen, Y.C.; Zeng, Y.X.; Kung, H.F.; Xie, D. Curcumin induces down-regulation of EZH2 expression through the MAPK pathway in MDA-MB-435 human breast cancer cells. *Eur. J. Pharmacol*. 2010, 637, 16–21.
5. Lekha Nair, K.; Thulasidasan, A.K.T.; Deepa, G.; Anto, R.J.; Vinod Kumar, G.S. Purely aqueous PLGA nanoparticulate formulations of curcumin exhibit enhanced anticancer activity with dependence on the combination of the carrier original. *Int. J. Pharm*. 2012, 425, 44–52.
6. Wena, Y.D.; Ho, Y.L.; Shiau, R.J.; Yeh, J.K.; Wua, J.Y.; Wanga, W.L.; Chiou, S.J. Synergistic antitumor effect of curcumin and dinitrosyl iron complexes for against melanoma cells. *J. Organomet. Chem*. 2010, 695, 352–359.
7. Dhillon, N.; Aggarwal, B.B.; Newman, R.A.; Wolff, R.A.; Kunnumakkara, A.B.; Abbruzzese, J.L.; Ng, C.S.; Badmaev, V.; Kurzrock, R. Phase II trial of curcumin in patients with advanced. *Clin. Cancer Res*. 2008, 14, 4491–4499.

8. Dhillon, N.; Wolff, R.A.; Abbruzzese, J.L.; Hong, D.S.; Camachi, L.H.; Li, L. Phase II clinical trial of curcumin in patients with advanced pancreatic cancer. *J. Clin. Oncol*. 2006, 24, 14151.
9. Shi, S.; Manjul, M. Comparative bioavailability of curcumin, turmeric and BiocurcumaxTM in traditional vehicles using non-everted rat intestinal sac model. *J. Funct. Foods* 2010, 2, 60–65.
10. Yang, F.; Jin, C.; Jiang, Y.; Li, J.; Di, Y.; Ni, Q.; Fu, D. Liposome based delivery systems in pancreatic cancer treatment: From bench to bedside. *Cancer Treat. Rev*. 2011, 37, 633–642.