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Research Article

THE APPLICATION OF CENTRAL COMPOSITE DESIGN FOR OPTIMIZATION OF KETOPROFEN NIOSOMES

***ABDELAZIZ E. ABDELAZIZ, AHMED M. SAMY, ELSHERBINY A. ELABD, MOHAMED A. RASLAN**

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.
Email: aazziizzoo@yahoo.com

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ABSTRACT

The objective of the present study was to investigate the effect of important formulation variables on drug entrapment in and drug release from niosome formulations of ketoprofen to obtain an optimized formula of Ketoprofen niosomes using central composite design. Contour and response surface plots were depicted based on the equation given by the model of the form $Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_1^2X_{11}+b_2^2X_{22}+b_3^2X_{33}+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{123}X_1X_2X_3$ where Y is the measured response associated with each factor level combination. Niosomes were prepared by a lipid hydration method using central composite design with three different variables include; Surfactant cholesterol ratio (X_1), HLB (X_2) and total lipid concentration (X_3). The optimization procedure generated the maximum overall desirability value. Central composite design succeeded in optimization of the formulation ingredients on the entrapment efficiency and in vitro release of Ketoprofen niosomes. Response surface methodology gave a mean to understand the effect of variables for the development of Ketoprofen niosomes. Finally the optimization process provides a formula having optimum level of factors as 0.66:1 from X_1, 7.86 from X_2, and 34.18 from X_3. This optimized formula produces entrapment efficiency (Y_1)equal to 42.22 % and release after 1 h (Y_2), 6 h (Y_3), and 12 h (Y_4), 28.89 %, 71.64 % and 91.31 % respectively and these observed values of the optimized formula were close to the predicted values. Our study proved that experimental design methodology could efficiently be applied for characterization and optimization of formulation parameters affecting entrapment efficiency and drug release from ketoprofen niosomes.

Keywords: Ketoprofen; Noisome; Central composite design; Optimization

INTRODUCTION

Niosomes are vesicles made up of nonionic surfactant in aqueous media resulting in closed bilayer structures. Niosomes are also able to entrap hydrophilic substances in the inner aqueous phase or hydrophobic drugs by partitioning of these molecules into their hydrophobic domains. Moreover, compared to liposomes, niosomes offer higher chemical and physical stability [1]with lower cost and greater availability of surfactant classes [2]. Different types of surfactants are proposed as starting material to prepare niosomes, i.e. the SPAN® series and the Brij® series, and their physico-chemical properties can modulate the stability and the features of vesicular systems because they are able to influence the fluidity of bilayers [3].

Niosomes have been reported to enhance the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of the drug and improve penetration of the trapped substances across the skin. In addition, these systems have been reported to decrease side effects and to give a considerable drug release [4].

In addition to traditional experimentation, factorial design, first reported by Box and Wilson is a very useful tool for the identification of critical process parameters and to optimize the respective process conditions [5].Some strategies are frequently used to achieve optimization such as full factorial, Box-Behnken, central composite designs, Plackett-Burman Designs, etc., which is a set of statistical techniques that allows the formulator to select the most influential factors on an experimental response and to obtain their optimum values[4]. Response surface methodology explores the relationships between several control variables and one or more response variables. Response surface methodology has been successfully utilized in several studies to optimize process and formulation variables and to obtain product with desired properties [6].

Using optimization designs and analysis of the response surfaces are powerful, efficient, and systematic tools that shorten the time required for the development of pharmaceutical dosage forms and increase research output [8].Optimization may be considered as the search for a result that is satisfactory and at the same time the best possible within a limited field of search. Thus, the type and components of a formulation may be selected, according to previous experience, by expert knowledge (possibly using an expert system) or by systematic screening. Then the relative and/or total proportions of the excipients are varied to obtain the best endpoint, or a process is chosen, and a study is carried out to determine the best operating conditions to obtain the desired formulation properties[8].

Ketoprofen is a poorly water-soluble non-steroidal anti-inflammatorydrug, broadly used as analgesic and for the treatmentof rheumatoid arthritis and osteoarthritis. Its oral administration is associated with a high risk of adverse gastro-intestinaleffects; it is therefore considered a good candidate for transdermal administration [9].

MATERIALS AND METHODS

Ketoprofen was kindly provided by El-Amyria Drug Company,Cairo, (Egypt),Span 20and Span 60from Sigma Chemical Co., Steinheim (Germany),Cholesterol from Sigma Chemical Co., St. Louis, MO, (USA),Sodium hydroxide and Potassium dihydrogen phosphate form El-Nasr Pharmaceutical Chemical Co., Cairo, (Egypt), andChloroform from Labscan Ltd, Dublin, (Ireland).

Software

The mathematical relationship in the form of polynoimal equation for the measured responces obtained with the statistical package Statgraphics(R)*plus* (vesrsion 4, Manugistics Inc., Rockville, MD, USA).

Equipment

An electric balance (Mettler AJ100, Switzerland), Ultraviolet spectrophotometer (Jenway 6305 uv/vis, UK), Buchi rotavapor (R-3000, Switzerland), Magnetic stirrer (Type MMS, Germany), Bath Sonicator (Model 275T, Crest Ultrasonic Corp, New York, USA), Dissolution apparatus (Erweka TD6R, Germany), Shaker water bath (Julabo SW-20 C, Germany),Centrifuge (Biofuge, primo Heraeus, Germany), and JEOL Transmission Electron Microscope (JTEM model 1010, Japan).

Preparation of Ketoprofen niosomes using central composite design

Niosomes were prepared by lipid hydration method using three different variables include: Surfactant cholesterol ratio (X_1), HLB

(X_2), and total lipid concentration (X_3). Central composite design was established to prepare sixteen different formulae of Ketoprofen niosomes. Surfactantcholesterol ratio was used in different values (0.65:1, 1:1, 1.5:1, 2:1, and 2.3:1).Total lipid concentration was used in different values (26.5, 30, 33.5, 40, and 43.4). Mixed Span 20 and Span 60 surfactants were used in different HLB values (5.31, 6, 7, 8, and 8.68) which were calculated according to equation:

$$\% \text{ span } 20 = (RHLB\text{-}HLB_{low})/ (HLB_{high} - HLB_{low})$$

Mixed Span 20 and Span 60 surfactantswith the required HLB values (5.31, 6, 7, 8, and 8.68) and cholesterol were dissolved in 15 ml of chloroform. The solvent was evaporated using a rotary flash evaporator at speed 120 rpm, under low pressure at 60°C for preparing niosomes. Niosomes were formed by adding phosphate buffered saline, PBS (pH 7.4) containing Ketoprofen concentration 2.5 % slowly to the dried thin film formed on the walls of the round-bottom flask, with gentle agitation. Dispersion of the mixture was carried out at 25°C using a sonicator, 20-KHz, and 500-W vibra cell at 1-min intervals for a period of 15 min.

Ketoprofen entrapment efficiency

The non-encapsulated drug was separated from the niosomal dispersions by centrifugation of the dispersion at 15,000 rpmfor 45 min [10]. The supernatant was separated, diluted to 100 ml with PBS pH 7.4, filtered using a membrane filter (0.2 µm pore size), and measured using a spectrophotometer at 262 nm [11].The percentage of drug encapsulation (EP (%))was calculated by the following equation:

$$EP\ \% = [(C_t - C_r \backslash C_t)] \times 100\%$$

Where C_t is the concentration of total Ketoprofen and C_r is theconcentration of free Ketoprofen.

In-vitro release of Ketoprofen

This study was carried out using a USP dissolution tester (Apparatus I). After separation of the un-entrapped and adsorbed drug, the Ketoprofen niosomal suspension (5ml) was placed in cylindrical tubes (2.5 cm in diameter and 6 cm in length). Each tube is tightlycovered with a Spectra por© molecular porous membrane tubingfrom one end and attached to the shafts of the USP Dissolutiontester apparatus, instead of the baskets, from the other end [12]. Theshafts were then lowered to the vessels of the dissolution apparatuscontaining 250 ml of phosphate buffer (pH 7.4)so that the dissolution medium outside and the vesicles preparationinside were adjusted at the same level. The release study wascarried out at 37±0.5 ºC, and the stirring shafts were rotatedat a speed of 50 rpm. Five milliliter samples were withdrawnperiodically at predetermined time intervals of 1, 2, 3, 4, 6, 8, 10, and finally 12 h.Every withdrawalwas followed by replacement with fresh medium to maintain aconstant volume. The samples were analyzed spectrophotometricallyat 262 nmand the results were the mean values of three runseach representing one batch.

Statistical analysis

The significance of estimation was determined by Student`s t-test.

Optimization of the formulation ingresients

In this study a three factors, three levels central composite design [13] was used for the optimization procedure. This design is suitable for exploration of quadratic response surface and constructs a second order polynomial model, thus helping in optimizing a process using a small number of experimental runs. The model constructed was as follow;

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_2X_3 + a_6X_1X_3 + a_7X_1^2 + a_8X_2^2 + a_9X_3^2 \text{ ----------} + E$$

Where a_0 to a_9 are the regression coefficient, X_1, X_2 and X_3 are the factors studied, Y is the measured response associated with each factor level combination and E is the error term.

After generating the polynomial equations relating the dependent and independent variables, the process was optimized for the response Y_1 (entrapment efficiency), Y_2 (in-vitro release after one hour), Y_3 (in-vitro release after six hour) and Y_4 (in-vitro release after twelve hour). Optimization was performed to obtain the levels of X_1, X_2 and X_3, which give optimum values of Y_1, Y_2, Y_3 and Y_4 at constrained conditions. To verify these values, a new formulation was prepared according to the predicted levels of X_1, X_2 and X_3. The obtained responses (Y_1, Y_2, Y_3 and Y_4) were calculated.

Formulation of the optimized formula

The preparation, entrapment efficiency, in vitro release, and kinetic study (as described before) of the optimized formula were studied and the optimized formula was then characterized by TEM

RESULTS AND DISCUSSION

Preparation of Ketoprofen niosomes using central composite design

Three different variables include: Surfactant cholesterol ratio (X_1), HLB (X_2), and total lipid concentration (X_3) as shown in Table 1) were screened using central composite design and sixteen different formulae of Ketoprofen niosomes were obtained as shown in Table 2). Using equation to obtain a second order polynomial equation carried out mathematical modeling.

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_1^2X_{11}+b_2^2X_{22}+b_3^2X_{33}+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3 +b_{123}X_1X_2X_3$$

Where, Y is the dependent variable while b_0 is the intercept, bi (b_1, b_2 and b_3), bij (b_{12}, b_{13} and b_{23}) and bijk (b_{123}) represents the regression coefficient for the second order polynomial and Xi represents the levels of independent formulation variables.

Table 1: Formulation factors for central composite design

Independent factors	Levels	
	Low	High
X_1 = Surfactant Cholesterol ratio	1:1	2:1
X2= HLB	6	8
X_3 = Total lipid concentration %	30	40

Table 2: The designed Ketoprofen niosomes formulae

Formulae	X_1	X_2	X_3
F1	2.34:1	7	35
F2	0.65:1	7	35
F3	1:1	8	30
F4	2:1	6	30
F5	1.5:1	7	43.40
F6	1.5:1	7	33
F7	1:1	8	40
F8	1.5:1	7	26.59
F9	1.5:1	7	35
F10	1.5:1	8.68	35
F11	2:1	8	40
F12	1:1	6	40
F13	2:1	6	40
F14	1.5:1	5.31	35
F15	1:1	6	30
F16	2:1	8	30

Ketoprofen entrapment efficiency

The range of the entrapment efficiency of the prepared niosomes was found to be between 19.61 ± 0.26 % and 42.51 ± 0.75 % as shown in Table (3) and figure (1). The best value was ovserbed in formula F12 while the worst value was observed in formula F1.

Table 3: Entrapment efficiency of Ketoprofen niosomes

Formula no.	Entrapment efficiency (%) ± S.D.
F1	19.61 ± 0.26
F2	36.12 ± 0.42
F3	32.14 ± 0.66
F4	22.69 ± 0.70
F5	38.92 ± 0.31
F6	26.88± 1.01
F7	40.35± 1.21
F8	20.47 ± 0.91
F9	28.86 ± 1.35
F10	23.42 ± 1.00
F11	25.70 ± 1.11
F12	42.51 ± 0.75
F13	30.95±0.90
F14	30.04±0.65
F15	34.78±0.89
F16	21.59±0.35

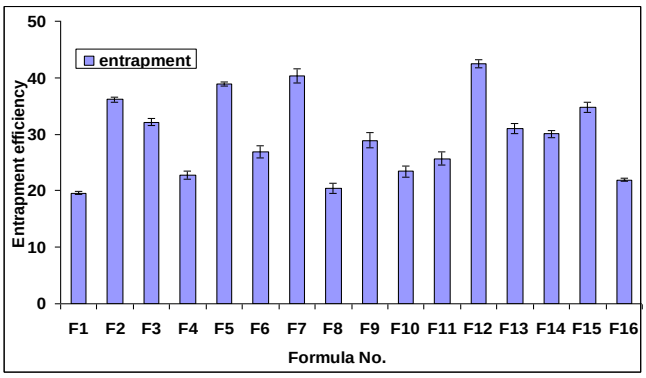

Fig. 1: Entrapment efficiency of Ketoprofen niosomes

Figures (2-4) showed the effect of the different independent variables on entrapment efficiency of Ketoprofen using STATGRAPHIC plus computer program.

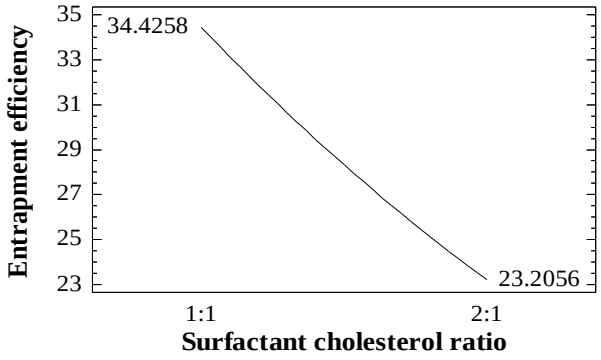

Fig. 2: Main effect plot showing the effect of Surfactant cholesterol ratio (X1) on the entrapment efficiency of Ketoprofen niosomes

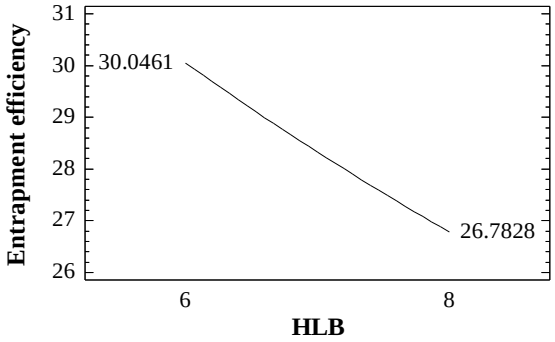

Fig. 3: Main effect plot showing the effect of HLB (X2) on the entrapment efficiency of Ketoprofen niosomes

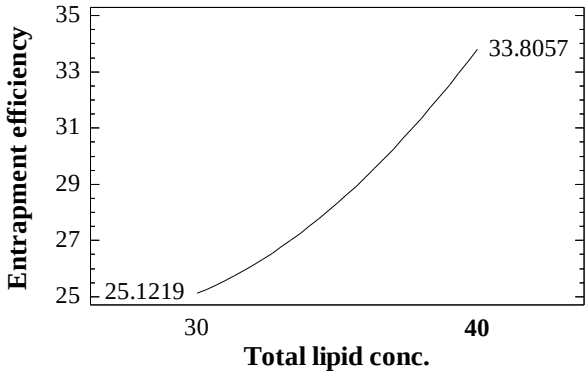

Fig. 4: Main effect plot showing the effect of total lipid concentration (X3) on the entrapment efficiency of Ketoprofen niosomes

As shown in Fig. 2 and 3), by increasing surfactant-cholesterol ratio (X_1) from 1:1 to 2:1, the entrapment efficiency was decreased from 34.42 to 23.20 % while by increasing the HLB (X_2) form 6 to 8, the entrapment efficiency was decreased from 30.04 to 26.78 %. Similar observations were previously reported [14].

As shown in Fig. 4), by increasing total lipid concentration (X_3) from 30 to 40 %, entrapment efficiency was increased from 25.12 to 33.80 %. This was agreed with Abd-Elbary et al. *[15]* who had reported that formula N3 containing the highest percent had resulted in significant ($P < 0.05$) increase in the mean niosome particle size, drug entrapment efficiency percentage and $t_{1/2}$ % value of the release profiles than other formulae. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the lipid bilayer of the niosomes [14]. Another possible explanation of these findings is related to the ability of cholesterol to abolish the gel to liquid phase transition of niosomal systems and thus improves the encapsulation of hydrophilic drugs. Moreover, it enhances the membrane rigidity by condensing the packing of surfactants in the bilayer membranes [15].

In vitro release of Ketoprofen

Figures (5-8) showed the release profiles of Ketoprofen from the investigated niosomes which occurred in two distinct phases (biphasic release processes), an initial phase in which rapid drug leakage was observed and stayed for about 8 hours, followed by slow phase but continued and stayed for at least 4 hours. This was agreed with Mehta et al.[16] who had reported that the release of anti-tuberculosis drugs was found to be biphasic in which initially a faster release rate is seen followed by a steady or a slower release rate after a certain period of time. The initial phase was due to desorption of drug from the surface of niosomes while the drug release in the slower phase was regulated by diffusion through the swollen niosomal bilayers [17].

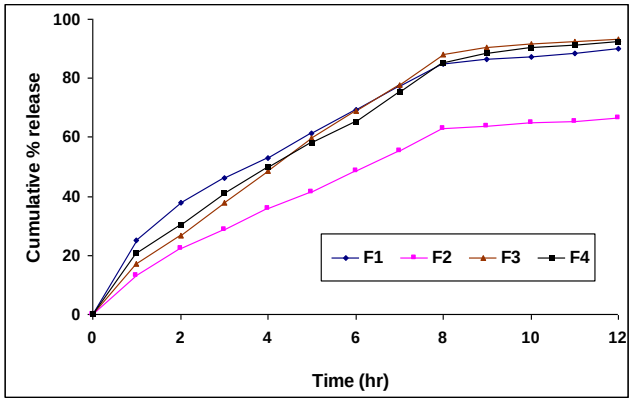

Fig. 5: In-vitro Release of Ketoprofenniosomes (F1-F4)

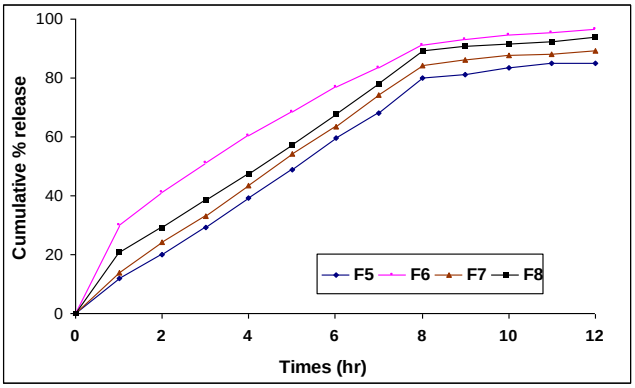

Fig. 6: In-vitro Release of Ketoprofenniosomes (F5-F8)

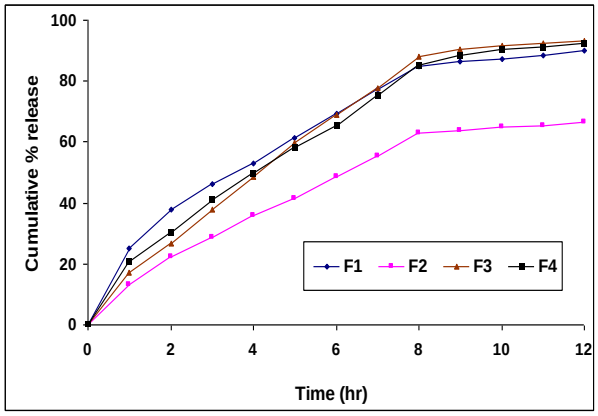

Fig. 7: In-vitro Release of Ketoprofenniosomes (F9-F12)

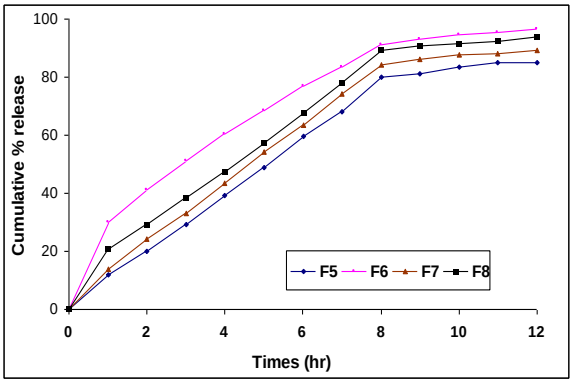

Fig. 8: In-vitro Release of Ketoprofenniosomes (F12-F16)

From the in vitro release profiles, we can indicate that; the slow release of Ketoprofen from the prepared niosomes ensures that the drug is available for a longer period of time and that too without degradation. Moreoverthe release data indicate that niosomes are at least as effective in their release characteristics, and may therefore offer improved bioavailability of some drugs with poor solubility, controlled release formulations. By comparison the entrapment efficiency and release data it was obvious that there was inversely proportional relationship the entrapment efficiency and the drug release. Entrapment efficiency is a measure of the vesicle ability to retain the drug; thus, the more the drug is retained in the vesicle, the slower the release profile will be [18]. The niosomal of higher entrapment efficiency, showed the lowest drug release % [19].

Furthermore, high values of X_1, X_2 and low value of X3 increased the release % of Ketoprofen. The general features of the release profile of the niosomes prepared using conventional surfactants revealed significant increase ($p < 0.01$) in the percentage drug released with the increase in HLB [20]. This may be attributed to the hydrophilic nature of Span surfactants which makes it act as a solubilizing agent for the drug, thus, facilitating drug release from the gel base [1].

The incorporation of cholesterol into niosomes delayed in vitro release of drug. Therefore, it induces a limited drug release through the membrane out of the vesicles [21][22]. This may be attributed to the increase in the viscosity of the niosomal dispersions containing higher total lipid % [23].

Statistical analysis

Tables (4-7) illustrated the ANOVA analysis partitions the variability in Y_1, Y_2, Y_3 and Y_4 into separate pieces for each of effect. It then tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error. The effects of all the tested independent variables have P-values less than 0.05, indicating that they are significantly different from zero at 95% confidence level.

Table 4: Analysis of variance for entrapment efficiency (Y_1)

Source	Sum of squares	D F	Mean square	F-Ratio	P-value
A: Surfactant-cholesterol ratio (X1)	429.82	1	429.82	46.63	0.0005
B: HLB (X2)	36.35	1	36.35	3.94	0.004
C: Total lipid % (X3)	259.05	1	259.05	28.11	0.001
AA	2.14	1	2.14	0.23	0.648
AB	0.300	1	0.300	0.03	0.862
AC	1.59	1	1.59	0.17	0.692
BB	0.066	1	0.066	0.01	0.935
BC	1.68	1	1.68	0.18	0.864
CC	11.52	1	11.52	1.22	0.310
Total error	55.30	6	9.21		
Total (Correlation)	803.189				

R-squared = 93.1145 percent; R-squared (adjusted for d.f.) = 82.7862 %; Standard Error of Est. = 3.036; Mean absolute error = 1.43384

Durbin-Watson statistic = 2.156

Table 5: Analysis of variance for Ketoprofen release after 1 h (Y_2)

Source	Sum of squares	D F	Mean square	F-Ratio	P-value
A: Surfactant-cholesterol ratio (X1)	255.76	1	255.76	10.39	0.01
B: HLB (X2)	136.21	1	136.21	5.54	0.004
C: Total lipid % (X3)	50.74	1	50.74	2.06	0.002
AA	41.48	1	41.48	1.69	0.24
AB	0.001	1	0.001	0.00	0.99
AC	18.21	1	18.21	0.74	0.42
BB	43.70	1	43.70	1.78	0.23
BC	0.10	1	0.10	0.00	0.98
CC	82.62	1	82.62	3.36	0.11
Total error	47.63	6	14.60		
Total (Correlation)	712.747				

R-squared = 79.2862 percent; R-squared (adjusted for d.f.) = 48.2154 percent; Standard Error of Est. = 2.96046; Mean absolute error = 1.35289

Durbin-Watson statistic = 2.86588

Table 6: Analysis of variance for Ketoprofen release after 6 h (Y_3)

Source	Sum of squares	D F	Mean square	F-Ratio	P-value
A: Surfactant-cholesterol ratio (X1)	322.15	1	322.15	12.05	0.03
B: HLB (X2)	235.25	1	235.25	8.80	0.02
C: Total lipid % (X3)	108.17	1	108.17	4.05	0.001
AA	65.41	1	65.41	2.45	0.16
AB	1.90	1	1.90	0.07	0.79
AC	24.85	1	24.85	0.93	0.39
BB	42.87	1	42.87	1.60	0.25
BC	0.15	1	0.15	0.01	0.94
CC	10.88	1	10.88	0.41	0.54
Total error	60.37	6	16.72		
Total (Correlation)	943.535				

R-squared = 83.0033 percent; R-squared (adjusted for d.f.) = 57.50 percent; Standard Error of Est. = 5.16995; Mean absolute error = 2.77786

Durbin-Watson statistic = 1.88436

Table 7: Analysis of variance for Ketoprofen release after 12 h (Y_4)

Source	Sum of squares	D F	Mean square	F-Ratio	P-value
A: Surfactant-cholesterol ratio (X1)	371.89	1	371.89	12.78	0.001
B: HLB (X2)	239.38	1	239.38	8.23	0.02
C: Total lipid % (X3)	116.84	1	116.84	4.02	0.04
AA	124.07	1	124.07	4.26	0.08
AB	10.32	1	10.32	0.35	0.57
AC	14.60	1	14.60	0.50	0.50
BB	20.41	1	20.41	0.70	0.43
BC	13.59	1	13.59	0.47	0.51
CC	0.97	1	0.97	0.03	0.86
Total error	74.85	6	19.09		
Total (Correlation)	823.2				

R-squared = 84.4565 percent; R-squared (adjusted for d.f.) = 61.14 percent; Standard Error of Est. = 5.39421; Mean absolute error = 2.84374

Durbin-Watson statistic = 1.17811

Optimization of the formulation ingresients

The dependent and independent variables were related using mathematical relationships obtained with the statistical package. The polynomial equation obtained was;

$Y_1 = 279.79 - 49.12X_1 - 5.061X_2 + 1.284X_3 + 2.330X^2_1 + 0.503X^2_2$

$Y_2 = -68.89 + 0.307X_1 + 3.92X_2 + 66.25X_3 - 1.079X^2_1 - 0.262X^2_2$

$Y_3 = 9.702 - 4.148X_1 - 0.8190X_2 - 7.38X_3 + 5.72X^2_1 + 16.87X^2_2$

The equation represents the effect of process variables (X_1, X_2 and X_3) and their interactions on the responses (Y_1, Y_2, Y_3 and Y_4). It is obvious from these equations that the interaction terms are not accounted. Only the main effects of the independent variable on responses are considered. The main effect is the effect of the factor averaged over all levels of the other factors. As an example, the main effect of X_1 on Y_1 can be calculated as

Main effect = (avg (Y_1{X_1= max (X_1)}) - (avg (Y_1{X_1= min (X_1)})

The three independent variables were optimized with a sixteen run central composite design as shown in Table 8), when mixing of HLB (7.86), total lipid concentration (34.62) and surfactant-cholesterol ratio (0.66:1), optimum response for the entrapment efficiency (43.18), for the in-vitro release after one hour (29.19), for the in-vitro release after six hours (73.86), and for the in-vitro release after twelve hours (90.34).

Table 8: Optimum desirability

Factor	Optimum	Response	Optimum
X_1	0.66:1	**(Y_1)**	43.18
X2	7.86	**(Y_2)**	29.19
X_3	34.18	**(Y_3)**	73.86
		(Y_4)	90.34

The relationship between the dependent and the independent variables was further elucidated using response surface plot and counter plot.

Effect of X_1, X_2, and X_3 on Y_1 (entrapment efficiency)

Figures (8 and 9) showed the contour plot and the response surface plot, which displayed the effect of surfactant cholesterol ratio (X_1) and HLB (X_2) on the entrapment efficiency (Y_1) at fixed value of the total lipid concentration percent (X_3) at 35%. By increasing X_1 up to 2:1 along with increasing X_2 up to 8 results in decreasing the entrapment of the formulation up to 25 % while decreasing X_1 up to 1:1 along with decreasing X_2 up to 6 results in increasing the entrapment of the formulation up to 34 %.

On the other hand, by increasing X_1 up to 2:1 along with decreasing X_2 up to 6 results in decreasing the entrapment of the formulation up to 23 % while decreasing X_1 up to 1:1 along with increasing X_2 up to 8 results in increasing the entrapment of the formulation up to 32 %. It was concluded that contour plot gave an idea about the exact percent of X_1 and X_2 at which the entrapment efficiency became at higher level at fixed percent of X_3 (35 %).

From the figures, it was concluded that, using X_1 at low level along with percent of X_2 ranging from 6-8 produces a formulation having entrapment efficiency from 33-35 % while using X_1 at high level along with percent of X_2 ranging from 6-8 produces a formulation having entrapment efficiency from 22-24%.

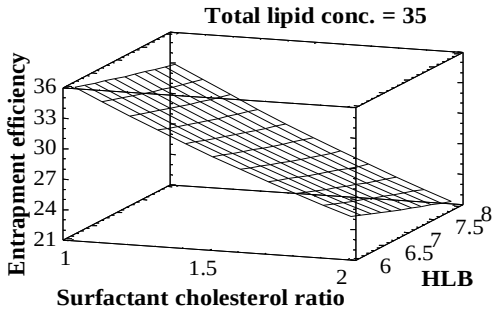

Fig. 9: Response surface plot showing the effect of X_1 and X_2 on Y_1

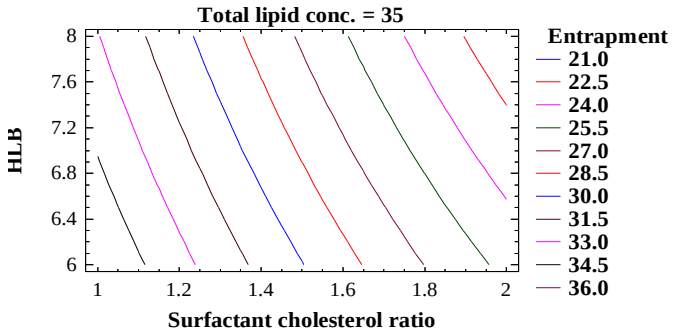

Fig. 10: Counter plot showing the effect of X_1 and X_2 on Y_1

Figures (10 and 11) showed the contour plot and the response surface plot, which displays the effect of HLB (X_2) and total lipid concentration percent (X_3) on the entrapment efficiency (Y_1) at fixed value of the surfactant cholesterol ratio (X_1) at 1.5:1. By increasing X_2 up to 8 along with increasing X_3 up to 40 results in increasing the entrapment of the formulation up to 32 % while decreasing X_2 up to 6 along with decreasing X_3 up to 30 results in decreasing the entrapment of the formulation up to 27 %. On the other hand, By increasing X_2 up to 8 along with decreasing X_3 up to 30 results in decreasing the entrapment of the formulation up to 24 % while decreasing X_2 up to 6 along with increasing X_3 up to 40 results in increasing the entrapment of the formulation up to 36 %. It was concluded that contour plot gives an idea about the exact percent of X_2 and X_3 at which the entrapment efficiency becomes at higher level at fixed percent of X_1 (1.5:1).

From the figures, it was concluded that, using X_2 at low level along with percent of X_3 ranging from 30-40 produces a formulation having entrapment efficiency from 27-36 % while using X_2 at high level along with percent of X_3 ranging from 30-40 produces a formulation having entrapment efficiency from 24-32%.

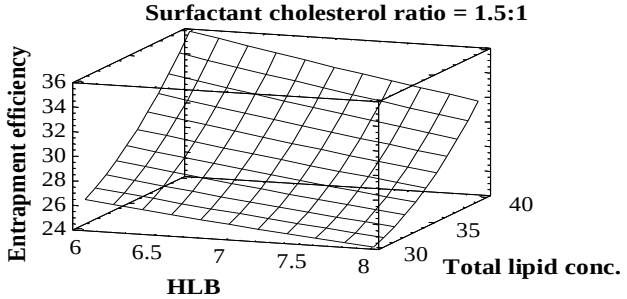

Fig. 11: Response surface plot showing the effect of X_2 and X_3 on Y_1

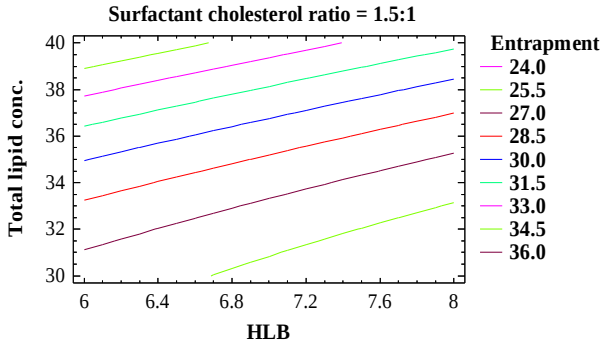

Fig. 12: Counter plot showing the effect of X_2 and X_3 on Y_1

Figures (12 and 13) showed the contour plot and the response surface plot, which displayed the effect of surfactant cholesterol ratio (X_1) and total lipid concentration percent (X_3) on the entrapment efficiency (Y_1) at fixed value of the HLB (X_2) at 7. By increasing X_1 up to 2:1 along with increasing X_3 up to 40, results in decreasing the entrapment of the formulation up to 28 %, while decreasing X_1 up to 1:1 along with decreasing X_3 up to 30 results in increasing the entrapment of the formulation up to 31 %. On the other hand, by increasing X_1 up to 2:1 along with decreasing X_3 up to 30 resulted in decreasing the entrapment of the formulation up to 21 %, while decreasing X_1 up to 1:1 along with increasing X_3 up to 40 results in increasing the entrapment of the formulation up to 39 %. It was concluded that contour plot gives an idea about the exact percent of X_1 and X_3 at which the entrapment efficiency becomes at higher level at fixed percent of X_2 (7).

From the figures, it was concluded that, using X_1 at low level along with percent of X_3 ranging from 30-40 produces a formulation having entrapment efficiency from 31-39 % while using X_1 at high level along with percent of X_2 ranging from 30-40 produces a formulation having entrapment efficiency from 21-28%.

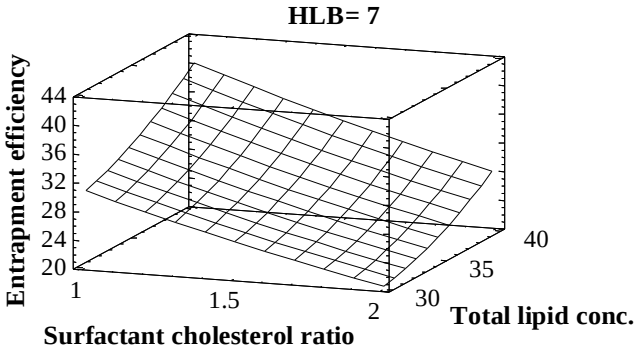

Fig. 13: Response surface plot showing the effect of X_1 and X_3 on Y_1

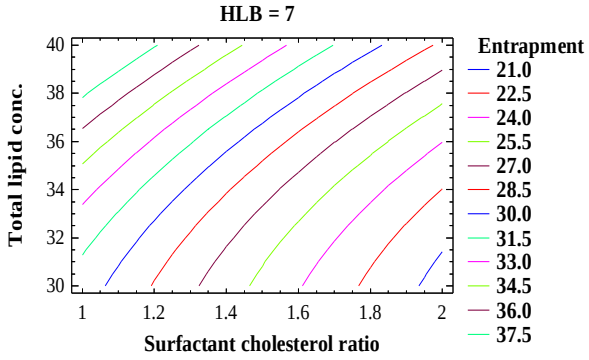

Fig. 14: Counter plot showing the effect of X_1 and X_3 on Y_1

Effect of X_1, X_2, and X_3 on Y_4 (release after 12 h)

Figures (14 and 15) showed the contour plot and the response surface plot, which displays the effect of surfactant cholesterol ratio (X_1) and HLB (X_2) on the in vitro release percent after 12 hr (Y_4) at fixed value of the total lipid concentration percent (X_3) at 35%. BY increasing X_1 up to 2:1 along with increasing X_2 up to 8 results in increasing the in vitro release percent of the formulation up to 96 % while decreasing X_1 up to 1:1 along with decreasing X_2 up to 6 results in decreasing the in vitro release percent of the formulation up to 76 %. On the other hand, by increasing X_1 up to 2:1 along with decreasing X_2 up to 6 results in increasing the in vitro release percent of the formulation up to 90 % while decreasing X_1 up to 1:1 along with increasing X_2 up to 8 results in decreasing the in vitro of the formulation up to 87 %. It was concluded that contour plot gives an idea about the exact percent of X_1 and X_2 at which the in vitro release percent becomes at higher level at fixed percent of X_3 (35 %).

From the figures, it was concluded that; using X_1 at low level along with percent of X_2 ranging from 6-8 produces a formulation having an in vitro percent from 76-87 % while using X_1 at high level along with percent of X_2 ranging from 6-8 produces a formulation having an in vitro release percent from 90-96 %.

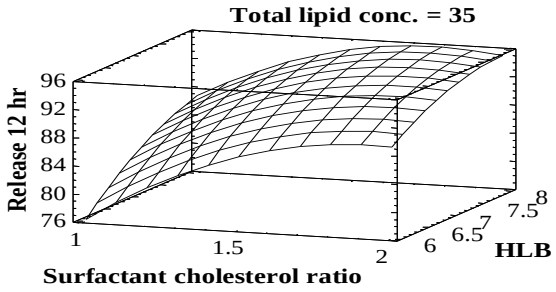

Fig. 15: Response surface plot showing the effect of X_1 and X_2 on Y_4

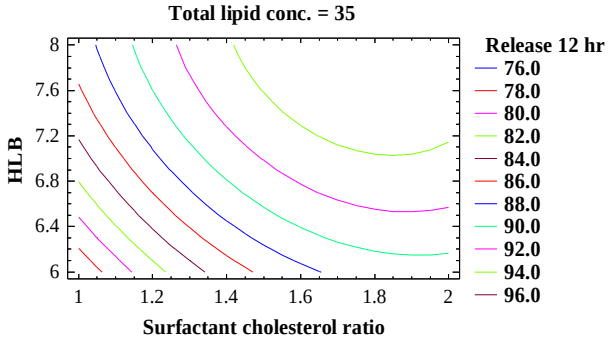

Fig. 16: Counter plot showing the effect of X_1 and X_2 on Y_2

Figures (16 and 17) showed the contour plot and the response surface plot, which displays the effect of surfactant cholesterol ratio (X_3) and total lipid concentration percent (X_3) on the in vitro release after 12 hr (Y_4) at fixed value of the HLB (X_2) at 7. By increasing X_1 up to 2:1 along with increasing X_3 up to 40, results in increasing the in vitro release percent of the formulation up to 92 % while decreasing X_1 up to 1:1 along with decreasing X_3 up to 30 results in decreasing the in vitro release of the formulation up to 88 %. On the other hand, by increasing X_1 up to 2:1 along with decreasing X_3 up to 30 results in increasing the in vitro release of the formulation up to 96 % while decreasing X_1 up to 1:1 along with increasing X_3 up to 40 results in decreasing the in vitro release percent of the formulation up to 79 %. It was concluded that contour plot gives an idea about the exact percent of X_1 and X_3 at which the in vitro release percent becomes at higher level at fixed percent of X_2 (7).

From the figures, it was concluded that; using X_1 at low level along with percent of X_3 ranging from 30-40 produces a formulation having in vitro release percent from 79-88 % while using X_1 at high level along with percent of X_2 ranging from 30-40 produces a formulation having in vitro release percent from 92-96 %.

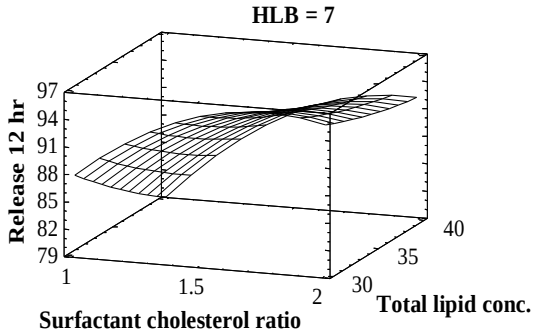

Fig. 17: Response surface plot showing the effect of X_1 and X_3 on Y_4

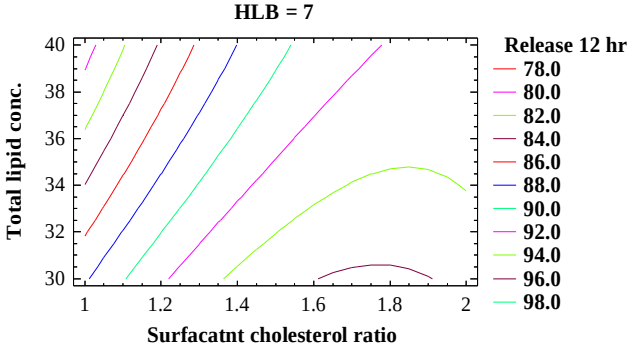

Fig. 18: Counter plot showing the effect of X_1 and X_3 on Y_2

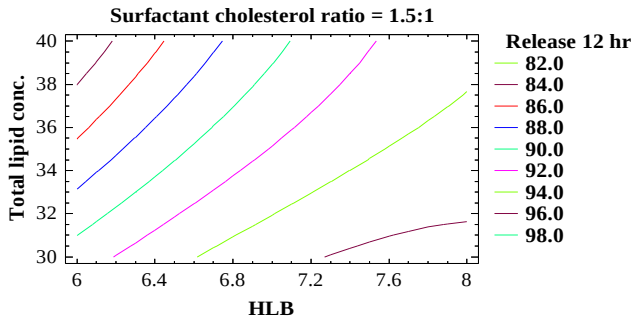

Fig. 19: Response surface plot showing the effect of X_2 and X_3 on Y_4

Figures (18 and 19) showed the contour plot and the response surface plot, which displays the effect of HLB (X_2) and total lipid concentration percent (X_3) on the in vitro release after 12 hr (Y_4) at fixed value of the surfactant cholesterol ratio (X_1) at 1.5:1. By increasing X_2 up to 8 along with increasing X_3 up to 40 results in increasing the in vitro release percent of the formulation up to 93 % while decreasing X_2 up to 6 along with decreasing X_3 up to 30 results in decreasing the in vitro release percent of the formulation up to 91 %. On the other hand, By increasing X_2 up to 8 along with decreasing X_3 up to 30 results in increasing the in vitro release percent of the formulation up to 97 % while decreasing X_2 up to 6 along with increasing X_3 up to 40 results in decreasing the in vitro release percent of the formulation up to 82 %. It was concluded that contour plot gives an idea about the exact percent of X_2 and X_3 at which the in vitro release percent becomes at higher level at fixed percent of X_1 (1.5:1).

From the figures, it was concluded that; using X_2 at low level along with percent of X_3 ranging from 30-40 produces a formulation having in vitro release percent from 82-91 % while using X_2 at high level along with percent of X_3 ranging from 30-40produces a formulation having in vitro release percent from 93-97 %.

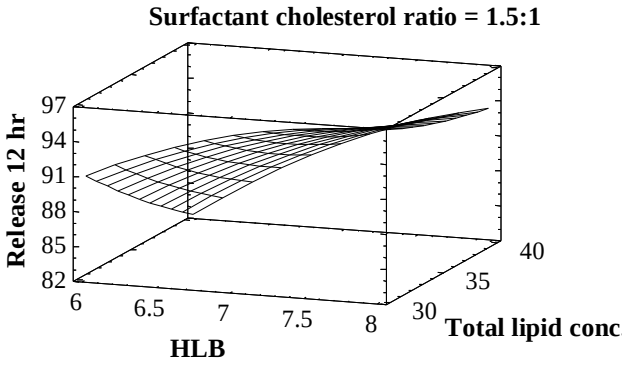

Fig. 20: Counter plot showing the effect of X_2 and X_3 on Y_4

Formulation of the optimized formula

The optimized formula prepared by lipid hydration method. The entrapment efficiency of the optimized formula was found to be equal 42.22 ± 0.52 %. The cumulative percent release of Ketoprofen from the optimized formula after one hour was 28.89%, after six hours was 71.64% and after twelve hours was 91.31%. The Kinetic models of the optimized formula were found to obey Higushi's diffusion model.

It was obvious that the observed values of the response were closed to the predicted values as shown in Table 9).

Table 9: Observed and predicted values of the responses for the optimized formula

Response	Observed	Predicted	Residual
Y_1	42.22	43.18	-0.96
Y_2	28.89	29.19	-0.3
Y_3	71.64	73.86	-2.22
Y_4	91.31	90.34	0.97

Transition electron micrographs revealed the formation of well identified niosomal vesicles as shown in Fig. 20). The examined niosomes appeared as spherical unilamellar nano vesicles with sharp boundaries. Particle size analysis of the optimized formula shows that the size range lied between 90.55 and 199.76 nm (mean 115.89 nm).

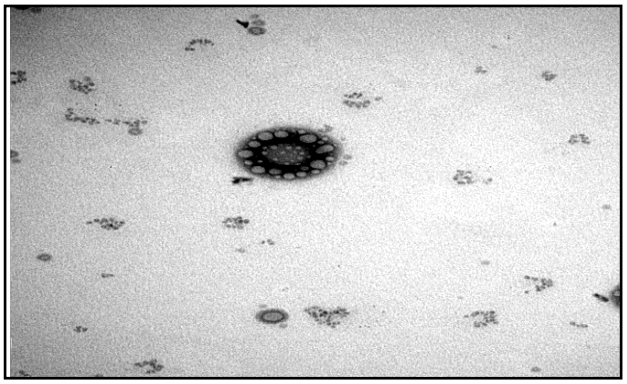

Fig. 21: TEM micrograph of the optimized formula

CONCLUSION

Central composite design succeeded in optimization of the formulation ingredients on the entrapment efficiency and in vitro release of Ketoprofen niosomes. Response surface methodology gave a mean to understand the effect of variables for the development of Ketoprofen niosomes. Finally the optimization process provides a formula having optimum level of factors as 0.66:1 from X_1, 7.86 from X_2, and 34.18 from X_3. This optimized formula produces entrapment efficiency (Y_1)equal to 42.22 % and release after 1 h (Y_2), 6 h (Y_3), and 12 h (Y_4), 28.89 %, 71.64 % and 91.31 %

respectively and these observed values of the optimized formula were close to the predicted values.

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