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Research Article

OPTIMIZATION OF PIROXICAM NIOSOMES USING CENTRAL COMPOSITE DESIGN

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ABSTRACT

Objective: The objective of the present study was to obtain an optimized formula of Piroxicam (PIR) niosomes using central composite design.

Method: PIR niosomes were prepared by lipid evaporation method and all vesicles were evaluated for their entrapment efficiency (EE%), and in-vitro drug release.

Results: EE% was found to be between 27.24% and 45.27%. The release profiles of PIR niosomes occurred in two distinct phases, an initial phase for about 8 hours, followed by slow phase for at least 4 hours. The in-vitro study suggests that higher EE% was related with slow release. The release pattern shown by these formulations were Higuchi diffusion controlled mechanism. The effects of all the tested independent variables have P-values < 0.05.

Conclusion: Central composite design succeeded in optimization of the formulation ingredients on EE% and invitro release of PIR niosomes. Finally the optimization process provides a formula having optimum level of factors.

Keywords: Central composite design, Entrapment efficiency (EE%), Niosomes, Optimization, Piroxicam (PIR).

INTRODUCTION

Niosomes or non-ionic surfactants vesicles are microscopic lamellar structures formed on the admixture of a non-ionic surfactant, cholesterol and phosphate with subsequent hydration in aqueous media [1]. PIR is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, rheumatoid arthritis, analgesic, and antipyretic activities. The mechanism of action of PIR, like that of other NSAIDS, is not completely understood but may be related to prostaglandin synthetase inhibition [2]. 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 [3]. Some strategies are frequently used to achieve optimization such as full factorial, Box-Behnken, central-composite, Plackett-Burman designs, etc. [4].

MATERI METHODS

Materials

PIR was provided by El-Mehan Drug Company, (Cairo,Egypt), Span 20 and Span 60 from Sigma Chemical Co., (Germany), Cholesterol from Sigma Chemical Co., (USA), Sodium hydroxide and Potassium dihydrogen phosphate, PureLab, Madison, USA, and Chloroform from Labscan Ltd, Dublin, (Ireland). All other chemicals used were of analytical grade.

Software

Statgraphics(R) plus (vesrsion 4, Manugistics Inc., Rockville, MD, USA).

Equipment

An electric balance (SARTORIUS AG, Germany), Shimadzu UV spectrophotometer (2401/PC), Japan, Buchi rotavapor (R-3000, Switzerland), Digital Sonifier (Branson,Danbury, USA), Dissolution apparatus (Erweka GmbH, Germany), Shaker water bath (Julabo SW-20 C, Germany), pH meter, JENWAY (England), Centrifuge (Biofuge, primo Heraeus, Germany), and JEOL Transmission Electron Microscope (JTEM model 1010, Japan).

Methods

Preparation of PIR niosomes

Niosomes were prepared by lipid hydration method using three variables include: HLB (X_1), total lipid (X_2), and surfactant cholesterol ratio (X_3). Central composite design was established to prepare sixteen different formulae of PIR niosomes. Mixed Span 20 and Span 60 surfactants were used in different HLB values which were calculated according to equation:

% span 20 = (RHLB-HLB_{low})/ (HLB_{high} – HLB_{low}).

Mixed surfactants and cholesterol were dissolved in 10 ml of chloroform. The solvent was evaporated using a rotary flash evaporator at speed 80 rpm, under low pressure at 60°C for preparing niosomes. Niosomes were formed by adding phosphate buffered saline, PBS (pH 7.4) containing 10mg PIR slowly to the dried thin film formed on the walls of the round-bottom flask, with gentle agitation. Dispersion of the mixture was carried using a sonicator for a period of 5 min.

Entrapment efficiency of niosomes (EE%)

The unentraped drug was separated from the niosomal dispersions by centrifugation at 15,000 rpm for 45 min. The supernatant was separated, diluted to 100 ml with PBS pH 7.4, filtered using a membrane filter (0.45µm pore size), and measured using a spectrophotometer at 354 nm. EE% was calculated by the following equation [5].

$$EE\% = [(Ct - Cr \backslash Ct)] \times 100\%$$

Ct is the concentration of total PIR.

Cr is the concentration of free PIR.

In-vitro release of PIR

This study was carried out using a USP dissolution tester (Apparatus I). Niosomal suspension (5ml) was placed in cylindrical tubes (2.5cm in diameter and 6cm in length). Each tube is tightly covered with a molecular porous membrane from one end and attached to the shafts of the USP Dissolution apparatus, instead of the baskets, from the other end. The shafts were then lowered to the vessels containing 250 ml of PBS (pH 7.4) at 37±0.5 ºC, and 50 rpm. 5ml samples were withdrawn at time intervals of 1, 2, 3, 4, 6, 8, 10, and 12 hr. followed by replacement with fresh medium. The samples were analyzed spectrophotometrically at 354 nm. The obtained data were subjected to kinetic treatment according to zero, first, and Higuchi diffusion models [6]. The correlation coefficient (r) was determined in each case.

Statistical analysis

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

Optimization of the formulation ingresients

Central composite 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 [7]. The model constructed was as follow;

$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_2X_3 + a_6X_1X_3 + a_7X_1^2 + a_8X_2^2 + a_9X_3^2$ ----------+ 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.

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.

Formulation of the optimized formula

The preparation, EE%, in vitro release, and kinetic study (as described before) of the optimized formula were studied and the optimized formula was then characterized by Transmission Electron Microscope (TEM).

RESULTS AND DISCUSSION

Preparation of PIR niosomes

Three different variables include: HLB (X_1), total lipid (X_2), and Surfactant -cholesterol ratio (X_3) as shown in table (1) were screened using central composite design and sixteen different formulae of PIR 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.

Entrapment efficiency of PIR (EE%)

As shown in figure (1) the range of the entrapment efficiency of the prepared niosomes was found to be between 27.24 % for F2 and 45.27 % for F9. Figures (2) showed the effect of the different independent variables on EE% of PIR using STATGRAPHIC plus computer program. By increasing (X_1); EE% was decreased from 42.61 to 29.67% while by increasing d (X_2); EE% was increased from 33.50 to 36.37%.

Table 1: **Formulation factors for central composite design**

Independent factors	Low	High
X_1 = HLB	4.7	8.6
X2= Total Lipid amount (mg)	30	50
X_3 = Surfactant : cholesterol	1:1	2:1

Table 2: The designed formulae of PIR Niosomes

Formulae	X_1	X_2	X_3
F1	6.65	40.0	1:1
F2	8.6	30.0	2:1
F3	6.65	40.0	1.5:1
F4	4.7	30.0	2:1
F5	8.6	50.0	2:1
F6	4.7	40.0	1.5:1
F7	6.65	40.0	1.5:1
F8	8.6	30.0	1:1
F9	4.7	50.0	1:1
F10	6.65	30.0	1.5:1
F11	4.7	30.0	1:1
F12	6.65	40.0	2:1
F13	6.65	50.0	1.5:1
F14	8.6	40.0	1.5:1
F15	4.7	50.0	2:1
F16	8.6	50.0	1:1

By increasing (X3), EE% was decreased from 36.04 to 32.99 %. 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 [8]. 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 [9].

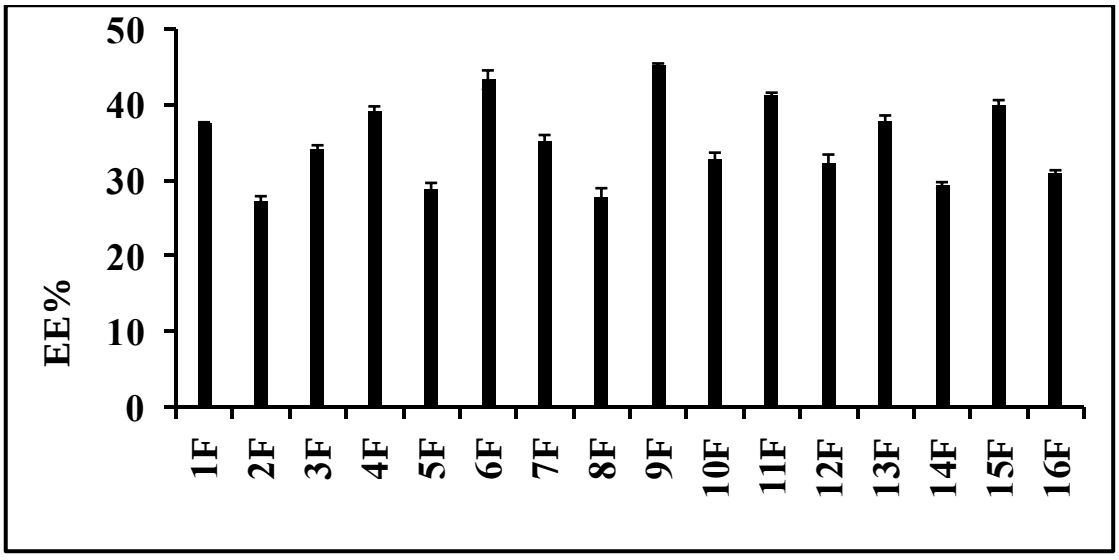

Fig. 1: EE% of PIR niosomes

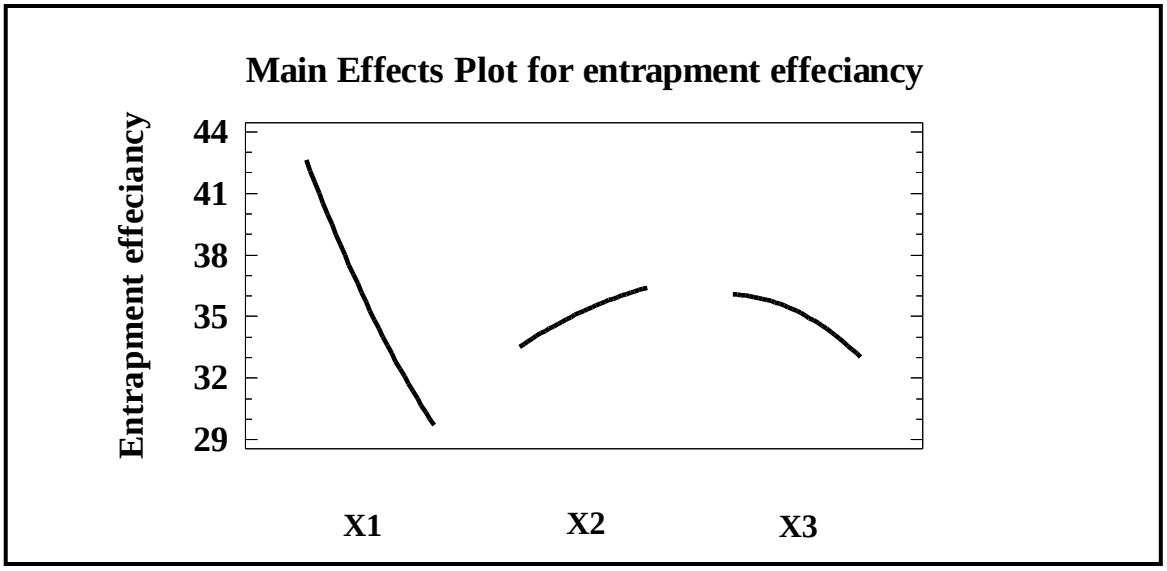

Fig. 2: Main effect plot showing the effect of (X1), (X2) and (X3) on EE% (Y1)

In-vitro release of PIR

Figures (3-6) showed the release profiles of PIR from the investigated niosomes which were occurred in two distinct phases, an initial phase in which rapid drug leakage was observed and stayed for about 8 hours, followed by slow phase stayed for at least 4 hours. 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 [10].

From figure (7-9), it was concluded that; there is inversely proportional relationship between EE% and the drug release. EE% 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 [11].

The rate of release of PIR niosomes was increased by increasing (X_1). 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 [12].

The rate of release was decreased as (X_2) was increased. This may be attributed to the increase in the viscosity of the niosomal dispersions containing higher total lipid [13]. The rate of release was increased by increasing the (X3). This may be attributed to the incorporation of cholesterol into niosomes delayed in-vitro release of drug [14].

As shown in table (3) the best kinetic order for the in-vitro release of PIR was calculated from the highest values of the obtained correlation coefficients.

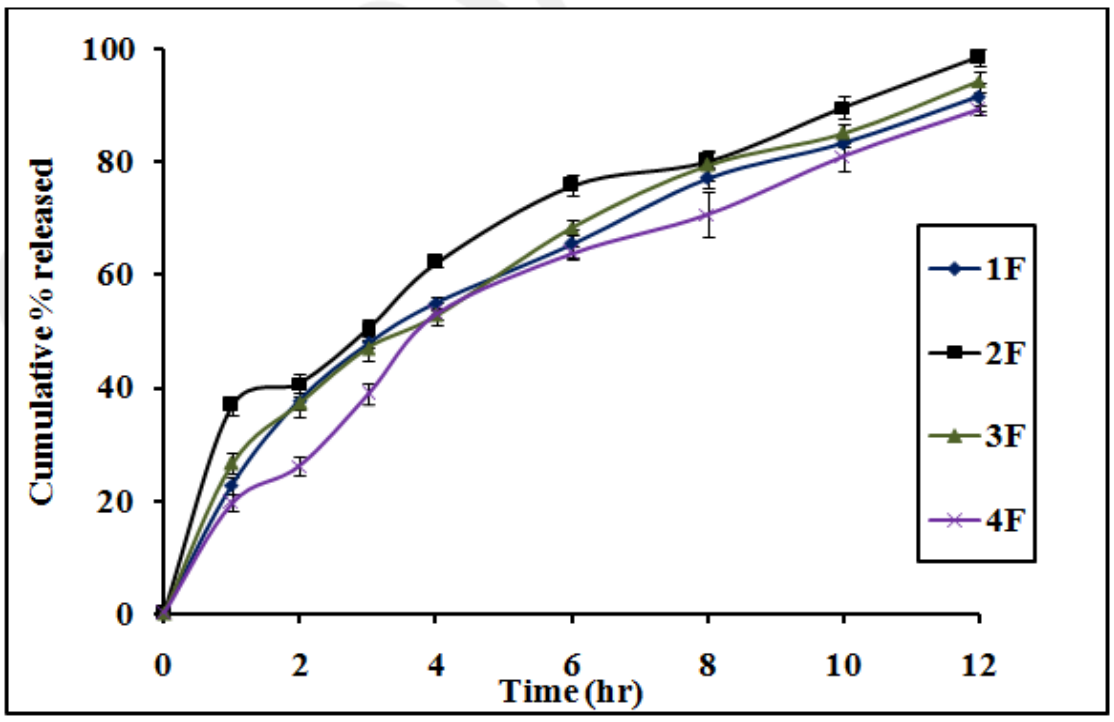

Fig. 3: In-Vitro Release of PIR Noisome (F1-F4)

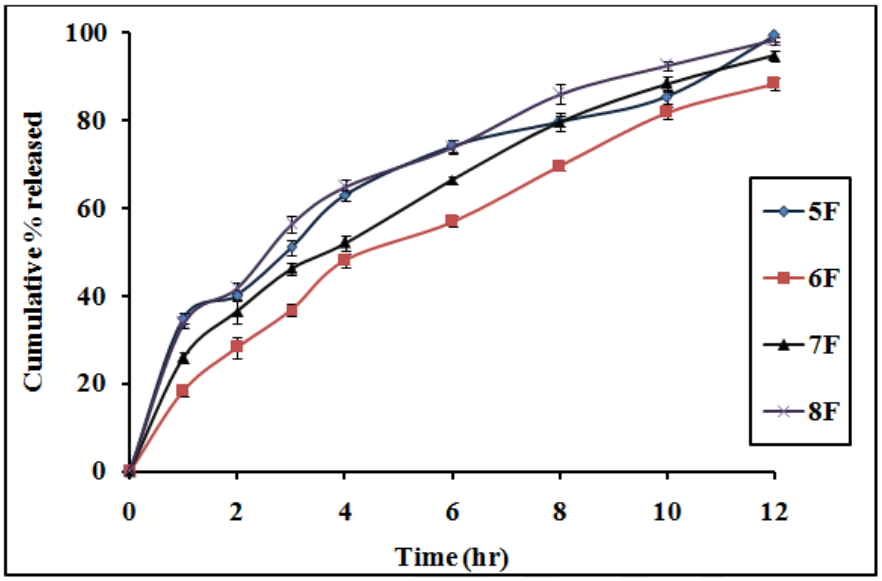

Fig. 4: In-Vitro Release of PIR Noisome (F5-F8)

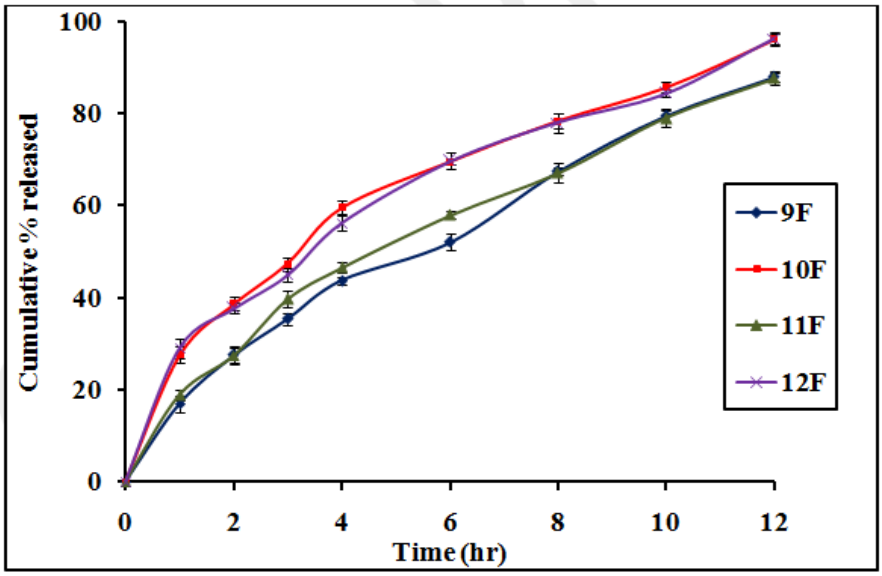

Fig. 5: In-Vitro Release of PIR Noisome (F9-F12)

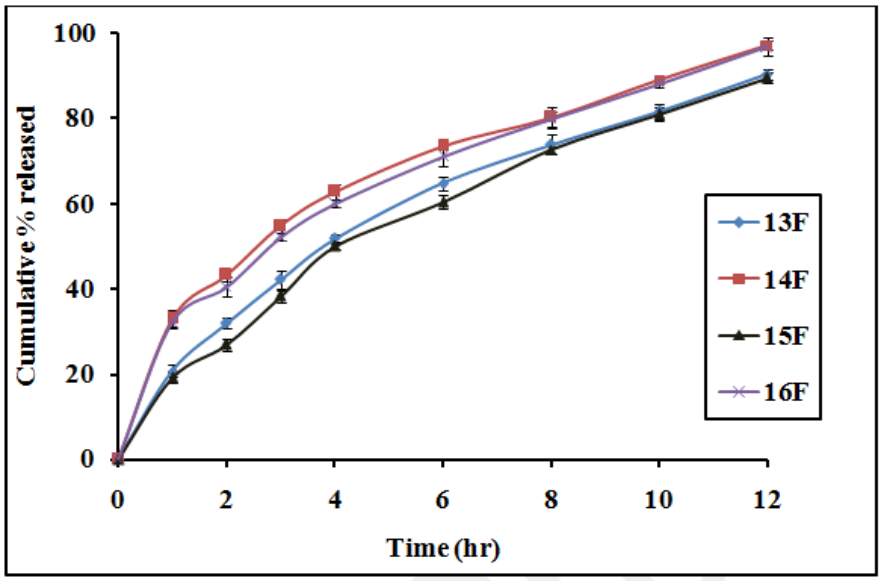

Fig. 6: In-Vitro Release of PIR Noisome (F13-F16)

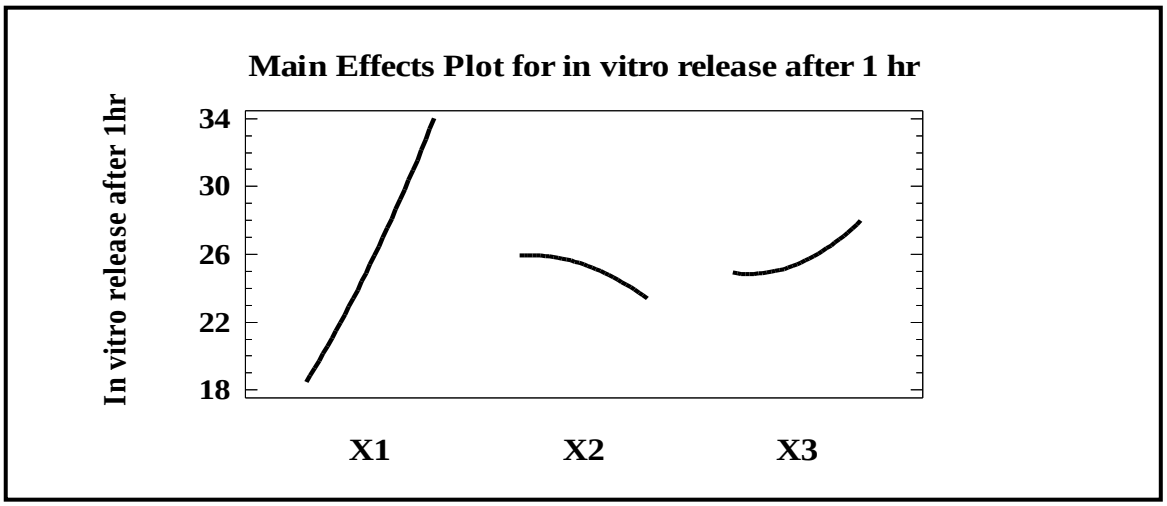

Fig. 7: Main effect plot showing the effect of (X1), (X2) and (X3) on (Y2)

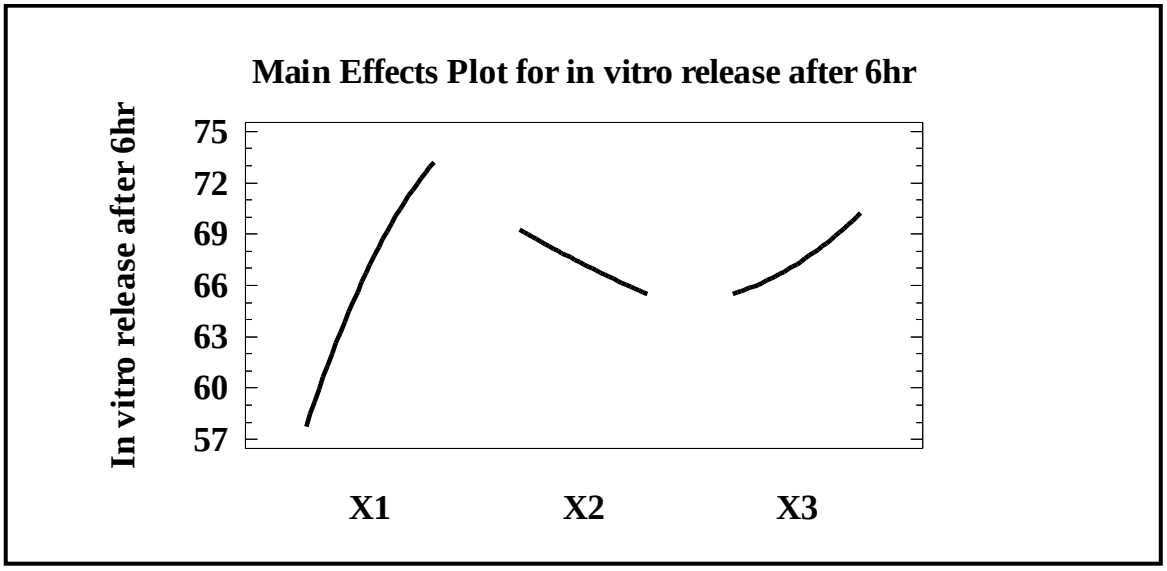

Fig. 8: Main effect plot showing the effect of (X1), (X2) and (X3) on (Y3)

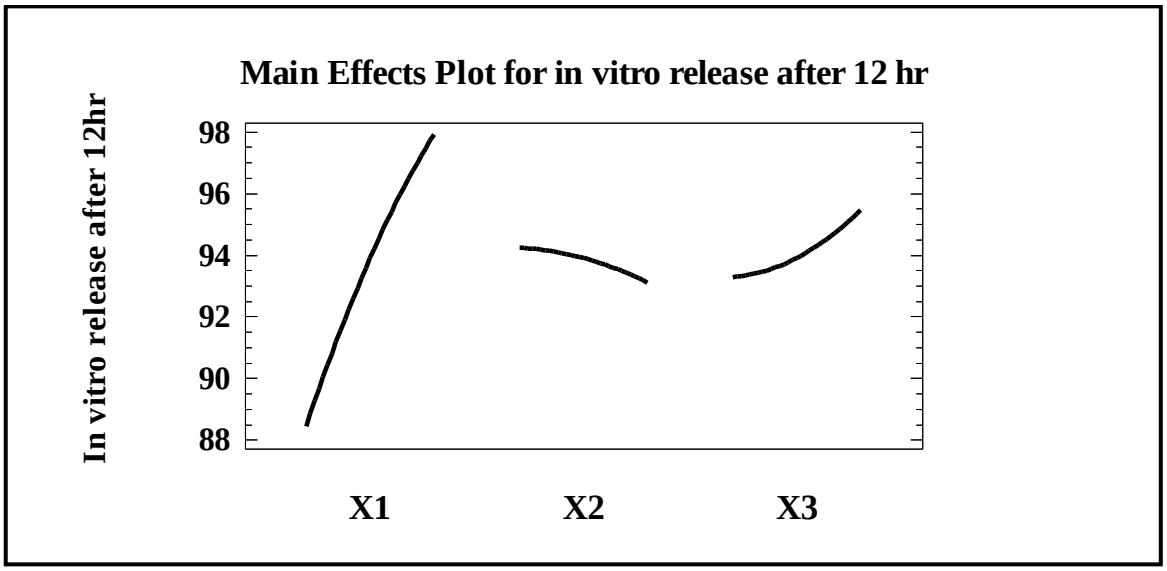

Fig. 9: Main effect plot showing the effect of (X1), (X2) and (X3) on (Y4)

Table 3: The Calculated Correlation Coefficients for the In-Vitro Release of PIR Noisome Employing Different Kinetic Orders or Systems

Formula No.	Correlation coefficient (r)		
	Zero	first	Higuchi diffusion model
F1	0.9750	0.9911	0.9968
F2	0.9814	0.9276	0.9923
F3	0.9842	0.9813	0.9983
F4	0.9749	0.9909	0.9929
F5	0.9779	0.8515	0.9907
F6	0.9899	0.9898	0.9980
F7	0.9882	0.9837	0.9988
F8	0.9723	0.9667	0.9943
F9	0.9948	0.9830	0.9960
F10	0.9801	0.9606	0.9971
F11	0.9893	0.9881	0.9985
F12	0.9860	0.9514	0.9968

Statistical analysis

Tables (4-7) illustrated the ANOVA analysis partitions the variability in Y1, Y2, Y3 and Y4 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	DF	Mean square	F-Ratio	P-Value
A: (X1)	419.127	1	419.127	274.09	0.0001
B: (X2)	20.7072	1	20.7072	13.54	0.0103
C: (X3)	3.1953	1	3.1953	15.17	0.0080
AA	2.16172	1	2.16172	1.41	0.2794
AB	0.00405	1	0.00405	0.00	0.9606
AC	2.53125	1	2.53125	1.66	0.2456
BB	0.236455	1	0.236455	0.15	0.7077
BC	3.05045	1	3.05045	1.99	0.2075
CC	1.36473	1	1.36473	0.89	0.3813
Total error	9.17483	6	1.52914		
Total (correlation.)	480.667	15			

R-squared = 98.0912 percent; R-squared (adjusted for d.f.) = 95.2281 percent; Standard Error of Est. = 1.23658; Mean absolute error = 0.646957; Durbin-Watson statistic = 1.76985

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

Source	Sum of squares	DF	Mean square	F-Ratio	P-Value
A: (X1)	604.351	1	604.351	164.17	0.0000
B: (X2)	16.0782	1	16.0782	4.37	0.0816
C: (X3)	23.4396	1	23.4396	6.37	0.0451
AA	1.75262	1	1.75262	0.48	0.5160
AB	0.132613	1	0.132613	0.04	0.8557
AC	1.13251	1	1.13251	0.31	0.5992
BB	1.46189	1	1.46189	0.40	0.5518
BC	0.148513	1	0.148513	0.04	0.8474
CC	2.79879	1	2.79879	0.76	0.4168
Total error	22.0874	6	3.68124		
Total (correlation.)	674.053	15			

R-squared = 96.7232 percent; R-squared (adjusted for d.f.) = 91.808 percent; Standard Error of Est. = 1.91866; Mean absolute error = 0.919057; Durbin-Watson statistic = 1.76442

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

Source	Sum of squares	DF	Mean square	F-Ratio	P-Value
A: (X1)	595.521	1	595.521	857.57	0.0001
B: (X2)	36.024	1	36.024	51.88	0.0004
C: (X3)	55.7432	1	55.7432	80.27	0.0001
AA	8.31749	1	8.31749	11.98	0.0135
AB	2.80845	1	2.80845	4.04	0.0910
AC	10.2605	1	10.2605	14.78	0.0085
BB	0.0437311	1	0.0437311	0.06	0.8102
BC	1.5488	1	1.5488	2.23	0.1859
CC	1.02586	1	1.02586	1.48	0.2699
Total error	4.16657	6	0.694429		
Total (correlation.)	715.027	15			

R-squared = 99.4173 percent; R-squared (adjusted for d.f.) = 98.5432 percent; Standard Error of Est. = 0.833324; Mean absolute error = 0.389125; Durbin-Watson statistic = 1.49028

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

Source	Sum of squares	DF	Mean square	F-Ratio	P-Value
A: (X1)	224.581	1	224.581	64.48	0.0002
B: (X2)	3.18096	1	3.18096	0.91	0.3761
C: (X3)	11.881	1	11.881	3.41	0.1143
AA	1.36211	1	1.36211	0.39	0.5548
AB	0.177013	1	0.177013	0.05	0.8291
AC	0.0000125	1	0.0000125	0.00	0.9985
BB	0.144102	1	0.144102	0.04	0.8455
BC	0.577812	1	0.577812	0.17	0.6979
CC	0.573011	1	0.573011	0.16	0.6991
Total error	20.8981	6	3.48302		
Total (correlation.)	263.287	15			

R-squared = 92.0626 percent; R-squared (adjusted for d.f.) = 80.1566 percent; Standard Error of Est. = 1.86628; Mean absolute error = 0.923017; Durbin-Watson statistic = 1.71596

Optimization of the formulation ingresients

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

$Y_1 = 53.4325 - 7.30646\ X_1 + 0.576409\ X_2 + 6.69125\ X_3 + 0.238137\ (X_1)^2 - 0.00115385\ X_1X_2 + 0.576923\ X_1X_3 - 0.00299483\ (X_2)^2 - 0.1235\ X_2X_3 - 2.87793\ (X_3)^2$

$Y_2 = 9.91477 + 0.820092\ X_1 + 0.471956\ X_2 - 12.9584\ X_3 + 0.214423\ (X_1)^2 - 0.00660256\ X_1X_2 + 0.385897\ X_1X_3 - 0.00744655\ (X_2)^2 + 0.02725\ X_2X_3 + 4.12138\ (X_3)^2$

$Y_3 = 30.195 + 10.697\ X_1 - 0.626892\ X_2 + 1.44071\ X_3 - 0.467116(X_1)^2 + 0.0303846\ X_1X_2 - 1.16154\ X_1X_3 + 0.00128793\ (X_2)^2 + 0.088\ X_2X_3 + 2.49517\ (X_3)^2$

$Y_4 = 69.9995 + 5.25143\ X_1 + 0.100737\ X_2 - 5.55596\ X_3 - 0.189032\ (X_1)^2 - 0.00762821\ X_1X - 0.00128205\ X_1X_3 - 0.00233793\ (X_2)^2 + 0.05375\ X_2X_3 + 1.86483\ (X_3)^2$

The equation represents the effect of process variables (X_1, X_2 and X_3) on the responses (Y_1, Y_2, Y_3 and Y_4).

These variables were optimized with a sixteen run central composite design as shown in table (8), when mixing of X_1 (6.036), X_2 (50 mg) and X_3 (1:1), optimum response for EE% (40.1123), for Y_2 (20.5208), for Y_3 (60.1140), and for Y_4 (90.6887).

Table 8: Optimum desirability

Independent variables	Low	High	*Optimum*
X_1	4.7	8.6	*6.036*
X2	30	50	*50*
X_3	1:1	2:1	*1:1*
Response	***Optimum***		
Y_1	*40.1123*		
Y_2	*20.5208*		
Y3	*60.1140*		
Y4	*90.6887*		

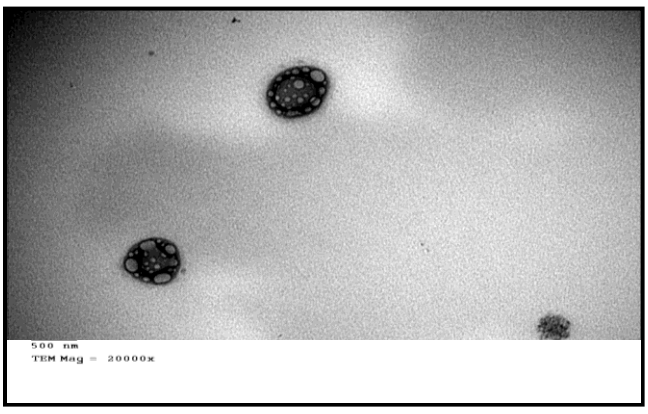

Fig. 10: TEM micrograph of the optimized formula

Formulation of the optimized formula

The optimized formula prepared by lipid hydration method. EE% of optimized formula was found to be 41.52 ± 0.42%. Y2 was 21.96%, Y3 was 61.84% and Y4 was 91.68%. The Kinetic models of the optimized formula were found to obey Higushi's diffusion model.

As shown in figure (10). The examined niosomes appeared as spherical unilamellar nano vesicles with size ranged between 85.79 and 176.84 nm (mean 118.43 nm).

CONCLUSION

Central composite design succeeded in optimization of the formulation ingredients on EE% and in vitro release of PIR niosomes. Finally the optimization process provides a formula having optimum level of factors as from 6.036 X_1, 50 from X_2, and 1:1 from X_3.This optimized formula produces EE% (Y_1), release after 1 h (Y_2), 6 h (Y_3), and 12 h (Y_4) equal to 41.52%, 21.96%, 61.84% and 91.68% respectively and these observed values of the optimized formula were close to the predicted values.

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