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FORMULATION AND OPTIMIZATION OF CEFPODOXIME PROXETIL LOADED SOLID LIPID NANOPARTICLES BY BOX-BEHNKEN DESIGN

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ABSTRACT
The objective of this study was to develop and evaluate solid lipid nanoparticles of Cefpodoxime Proxetil for enhancement of bioavailability via its lymphatic absorption. The solid lipid nanoparticles (SLNs) were prepared by solvent evaporation method using Precirol as a lipid carrier. A Box Behnken design has been applied to study the effect of independent variables i.e. lipid concentration, span 60 concentration and stirring speed on dependent variables i.e. particle size and entrapment efficiency. Response surface plots and counter plots were drawn and optimum formulations were selected based on feasibility search method. Validation of optimized study performed using four confirmatory runs indicated very high degree of prognostic ability of response surface methodology, with mean percentage error as +0.02. Optimized SLN formulations were freeze dried and its effect on particle size was evaluated. Optimized solid lipid nanoparticles were evaluated for EE, Drug content, FTIR, DSC, SEM and in vitro drug release study.

Keywords: Solid lipid nanoparticles, Cefpodoxime Proxetil, Box Behnken design, Precirol, Lymphatic absorption and Solvent diffusion-evaporation method

INTRODUCTION

Cefpodoxime Proxetil (1-[(isopropoxycarbonyl) oxy] ethyl ester of (Z)-7-[2-(2-amino-1,3-thiazol-4-yl)-2- methoxyiminoacetamido]-3-methoxymethyl-3- cephem-4-carboxylic acid) is the orally active ester prodrug of third generation Cephalosporin (CP). CP is used orally for the treatment of mild to moderate respiratory tract infections, uncomplicated gonorrhea and urinary tract infections. Cefpodoxime Proxetil is a prodrug and gets hydrolyzed to its parent moiety Cefpodoxime Acid (CA) by non specific Cholinesterase enzyme in the intestinal wall/plasma[1]. Although CP is designed to improve the permeability, its parent moiety CA has 50% oral bioavailability. The reasons for this poor bioavailability of CP are low aq. solubility and preabsorption luminal conversion of CP into CA by action of digestive Cholinesterase[2]. Now bioavailability of CP can be improved simply by increasing solubility or by eliminating preabsorption conversion of CP to CA[2,3]. Hence formulation needs to be prepared for CP, which can bypass the passage of drug through epithelial cells and provide sufficient protection to the drug from the luminal cholinesterase. Hence in order to improve bioavailability formulation enabling lymphatic absorption such as nanoparticles, nanoemulsions can be prepared[2, 4]. Such a lymphatic targeting can be achieved through lipid based carrier systems such as lipid solutions, micellar solutions, microemulsions, nanoemulsions, liposomes, self-emulsifying drug delivery systems and recently Solid lipid nanoparticles (SLNs).[5]

SLNs were introduced in 1991, have attracted increasing attention as an alternative colloidal carrier system to traditional polymeric nanoparticles for controlled drug delivery because of their good tolerability and biodegradability, lack of acute and chronic toxicity of the carrier, physical stability, possibility of large scale production and feasibility to incorporate lipophilic and hydrophilic drug[6, 7]. The SLNs-based systems have characteristics of conventional carriers as well as some additional characteristics as elaborated above that obviate drawbacks associated and reported for conventional systems. Therefore they are considered to be, better alternative than liposomes, microemulsions, nanoemulsions, polymeric nanoparticles, self-emulsifying drug delivery systems and among others[8, 9].

Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery devices. Based on the principles of design and experiments (DOE) the methodology encompasses the use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to determine the optimum formulations[10-12].

In the present work, CP-loaded SLNs were successfully prepared by solvent evaporation technique. The formulation was optimized by using Box Behnken design with the help of design expert software. The optimized SLNs formulation were evaluated for various parameters like mean particle size, drug content, entrapment efficiency, zeta potentials and in vitro drug release study.

MATERIALS AND METHODS

Cefpodoxime proxetil and Precirol ATO 5 were obtained as a gift sample from Maxim Pharmaceuticals, Pune and Colorcon Asia Ltd. (Goa, India) respectively. All other chemicals and reagents were of analytical grade.

Compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

The CP and Precirol ATO 5 (1:1) were kept at room temperature for 30days. Then samples were subjected to the FTIR studies by using KBr as a blank.

Preparation of Solid Lipid Nanoparticles

Solvent Evaporation Method

Among the various techniques available CP-loaded SLNs were prepared by using solvent evaporation technique. Cefpodoxime Proxetil 100 mg was weighed accurately and dissolved in 10 ml of dichloromethane AR grade. Different proportions of Precirol ATO 5 and lipophilic surfactant Span 60 was dissolved to this solution (organic phase). In aqueous phase 1 gm of Tween 80 was added to 100 ml of purified water. Aqueous phase was stirred with speed of 2000 rpm for 15 minutes. Then organic phase was added to aqueous phase and obtained pre-emulsion was stirred with 13000 rpm for 3 hrs. Composition for different batches is shown in Table 1[5, 8].

Experimental design

From the preliminary trials in the present study a 3^3 Box Behnken Design was employed to study the effect of independent variables, i.e. Drug to Lipid ratio (X1), Span 60 concentrations (X2) and Stirring Speed (X3), on dependent variables like Particle size (Y_1), Entrapment efficiency (Y_2). Effects of independent variables were studied at three different levels. The factors and levels were suitably coded and as indicated in Table 2. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative[10-12].

Optimization Data Analysis

Optimization of preparation of CP-SLN was done by Design Expert Software (Version 7.1.6, Stat-Ease Inc., Minneapolis, MN). The CP-SLNs were prepared with different independent variable at different levels and responses, average particle size and % entrapment efficiency were obtained. The data was substituted to design expert software and polynomial equations were obtained[12, 13].

The polynomial regression results were expressed using 3-D graphs and contour plots. Finally, the optimum formulations were selected. Four optimum formulations were picked by the critical evaluation from desirability factors. The criterion for selection of optimum formulations was primarily based on the highest possible values of drug entrapment efficiency (%) and smallest value of particle size for the SLNs.

Validation of the Response Surface Methodology (RSM)

Four optimized formulations were selected as check points to validate RSM. The SLNs were formulated using the chosen optimal composition and evaluated for particle size and entrapment efficiency. Plots between predicted and observed responses were critically compared, and the percent error calculated with respect to the observed responses. Correlation plots were also constructed separately for four optimized formulations[13].

Evaluation of SLNs

From four optimum formulations one was selected having minimum residual error and were evaluated for particle size, zeta potential, drug content, entrapment efficiency and in-vitro drug release study.

The average particle size and size distribution of selected CP-SLN dispersions were recorded using Malvern Mastersizer 2000 MS (Malvern Instruments, Worcestershire, UK)[14,15]. Zeta potential measurement was carried out by using Zeta potential analyzer (Delsa 4405X; BECKMAN COULTER) at 25^0C[15,16]. Drug content from CP-SLN was analyzed by UV spectroscopy method using 0.1 N HCl as a solvent at 263 nm[16]. Drug entrapment efficiency (EE) in the SLNs was expressed as percent of the added drug actually entrapped into solid lipid nanoparticles. For this the 25% NaCl solution (5 ml) were added into the 100 ml of dispersion and centrifuged. Precipitate was dissolved in methanolic 0.1 N HCl, filtered and analyzed with UV spectroscopy. % entrapment efficiency is calculated by following formula:

EE (%): (Total drug content - unentrapped drug) ×100/ Total drug content[9,16]

In vitro release studies of optimized CP-SLN formulations were performed using modified Franz diffusion cell. Dialysis membrane (Himedia, Mumbai) having pore size 2.4 nm, molecular weight cut off between 12,000–14,000, was used. SLN formulations equivalent to 10 mg of CP was placed in the donor compartment and the receptor compartment was filled with dialysis medium (0.1 N HCl) (7 ml). At fixed time intervals, 0.1 ml of the sample was withdrawn from receiver compartment through side tube. Fresh dialysis medium was placed to maintain constant volume. Sample dilution were made and analyzed by UV spectrometry at 263 nm[9, 14, 16].

Freeze drying of CP-SLNs dispersion

Aliquots of four different batches of the optimized formulations were freeze-dried and converted into solid form to increase the stability of CP-SLNs and improve the palatability of dosage form. Lactose (5% w/v) was added as a cryoprotectant to 50 mL aliquots of samples, which were frozen in liquid nitrogen and lyophilized (Heto Drywinner, Thermo Scientific, USA) for 48 h at -70 ˚C, at a 0.05 mm Hg pressure. Freeze-dried samples stored at room temperature.

From the results of above parameters optimized formulation is selected and evaluated for FTIR, DSC and SEM study.

RESULTS AND DISCUSSION

Compatibility studies

After 30 days, samples of drug with excipients (1:1 ratio) stored at room temperature, were observed for physical changes. No physical changes were observed in the sample. Figure 1 shows infrared spectrum of CP (M1) and CP-Precirol (M2).

Experimental design

The experiments were designed to study the effect of three independent variables i.e. drug: lipid ratio, concentration of Span 60 and Stirring speed at three levels on particle size and % drug entrapment efficiency(EE). Among all the Response Surface Methods designs, Box-Behnken design requires fewer runs and reduces number of experiments in a 3-factor experimental design. A Box Behnken experimental design was used to reduce the number of experiments to 15 with 2 repetitions. The selection of formulation variables such as drug: lipid ratio, concentration of Span 60 and stirring speed and their levels in the design were decided from pre-optimization study on pre-emulsion.

Optimization Data Analysis

Analysis of experimental results was done by using the Stat-Ease Design Expert software. After filling the data in the design, cubic (Stepwise) model was suggested to run the design (Table 3). F-values, P-value and model F-value for average particle size and entrapment efficiency was obtained from ANOVA. The selection of model, polynomial equations and other statistical data generated for average particle size and %EE of CP-SLN dispersion are given in Table 4. Nine coefficients (B_1 to B_9) were calculated with β_0 as the intercept.

$$Y= \beta_o+\beta_1A+\beta_2B+\beta_3C+\beta_4AB+\beta_5AC+\beta_6BC+\beta_7A^2{}_1+\beta_8B^2{}_2+\beta_9C^2{}_3$$

Where β_0, the intercept, is arithmetic average of all quantities outcomes of 15 runs, β_1 to β_9 are the coefficient computed from the observed experimental values of Y, and A, B and C are the coded levels of the independent variable(s). The equation can be used to obtain estimates of the responses.

The coefficients of the polynomial equations were generated using multiple linear regression analysis (MLRA) for particle size and % entrapment efficiency. The coefficients (β_0 to β_9) were calculated with β_0 as the intercept.

All the data of Summary output of regression analysis for effect of A, B and C on Y_1 and Y_2 respectively are enlisted in Table 4

Concerning Y_1, the equation of multiple linear regression analysis is as per below:

$$Y_1=326+49A-8.50B-4.62C-34.25BC-104.87A^2-30.12B^2-42.63C2+65.25BC^2.. \quad (1)$$

For particle size response, the Y_1 value for all the batches showed good correlation co-efficient 0.9917. As the variables A, B and C have p value less than 0.05; all significantly affect the particle size. The equation suggests that factor A has positive effect on particle size. As lipid ratio increases particle size also increases. B and C had negative effect on the particle size that leads to decrement in the particle size as concentration of span 60 increases whereas increase in stirring speed leads to initial decrease in particle size up to certain limit and beyond that no significant effect on particle size is observed. The interaction terms (AB, AC, BC, A^2, B^2, and C^2) show how the average particle size changes when two variables are simultaneously changed. Their magnitude suggests the extent of effects. The 3-D plots shows that as the lipid ratio increases the particle size get increased significantly and as the concentration of span 60 and stirring speed increases the particle size get decreased. (Figure 2 and 3)

From the figure 2 (a, b) and 3 (a, b) of 3 D and contour plots we conclude that as the lipid concentration increases particles size increased significantly, Span 60 concentration shows no significant decrease in particle size with increase in proportion whereas with increase in stirring speed particles size decreased initially and then increased.

Concerning Y_2, the equation of multiple linear regression analysis is as per below:

$$Y_2=80.37+4.34A+0.20B-1.19C+0.27AB-2.17AC+2.85BC+4.34A^2+1.11B^2+1.36C2-1.37A^2C-1.11BC^2 \quad \text{.....(2)}$$

For %EE response, the Y_2 value for all the batches showed good correlation co-efficient 0.9997. As the variables A, B and C have p value less than 0.05; all significantly affect the % entrapment efficiency. Equations suggest that factor A and B have positive effect on EE. As Drug : lipid ratio and span 60 concentration increases, % EE also increases. But magnitude suggests the extent. Factor A showed more effect than factor B on EE. Factor C had negative effect on the EE to less extent that leads to decrement in the EE as stirring speed increases. The interaction terms (AC, BC, A^2, B^2, and C^2) showed how the % EE changes when 2 variables are simultaneously changed. The 3-D plots show that as the drug : lipid ratio and the concentration of span 60 increases the EE get increased significantly and as and stirring speed increases the EE get decreased. (Figure 4 and 5)

From the figure 4 (a, b) and 5 (a, b) of 3 D and contour plots we conclude that as the lipid concentration increases EE was increased significantly Span 60 concentration showed no significant effect on EE whereas increase in Stirring speed showed no significant decrease in EE.

Search for optimum formulations

Among the various formulations optimum formulations were selected on the basis of Desirability factor. Formulations having highest desirability factor were selected among the obtained solutions (Table 5). Criteria for the selection were primarily based upon the highest possible values of %EE (> 80.00%) and lowest possible values of particle size (< 150nm).

Validation of Response Surface Methodology

For all 4 checkpoint formulations, the results were found to be within limits. Table 6 lists the checkpoints, the predicted and experimental values of all the response variables, and the percentage error in prognosis. Linear correlation plots between the observed and predicted values of drug entrapment efficiency and particle size demonstrated higher values of R^2, indicating excellent fitting of model (figure 6 and 7). Upon comparison of the observed responses with that of the anticipated responses, the prediction error varied between -1.34 and 2.16% and the values of r^2 ranged from 0.9735 to 0.9851. Thus, the low magnitudes of error as well as the significant values of R^2 in the current study indicate a high prognostic ability of the experimental design to predict entrapment efficiency and particle size of prepared lipid nanoparticles dispersions of CP is validated

Evaluation of SLNs

From the comparison study it is observed that formulation F_1 showed least error among optimized formulations hence it was selected for further evaluation.

A particle size distribution curve of sample F_1 is shown in Figure 8. It shows average particle size of 118 nm. The polydispersity index was slightly greater than 0.157. PI is measure of width of dispersion. Narrow dispersion comprises PI values from 0.1 to 0.2 and broad dispersion comprises PI value from 0.2 to 0.3. Above figure of 0.157 for PI indicates no wide difference in size among the particles. Zeta potential is key factor to evaluate the stability of colloidal dispersions. In general particle could be dispersed stably when absolute value of zeta potential was above 30 mV due to the electric repulsion between particles. As shown in Figure 9 zeta potential of F_1 formulation was -22.9 mV which indicate instability of SLNs in aqueous system. Due to lower zeta potential, surfactant molecules rearrange onto the surface of particles to form loops and tails leading to the bridging between the nanoparticles, which leads aggregation of particles. Entrapment efficiency of F_1 was found to be 90.71 %. A high amount of drug could be incorporated in nanoparticle dispersion. Such high incorporation was possible because of lipid solubility of CP and also Span 60 as a lipid surfactant helps to solubilise the CP into lipid which further increases entrapment of drug. Drug content study of freeze dried Optimized formulation F_1 was done to detect the actual concentration of CP present in freeze dried powder. The drug content of F_1 formulation was found to be 88.12%. Franz diffusion cells with dialysis membrane (pore size 2.4 nm) was used to study drug release from SLNs. Dialysis membrane retained nanoparticles and allowed the transfer of the drug immediately into the receiver compartment. Figure 10 shows the percentage release of CP from four optimized formulations. Percentage of CP released from SLNs up to 24 h were in the following order: Formulation F_3 (75.07%) < Formulation F_4 (78.04%) < Formulation F_2 (79.08 %) < Formulation F_1 (82.55%). There exists an inverse relation between the percent drug released and particle size. Because large particle size causes increase in diffusion path for release of drug leads to lower drug release. *In-Vitro* drug release parameters of optimized formulations are shown in Table 7.

Freeze drying of CP-SLNs dispersion:

The particle size of the four optimized formulations after re-dispersion were from 115-120 nm which indicates no significant difference in particle size after freeze drying than that before freeze drying. The cryoprotectant effect of these sugars (e.g. Lactose, Mannose), observed here, may arise from the formation of a protective capping layer around the SLNs, sustained by hydrogen bonds between the polar function of the drug molecules, exposed at the surface of the SLNs, and the hydroxyl functions of the sugar. The formation of a hydrosoluble matrix, where the particles are embedded in, may facilitate the reconstitution of the dispersions. This solved the issue of instability of SLNs dispersion. Variations observed in the size of the SLNs after reconstitution of freeze dried product were shown in Table 8.

The FTIR spectral observations indicated that there is no strong interaction between the drug and the Precirol ATO 5. As IR spectra of CP-SLN showed no such a characteristic peak which indicates that drug is completely entrapped in the lipid matrix. Combined IR spectra of CP, CP-Precirol and CP-SLN are shown in Figure 11. The DSC thermogram of CP exhibited a single sharp endothermic peak at 89° corresponding to its melting transition temperature. The thermograms of the CP-SLN showed no such characteristic peak, indicating that the drug was uniformly dispersed at the lipid matrix. Figure 12 depict the DSC thermogram of pure CP (a) and CP-SLN (b). These studies also support the hypothesis of formation of an envelope surrounding the bitter drug particles thereby masking bitter taste. The SEM image of CP-SLN (Figure 13) revealed that the particle size was in nanometric range (110 nm) and the particles had spherical morphology.

CONCLUSION

From the results it can be concluded that Precirol ATO 5 can be used to formulate an efficient SLNs for CP with smallest particle size and maximum entrapment efficiency. Floating microspheres also showed Spherical shape in nanometric range (< 120 nm), as revealed by the scanning electron microscopic studies which is helpful for its lymphatic absorption.

The application of Box-Behnken design demonstrates a useful tool for optimization of CP-SLNs. The results of multiple regression analysis led to a statistical model that described adequately the influence of the selected variables at different levels on the chosen response

Using the 3 D plots and contour plots, data from statistical design one can select suitable composition of formulation to obtain SLNs with appropriate particle size and % entrapment efficiency on the application of the system. Thus, the current study is useful for the successful design, development and optimization of SLNs for Cefpodoxime proxetil.

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Table 1: Composition of pre-emulsion during initial studies

Ingredient	Concentration (%)
CP	1
Precirol ATO 5	5-20
Lipophilic surfactant (Span 60)	2-6
Hydophilic surfactant (Tween 80)	1-2
Dichloromethane	10 ml
Water	100 ml

Table 2: Coded factors with their levels

Factors	Levels		
	Minimum (-1)	Intermediate (0)	Maximum (+1)
Drug: Lipid **(X1)**	1:5	1 : 10	1:20
Span 60 **(X2)**	2%	4%	6%
Stirring speed(X3)	11000 rpm	13000 rpm	15000 rpm

Table 3: Values of particle size and entrapment efficiency for PC-SLN dispersion prepared as per Box Behnken experimental design

Batch No.	Drug: Lipid (A)	Span 60 (B)	Stirring Speed (C)	Particle Size (nm) (Y1)	EE (%) (Y2)
1	0.00	0.00	0.00	326	80.37
2	1.00	-1.00	0.00	260	81.51
3	0.00	1.00	-1.00	350	79.87
4	1.00	0.00	1.00	210	76.87
5	1.00	0.00	-1.00	235	86.34
6	-1.00	1.00	0.00	135	89.56
7	0.00	1.00	1.00	270	83.19
8	0.00	-1.00	-1.00	168	88.19
9	0.00	0.00	0.00	326	80.37
10	1.00	1.00	0.00	230	81.66
11	0.00	0.00	0.00	326	80.37
12	-1.00	0.00	-1.00	129	90.9
13	-1.00	-1.00	0.00	139	90.5
14	-1.00	0.00	1.00	140	90.1
15	0.00	-1.00	1.00	225	80.1

Table 4: Summary of results of regression analysis for responses Y1 and Y2

Regression statistics for	Y_1	Y_2
Model	Cubic (Stepwise)	Cubic (Stepwise)
F value	89.30	850.85
Predicted R square	0.9532	0.9674
R Square	0.9917	0.9997
Adjusted R square	0.9806	0.9674
Adequate precision	26.714	84.015
Observations	15	15
Coefficients		
Coefficients	For Y_1	For Y_2
β_0	326	80.37
β_1	49	4.34
β_2	-8.5	-0.20
β_3	-4.62	-1.19
Equation		
$Y_1=326+49A-8.50B-4.62C-34.25BC-104.87A^2-30.12B^2-42.63C2+65.25BC^2$..... (1)		
$Y_2=80.37+4.34A+0.20B-1.19C+0.27AB-2.17AC+2.85BC+4.34A^2+1.11B^2+1.36C2-1.37A^2C-1.11BC^2$ ..... (2)		

Table 5: Solutions for optimum formulations

Formulation Code	Drug: Lipid	Span 60	Stirring Speed	particle Size	EE	Desirability
F_1	1: 5.19	2.00	13,056	114.21	90.70	0.827
F_2	1: 5.20	2.00	13,052	114.45	90.78	0.826
F_3	1: 5.19	2.00	13,058	115.45	90.82	0.825
F_4	1: 5.18	2.00	13,056	115.8	91.00	0.824

Table 6: Comparison of experimental results with predicted responses

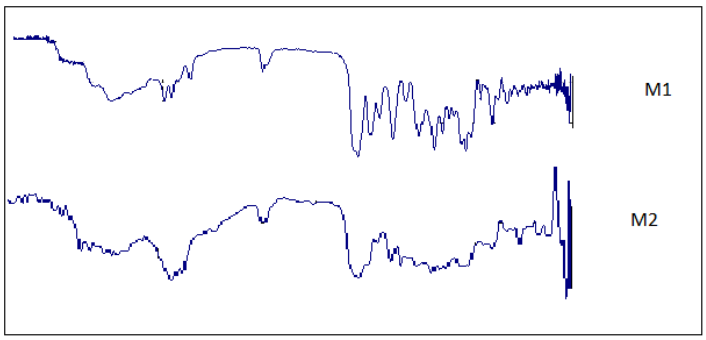
Formulation Code	Optimized Formulation (A:B:C)	Response variable	Experimental value	Predicted value	Percentage prediction error
F_1	1: 5.19: 13,056	**Y1** **Y2**	115 90.68	114.21 90.7	-0.69 +0.02
F_2	1: 5.20: 13,052	**Y1** **Y2**	116 90.71	114.45 90.78	-1.34 +0.08
F_3	1: 5.19: 13,058	**Y1** **Y2**	118 90.76	115.45 90.82	+2.16 +0.07
F_4	1: 5.18: 13,056	**Y1** **Y2**	119 91.02	115.8 91	-2.76 +0.02

Table 7: *In-Vitro* drug release parameters of optimized formulations

Time (h)	% Drug Release			
	Formulation O_1	Formulation O_2	Formulation O_3	Formulation O_4
0	0	0	0	0
1	8.77	5.05	7.87	4.51
2	16.77	11.24	11.61	6.57
3	19.61	17.56	17.03	13.54
4	29.02	26.40	29.02	15.12
5	33.41	32.84	33.67	19.22
6	33.66	34.55	37.44	24.12
7	40.37	38.53	42.31	26.53
8	45.78	42.65	45.67	37.82
22	67.47	69.73	69.92	71.72
23	72.37	77.31	74.95	76.22
24	82.55	79.09	75.07	78.04

Table 8: Particle size before and after freeze drying by aqueous re-dispersion

Optimized formulation	Before Freeze drying	After Freeze drying
	Particle size(nm)	Particle size(nm)
F_1	115	122
F_2	119	131
F_3	116	128
F_4	118	125



CP: (M1) and CP-Precirol: (M2)

Figure 1: IR Spectra ionof CP and CP-Precirol combination

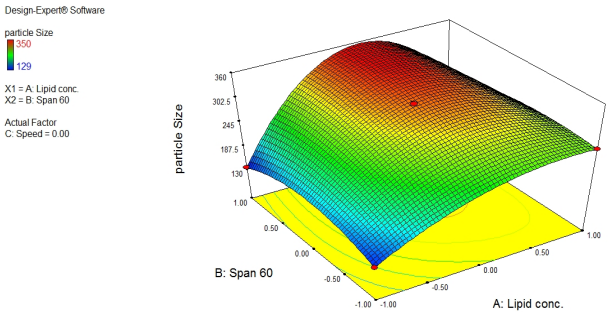


Figure 2 (a) : 3-D Response surface plot showing the influence of Span 60 and lipid concentration on the value of average particle size CP -SLN dispersion

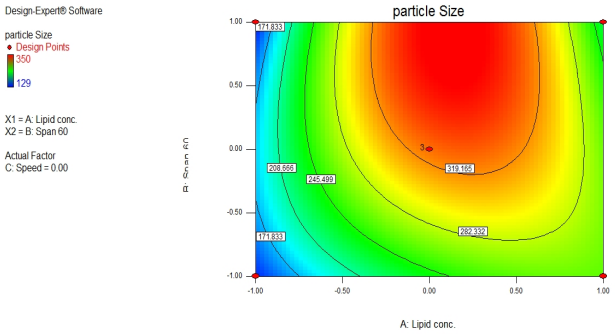


Figure 2 (b): Contour plot showing the influence of Span 60 and lipid concentration on the value of average particle size CP -SLN dispersion

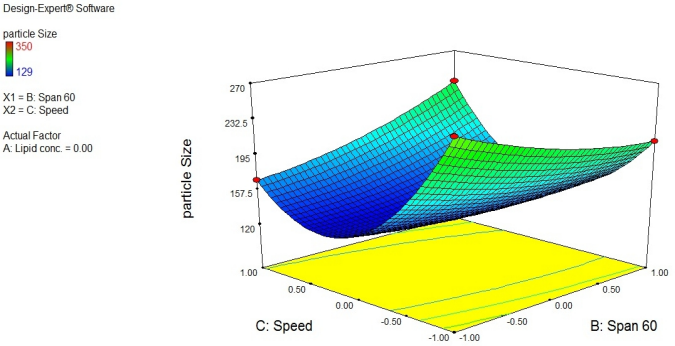


Figure 3 (a) : 3-D Response surface plot showing the influence of span 60 and stirring speed on the value of average particle size CP -SLN dispersion.

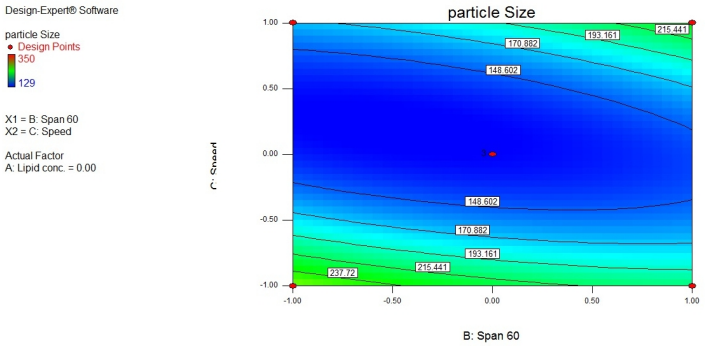


Figure 3 (b): Contour plot showing the influence of span 60 and stirring speed on the value of average particle size CP -SLN dispersion

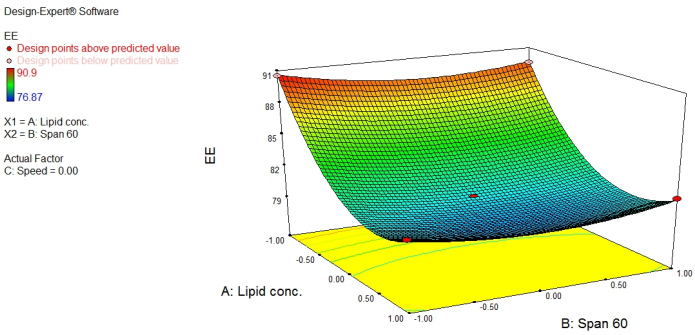


Figure 4 (a): 3-D Response surface plot showing the influence of span 60 and lipid concentration on the value of % EE of CP-SLN dispersion

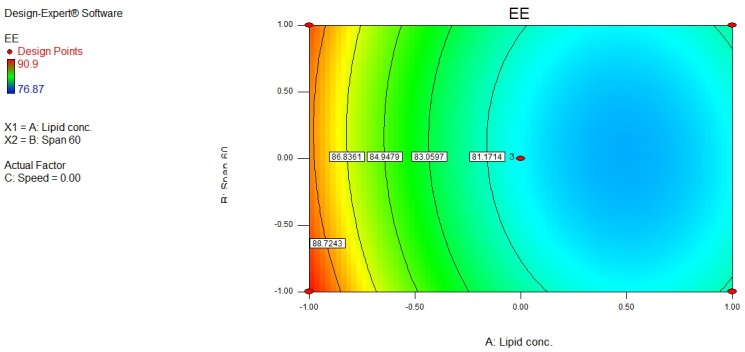


Figure 4 (b): Counter plot showing the influence of span 60 and lipid concentration on the value of % EE of CP-SLN dispersion

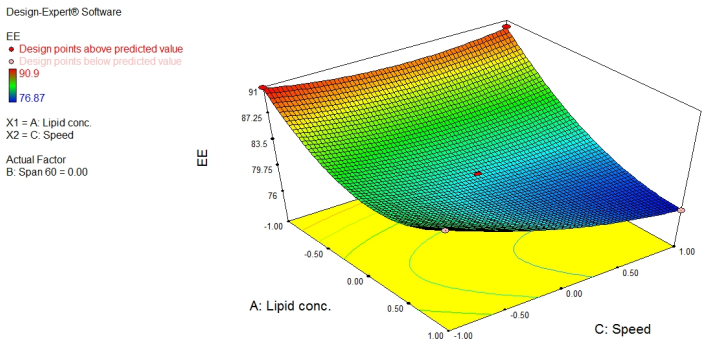


Figure 5 (a) : 3-D Response surface plot showing the influence of stirring speed and lipid concentration on the value of % EE of CP -SLN dispersion

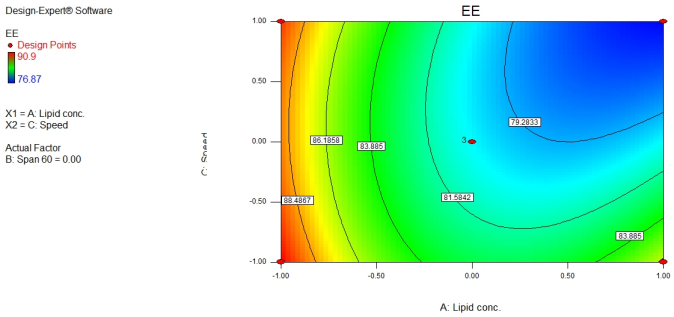


Figure 5 (b): Counter plot showing the influence of stirring speed and lipid concentration on the value of % EE of CP -SLN dispersion

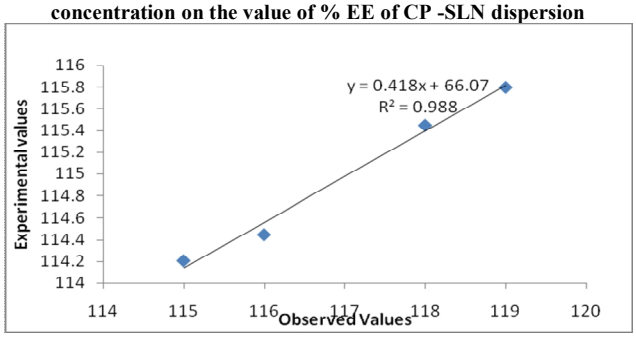


Figure 6: Linear plots between observed and predicted values of particle size.

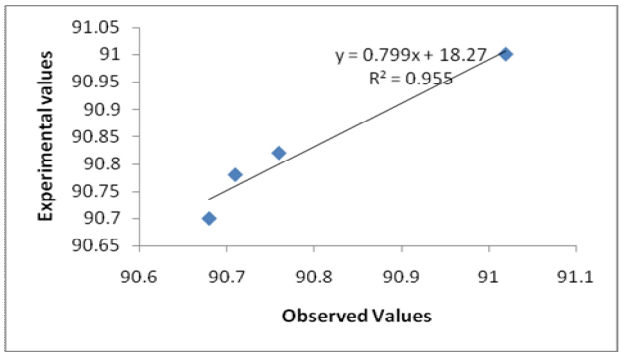
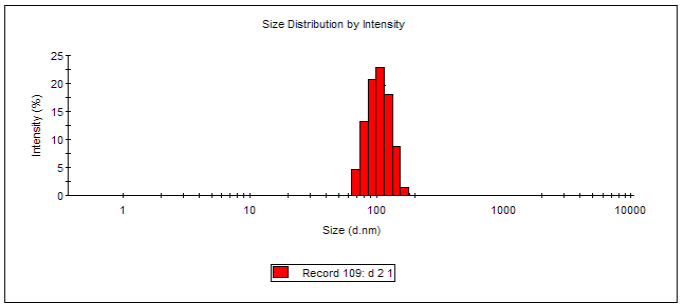


Figure 7: Linear plots between observed and predicted values of % EE



		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 113				
PdI: 0.157	Peak 1:	118	96.33	58.7
	Peak 2:	0.000	0.000	0.000
Intercept: 0.865				
Result Quality: Good				

Figure 8: Particle size distribution curve of Sample F_1.

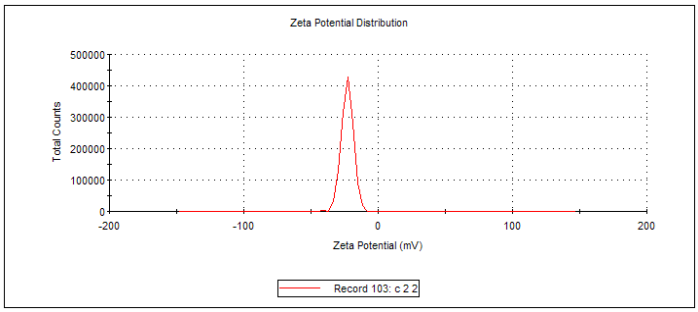


Figure 9: Zeta potential of Sample F_1

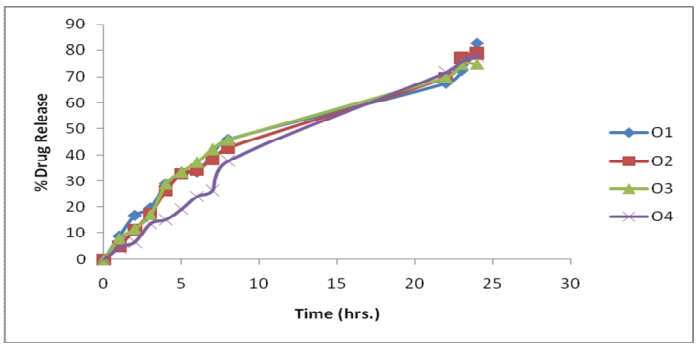


Figure 10: Drug release profile of optimized formulations

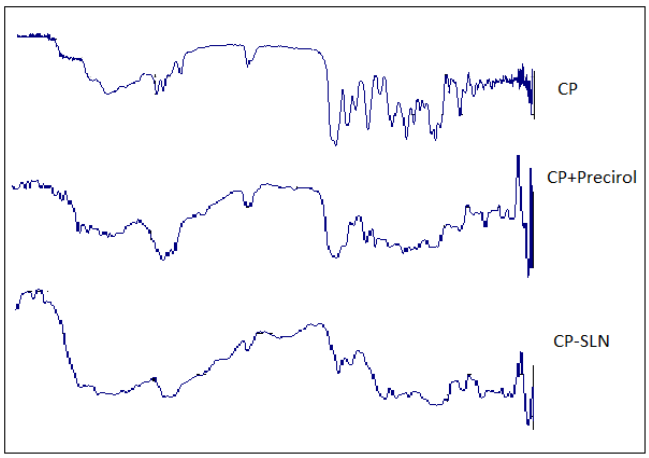
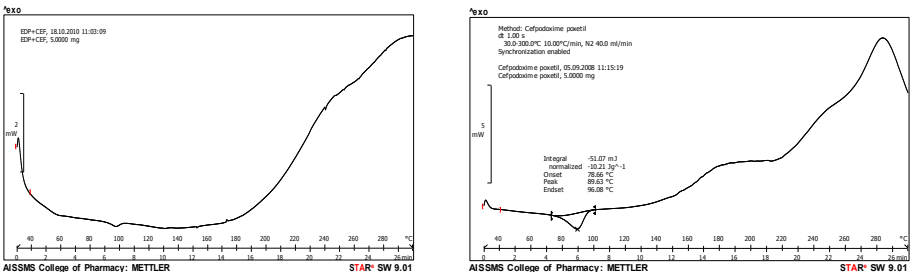


Figure 11: IR spectra of CP, CP-Precirol and CP-SLN



(a) (b)

Figure 12: DSC spectrum of a) CP and b) CP-SLN

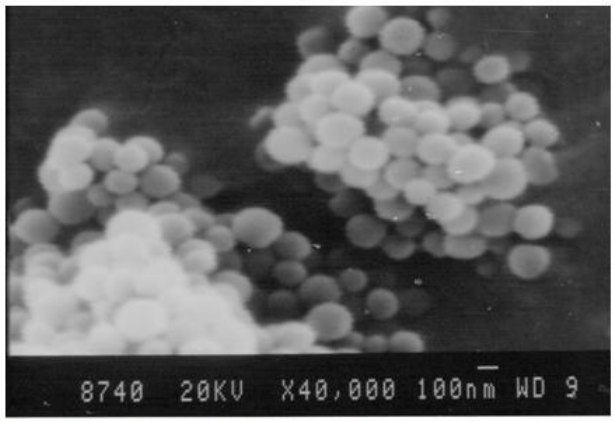


Figure 13: SEM image of solid CP-SLNs

Source of support: Nil, Conflict of interest: None Declared