



DESIGN AND OPTIMIZATION OF CEFTRIAXONE SOLID LIPID NANOPARTICLES BY USING BOX BEHNKEN DESIGN

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ABSTRACT

The purpose of the present study was to optimize a solid lipid nanoparticle (SLN) of Ceftriaxone by investigating the relationship between design factors and experimental data using response surface methodology. A Box-Behnken design was constructed using solid lipid (X(1)), surfactant (X(2)), and drug/lipid ratio (X(3)) level as independent factors. SLN was successfully prepared by a modified method of melt-emulsion ultrasonication and low temperature-solidification technique using glyceryl monostearate as the solid lipid, and poloxamer 188 as the surfactant. The dependent variables were entrapment efficiency (EE), drug loading (DL), and turbidity. Properties of SLN such as the morphology, particle size, zeta potential, EE, DL, and drug release behavior were investigated, respectively. As a result, the nanoparticle designed showed nearly spherical particles with a mean particle size of 248 nm. The polydispersity index of particle size was 0.277 ± 0.058 and zeta potential was -8.74 mV. The EE (%) and DL (%) could reach up to $83.29\% \pm 1.23\%$ and $10.11\% \pm 2.02\%$, respectively. In vitro release studies showed a burst release at the initial stage followed by a prolonged release of chloramphenicol from SLN up to 48 hours. The release kinetics of the optimized formulation best fitted the Peppas-Korsmeyer model. These results indicated that the Ceftriaxone-loaded SLN could potentially be exploited as a delivery system with improved drug entrapment efficiency and controlled drug release.

KEYWORDS: Ceftriaxone, solid lipid nanoparticle, Box-Behnken design.

INTRODUCTION

Solid Lipid Nanoparticles (SLN) were developed over the past decade as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. SLNs offer several advantages over other colloidal carrier systems. They are composed of physiological lipids, which reduce the risk of acute and chronic toxicity. SLNs can be manufactured economically and in a large scale production using high pressure homogenization without the use of organic solvents. SLNs are composed of lipophilic bioactives that is incorporated in a carrier lipid that remains solid at room and body temperature and are considered crystallized nanoemulsions.^[1-5]

Drug Profile: Ceftriaxone is a broad-spectrum third-generation cephalosporin antibiotic. It has a very long half-life compared to other cephalosporins and is high penetrable into the meninges, eyes, and inner ear. Ceftriaxone has broader and stronger gram-negative coverage than first or second-generation cephalosporins, but worse activity against methicillin-susceptible *S.aureus*. IUPAC name 2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(2-methyl-5,6-dioxo-1H-

1,2,4-triazin-3-yl) sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Figure 1 shows structure of Ceftriaxone. Molecular Weight of Ceftriaxone is 554.58.^[6] Ceftriaxone is a white to yellowish crystalline powder which is readily soluble in water, sparingly soluble in methanol and very slightly soluble in ethanol. The pH of a 1% aqueous solution is approximately 6.7.

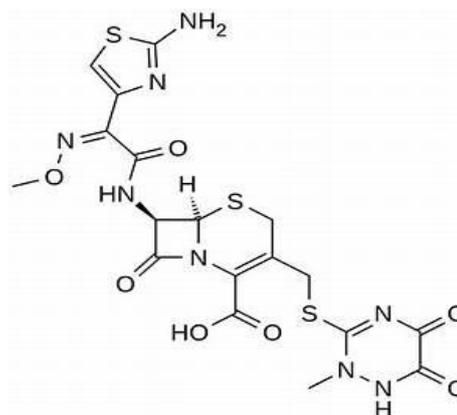


Figure 1: Chemical structure.

MATERIALS

The powered Ceftriaxone was supplied by Micro lab, Poloxamer 188 was purchased from Lobachem, Mumbai, Glyceryl Monostearate were purchased from Colorcon Asia Pvt. Ltd., Goa, Methanol was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical reagent grade.

METHODOLOGY

Preparation of SLN

SLN was prepared according to previous articles with some modification by the following melt-emulsion ultrasonication and low temperature-solidification methods. In brief, aqueous and oil phases were separately prepared in glass vials. Drug and a specified amount of GMS were dissolved in a specified volume of ethanol (2 mL) and heated above the melting temperature of GMS (70 °C). Hydrophilic surfactants and double distilled water were mixed at 70°C and added to the melted oil phase. The resulting suspension was continually stirred by mechanical agitation (DC-40, Hangzhou Electrical Engineering Instruments, China) at 400 rpm for 15 minutes at 70 °C. The original warm emulsion was further treated for 5 minutes (work 2 seconds and stand 3 seconds) by a Lab ultrasonic cell pulverizer (JY92-II, Ningbo Scientz Biotechnology Co., Ltd. China) at 600 W to form a nanoemulsion. This was rapidly cooled by immersing the beaker into icy water. Agitation continued until the nanoemulsion yielded a uniform dispersion of nanoparticles.

Experimental design

In this study, a 17-run, 3-factor, 3-level Box-Behnken design was employed to construct polynomial models for

the optimization process, because it requires few runs with 3 or 4 variables. This design was suitable for investigating the quadratic response surface and for constructing a second-order polynomial model using Design-Expert software (Trial Version 7.1.6, Stat-Ease Inc., MN). The design consisted of replicated center points and a set of points lying at the midpoints of each edge of the multidimensional cube, which defined the region of interest used to evaluate the main effects, interaction effects, and quadratic effects of the formulation ingredients, and to optimize the formulation. The non-linear quadratic model generated by the design was:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_2X_3 + A_6X_1X_3 + A_7X_1^2 + A_8X_2^2 + A_9X_3^2$$

in which Y is the measured response of the dependent variables associated with each factor-level combination; $A_0 - A_9$ are the regression coefficients of the respective variables and their interaction terms computed from the observed experimental values of Y; and X_1, X_2, X_3 are the coded levels of independent variables. The term X_1X_2 and X_i^2 ($i = 1, 2$ or 3) represent the interaction and quadratic terms respectively. Factors evaluated in this study were the amount of GMS (X_1), concentration of poloxamer (X_2) and the ratio of drug/lipid (X_3) as the independent variables which were represented by -1, 0 and +1, analogous to the low, middle, and high values respectively as described in Table 1. The studied dependent responses were entrapment efficiency (EE%) (Y_1), drug loading (DL%) (Y_2), turbidity (NTU) (Y_3) with constraints applied as described in Table 1. The experiment design matrix generated by the software was shown in Table 2.

Table 1: Variables and their levels in the Box-Behnken design.

	Levels		
	-1	0	1
Independent variables			
X1 □ Amount of lipid (w/v)	5%	7.5%	10%
X2 □ Amount of surfactant (w/v)	2%	5%	8%
X3 □ Drug/lipid ratio (w/w)	5%	10%	15%
Dependent variables			Constraints
Y1 □ Entrapment efficiency (EE%)			Maximize Maximize Minimize
Y2 □ Drug loading (DL%)			
Y3 □ Turbidity (NTU)			

Table 2: Box-Behnken experimental design.

Formulation	Lipid (w/v, X1)	Surfactant (w/v, X2)	Drug/lipid ratio (w/w, X3)
1	5%	2%	10%
2	10%	8%	10%
3	5%	8%	10%
4	5%	5%	15%
5	7.5%	5%	10%
6	10%	2%	10%
7	7.5%	5%	10%
8	7.5%	5%	10%
9	7.5%	2%	5%

10	7.5%	5%	10%
11	10%	5%	15%
12	7.5%	5%	10%
13	5%	5%	5%
14	7.5%	2%	15%
15	10%	5%	5%
16	7.5%	8%	5%
17	7.5%	8%	15%

Entrapment efficiency and drug loading determination

The content of Ceftriaxone was determined by the high performance liquid chromatography method. The chromatographic system consisted of a Shimadzu LC-10AT solvent delivery pump (Kyoto, Japan) equipped with a 20 μ L loop and a UV visible detector. A Kromosil (250 x 4.6 mm) analytical column was used. The eluate was monitored at 275 nm. The mobile phase was methanol and water (45:55, v/v) with a flow speed of 1.0 mL min⁻¹ at room temperature.

The calibration curve of peak area against concentration of Ceftriaxone is shown in equation (1):

$$A = 42832C + 3234.7$$

It fit over the range 3.2–32 mg. L⁻¹ with a correlation coefficient of $r = 0.9999$ (where A = peak area and C = Ceftriaxone concentration).

To determine entrapment efficiency and drug loading, at first, the appropriated SLN was precipitated by adding saturated NH₄SO₄ solution, and then collecting the serum and solid residue, respectively after centrifugation (CS120GXL, Hitachi, Japan) at 50,000 rpm for 15 minutes. The solid residue was dispersed in 40 mL phosphate buffered saline (PBS) solution (pH 7.4), in order to dissolve the free drug absorbed on the surface of nanoparticles, and then centrifuge-separated. Both the drug content in serum and that in PBS determined by HPLC were labeled as W_{free} . The efficiency of drug encapsulation (EE) and drug loading of nanoparticles were calculated according to equations (2) and (3),

$$EE = \frac{W_{total} - W_{free}}{W_{total}} \times 100\% \quad (2)$$

$$DL = \frac{W_{total} - W_{free}}{W_{total} - W_{free} + W_{lipid}} \times 100\% \quad (3)$$

where W_{total} were the weight of drug added in the system, and the weight of lipid added in the system, respectively.

Turbidity measurements

For the same samples used in the particle size analysis, measurements of turbidity represent the effect of suspended solids blocking the transmission of light through a body of water, which can be used as an indirect way to determine particle size in the dispersion

system. Dispersion turbidity in nephelometric turbidity units (NTU) was measured using a WZS-185 high turbidity Spectrometer (Shanghai Precision & Scientific Instrument Co., Ltd, China). All the containers used were cleaned thoroughly. All studies were repeated as duplicates, with good agreement being found among measurements.

Particle size and zeta potential analysis

The particle size and zeta potential of optimum SLN dispersions were measured by Zetasizer (3000SH, Malvern Instruments Ltd., UK). All samples were diluted with double distilled water to reach a suitable concentration before measurement.

Scanning electron microscopy (seM) analysis

Scanning electron micrographs were performed using a KYKY-1000B scanning electron microscope (KYKY technology development Ltd., China). A drop of the SLN dispersion was mounted on aluminium stubs covered with a glass lamella, air-dried, gold coated under vacuum, then examined.

In vitro release study

The formulation was subjected to in vitro release studies using 150 mL of artificial tear fluid (ATF) pH 7.4 as dissolution medium (maintained at 37 ± 0.5 °C), which is equivalent to the pH of the eye. In vitro release studies were performed using the dialysis bag method, modified to maintain a sink condition and achieve satisfactory reproducibility. Two mL of Ceftriaxone-loaded SLN dispersion was first poured into the dialysis bag (molecular weight cut off 12000–14000) with the two ends fixed by thread and placed into the pre-heated dissolution media. The suspension was stirred at 37 ± 0.5 °C, using a RCZ-8A Drug Dissolution Tester (Tianjin Medical Instrument Factory, China) with paddle rotating at 50 rpm. Five mL of the sample was withdrawn at fixed time intervals and the same volume of fresh medium was added accordingly. Samples were analyzed by HPLC as described above. The composition of ATF used was: sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g, and purified water q.s. 100 mL.20

RESULTS AND DISCUSSION

Statistical analysis of experimental data by Design-expert software

The results of the experimental design were analyzed using Design-Expert software, which provided considerable useful information and reaffirmed the utility

of statistical design for conduct of experiments. The selected independent variables including the amount of GMS, concentration of poloxamer 188, and drug/lipid ratio, significantly influenced the observed responses for EE (%), DL (%) and turbidity, which are presented in Table 3. Polynomial equations involving the main effect and interaction factors were determined based on estimation of statistical parameters such as multiple correlation coefficient, adjusted multiple correlation

coefficient, and the predicted residual sum of squares generated by Design-Expert software. The statistical validation of the polynomial equations was established by ANOVA provision available in the software. Therefore, the optimum values of the variables were determined according to the obtained experimental data using the Design-Expert software, based on the constrained criterion of desirability presented.^[9]

Table 3: Observed and predicted value of encapsulation efficiency (Y_1), drug loading (Y_2) and turbidity (Y_3) of formulations in the Box-Behnken design.

Formulation	Observed Y_1	Predicted Y_1	Observed Y_2	Predicted Y_2	Observed Y_3	Predicted Y_3
1	41.26	34.64	3.95	4.04	32	36.5
2	74.68	81.30	6.96	6.88	26	21.5
3	75	70.35	6.95	6.79	42	50
4	30.13	39.60	4.32	5.39	24	15.5
5	63.68	65.68	5.98	6.16	35	35.6
6	53.91	58.56	5.11	5.27	124	116
7	67.32	65.68	6.32	6.16	33	35.6
8	67.53	65.68	6.32	6.16	38	35.6
9	36.38	41.20	1.79	2.68	69	68.5
10	63.11	65.68	5.92	6.16	26	35.6
11	76.4	74.60	10.29	11.28	39	43
12	66.74	65.68	6.24	6.16	46	35.6
13	55.85	57.65	7.74	6.76	23	19
14	56.75	53.90	7.84	6.69	62	66
15	67.01	57.54	3.26	2.20	34	42.5
16	80.77	83.62	3.88	5.02	31	27
17	74.74	69.92	9.61	8.72	26	26.5

Response surface analyses plotted in three-dimensional model graphs for depicting the effects of the predetermined factors on the response of the entrapment efficiency, drug loading, and turbidity are shown in Figures 1 to 6, based on the model polynomial functions, to assess change in the response surface. The response surface plots were used to study the interaction effects of 2 independent variables on the responses or dependent variables, when a third factor is kept at constant level. When these plots were carefully observed, the qualitative effect of each variable on each response parameter could be visualized.^[10]

Effects on Entrapment efficiency (Y_1)

The entrapment efficiency varies from 30.13% (formulation 4) to 80.77% (formulation 16) for various factor level combinations (Table 3). The independent factors affecting the entrapment efficiency were the amount of GMS (X_1) and surfactant levels (X_2) ($P < 0.05$, Table 4 and Figure 1). The effect can be explained by the following quadratic equation:

$$Y_1 = 65.68 + 8.72X_1 + 14.61X_2 + 0.25X_3 + 3.24X_1X_2 + 8.78X_1X_3 + 6.6X_2X_3 + 4.64X_1^2 + 0.17X_2^2 + 3.69X_3^2 \quad (4)$$

The positive value before a factor in the regression equation indicates that the response increases with the factor and vice versa. The value of the correlation coefficient (r^2) of equation 4 was found to be 0.9396, indicating a

good fit. In Figure 1, the effect of varying the amount of lipid and emulsifier on the entrapment efficiency (Y_1) was studied when the drug/lipid ratio was kept constant. The lipid level (X_1) had a significant and positive effect on Y_1 as revealed by the positive value in the quadratic equation. The result showed that the entrapment efficiency rapidly increased as the amount of lipid increased. Increasing the amount of GMS, was bound to increase the % of EE because of the increased concentration of mono-, di-, and triglycerides, which act as solubilizing agents for highly lipophilic drugs and provide more and more spare space to accommodate excessive drugs. However increasing the amount of drug/lipid ratio while keeping the emulsifier level constant, as shown in Figure 2, would decrease the entrapment efficiency, probably because redundant drug becomes insoluble in GMS.

The entrapment efficiency could be increased with drug concentration (X_3) in the internal phase as more of the drug would be available for entrapment. The surfactant level (X_2) also has a significant and positive effect on the entrapment efficiency (Y_1). In Figure 3, the entrapment efficacy was significantly increased by increasing the amount of emulsifier and the amount of lipid. This effect was, probably due to the increased viscosity of the medium, because increasing the amount of lipid resulted in faster solidification of the nanoparticles. This would

also prevent drug diffusion to the external phase of the medium. As the percentage of emulsi- fier increased, part of the Ceftriaxone was incorporated in the surfactant

layer at the surface of the SLN, leading to a high entrapment efficacy.

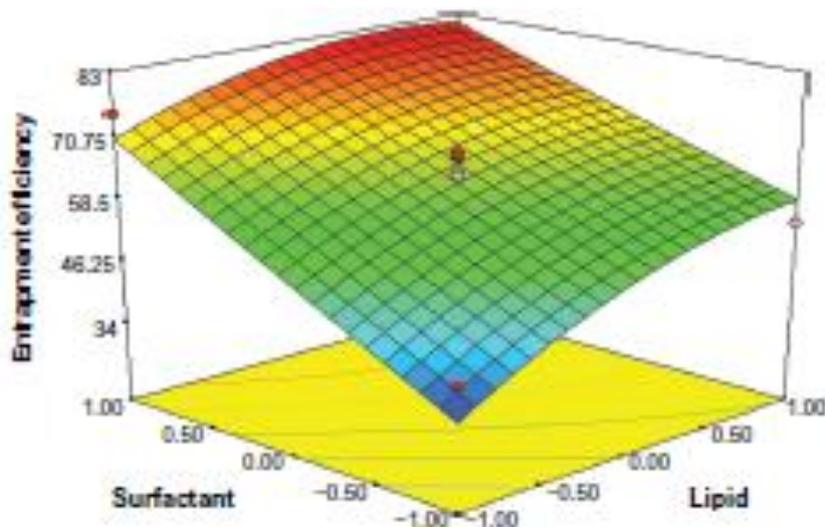


Figure 1 response surface plot showing effect of the amount of lipid (X_1) and surfactant levels (X_2) on entrapment efficiency (Y_1).

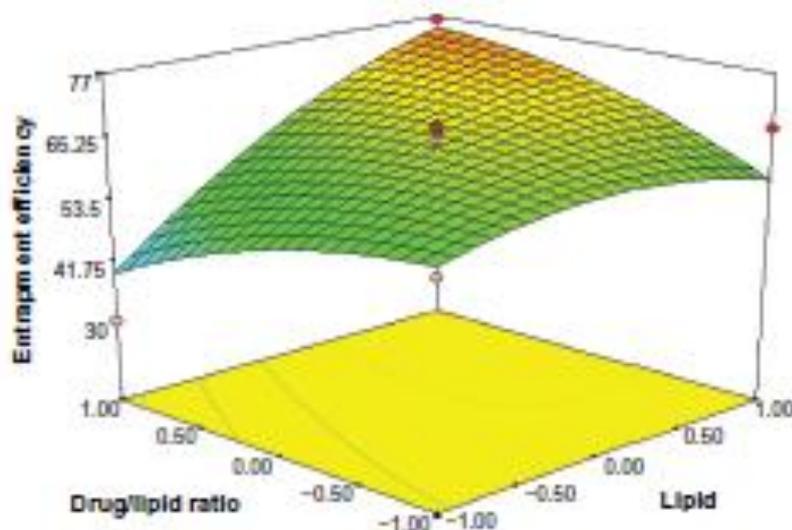


Figure 2 response surface plot showing effect of the amount of lipid (X_1) and drug/ lipid ratio (X_3) on entrapment efficiency (Y_1).

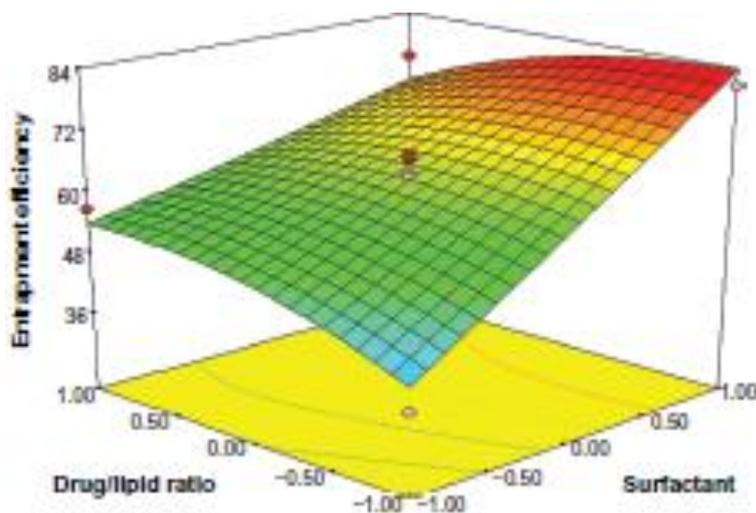


Figure 3 response surface plot showing effect of the amount of surfactant (X₂) and drug/lipid ratio (X₃) on entrapment efficiency (Y₁).

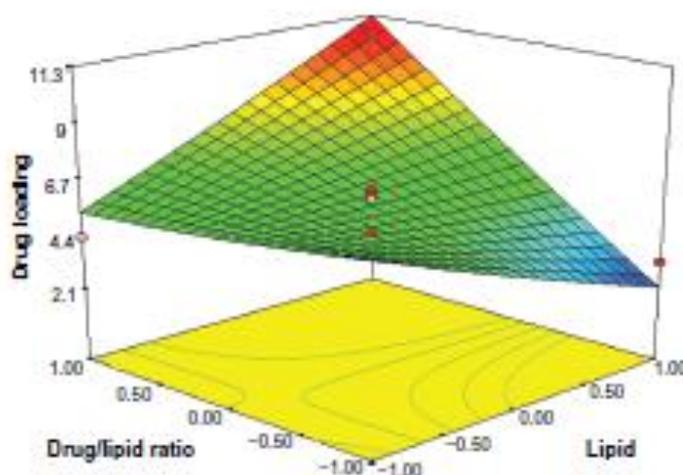


Figure 4 response surface plot showing effect of the amount of lipid (X₁) and drug/lipid ratio (X₂) on drug loading (Y).

Effects on drug loading (Y₂)

According to the results obtained from the experiments for drug loading, the value of drug loading varied from 1.79% (formulation 9) to 10.29% (formulation 11) (Table 3). The %DL was clearly influenced by surfactant concentration and the drug/lipid ratio as seen in Table 4. The following equation can explain the effect of factor levels on %DL:

$$Y_2 = 6.16 + 0.33X_1 + 1.09X_2 - 1.93X_3 - 0.28X_1X_2 + 2.61X_1X_3 - 0.079X_2X_3 + 0.11X_1^2 - 0.52X_2^2 + 0.14X_3^2$$

The main effects of X₁, X₂, and X₃ represent the average result of changing 1 variable at a time from its low level to its high level. The negative coefficients before independent variables indicate an unfavorable effect on the %DL, while the positive coefficients indicate a favorable effect on the %DL. The interaction term shows how the %DL changes when 2 variables are simultaneously changed. Analyzing these coefficients in the above second-order polynomial model shows how the increase of drug/lipid ratio and emulsifier level enhances the drug

loading of SLN, making it more efficient than for GMS because of the positive and large coefficient of these particles. The cross-interaction of GMS and drug/lipid ratio on %DL of SLN was significant because the coefficient of X₁X₃ was larger than others. Furthermore, the F-value for the full quadratic equation for the %DL of SLN was, indicating that the second-order response surface model was significant at the 5% level. Among the 3 independent variables, the lowest coefficient value is for X₁ (A₁=0.22 and P=0.05) indicating that this variable is insignificant in prediction of drug loading.

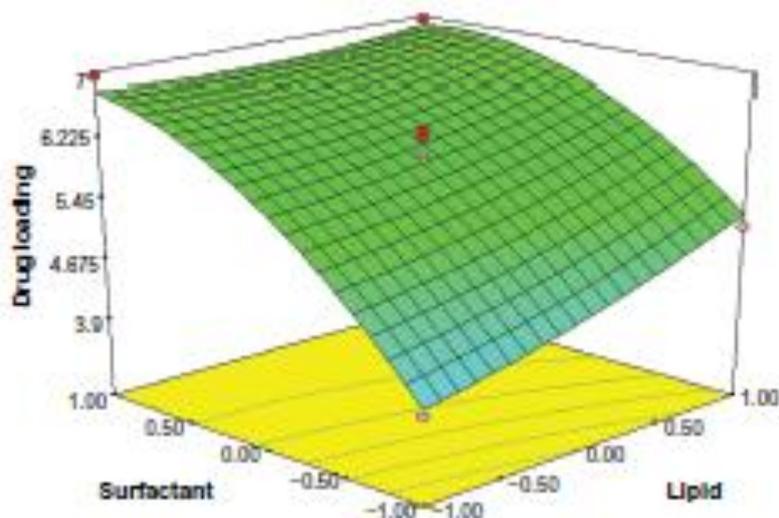


Figure 5 response surface plot showing effect of the amount of lipid (X_1) and surfactant levels (X_2) on drug loading (Y_2).

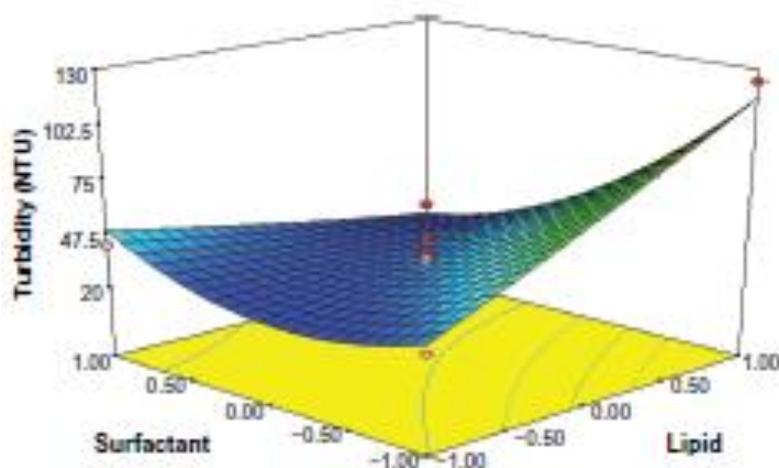


Figure 6 response surface plot showing effect of the amount of lipid (X_1) and surfactant levels (X_2) on turbidity (Y_3).

By analyzing the response surfaces for drug loading, it was obvious that the level of drug/lipid ratio and emulsifier exert positive influence on drug loading. As Figure 4 shows, an increase of lipid and emulsifier concentration leads to a non-linear increase in drug loading when the drug/lipid ratio is fixed. When the amount of surfactant is increased, the percent of DL is thought to increase because of enhanced entrapped drug; the reason why DL% is increased on the enhanced lipid is that the increased content of lipids can provide more and more spare space to accommodate excessive drugs. At the same time, according to equation (3) and Figure 5, for constant amount of surfactant concentration, when drug concentration increases, the amount of excipients increases, which results in reduced drug loading. effects on turbidity (Y_3)

Turbidity could have caused some obstruction when light was transmitted to a suspension. Turbidity value depends significantly on the particle size and thus can be used indirectly to reflect particle size. The turbidity of SLN

ranged from^[12] NTU (formulation 14) to NTU (formulation 8) (Table 3) with the selected levels of variables.^[13] Small-sized particles are highly desirable in order to increase the permeability through cornea surface. Results in Table 4 demonstrated that turbidity was significant but negatively dependent upon the surfactant level. The full second-order polynomial model for turbidity obtained by the response surface regression procedure using Design-Expert software is given by:

$$Y_3 = 35.6 + 12.75X_1 - 20.25X_2 - 0.75X_3 - 27X_1 X_2 + X_1 X_3 + 0.5X_2 X_3 + 1.7X_1^2 + 18.7X_2^2 - 7.3X_3^2$$
 ($r = 0.969$)

By analyzing these coefficients in the above second-order polynomial mode, turbidity is distinctly influenced by surfactant concentration and lipid ratio as seen in Table 4; the cross-interaction of poloxamer 188 and GMS on the size of SLN was significant because the coefficient of X_1X_2 was far greater than others. Furthermore, the F-value for the full quadratic equation for the SLN particle size was 12.07, indicating that the

second-order response surface model was significant at the 5% level.

As Figure 6 shows, the increase in emulsifier could efficiently reduce the particle size of SLN. On increasing the concentration of poloxamer 188, the particle size was decreased. This might be due to the surfactant-induced reduction in surface tension between the aqueous phase and organic phase. In addition, surfactant helps to stabilize the newly generated surfaces and prevents particle aggregation.^[14] The increase in GMS concentration would lead to the increase in size. The fact that the size of lipid nanoparticles is highly dependent on lipid concentration can be explained in terms of the tendency of the lipid to coalesce at high lipid concentration. According to Stoke's law, this behavior can be explained by a difference in density between the internal and external phases.^[15]

Optimization and validation

The desirability function was probed using Design-Expert software to acquire the optimized formulation.

The optimum formulation was based on the set criteria of maximum entrapment efficiency, maximum drug loading, and minimum particle size. Therefore a new batch of SLN with the predicted levels of formulation factors was prepared to confirm the validity of the optimization procedure. The composition of optimized formulation was achieved with 10% (w/v) GMS, 8% (w/v) surfactant and 13.5 mg/mL Ceftriaxone, which fulfill the requirements of optimization. The optimized formulation has entrapment efficiency $83.29\% \pm 1.23\%$, drug loading $10.11\% \pm 2.02\%$, and turbidity 21 ± 3 NTU, which were in good agreement with the predicted values.

Physicochemical characterization of nanoparticles

SEM studies revealed that Ceftriaxone -loaded SLN were almost spherical in shape with smooth morphology which appeared as white bright dots (Figure 7). However, in order to obtain more precise information on the size distribution, particle size and zeta potential, measurements were performed in the following study.

Table 4: Statistical analysis results of entrapment efficiency, drug loading, and turbidity entrapment efficiency(Y_1) drug loading (Y_2) turbidity(Y_3)

	Coefficient	P-value		Coefficient	P-value	Coefficient	P-value
Intercept	5.8663	0.0147*	6.25	0.0123*		12.07	0.0017*
X_1 (5%, 10%)	10.7146	0.0136*	0.72	0.4257		15.41	0.0057*
X_2 (2%, 8%)	30.0828	0.0009*	7.76	0.0271*		38.88	0.0004*
X_3 (5%, 15%)	0.0087	0.9282	24.20	0.0017*		0.05	0.8240
$X_1 \square X_2$	0.7408	0.4179	0.26	0.6235		34.56	0.0006*
$X_1 \square X_3$	5.4282	0.0526	22.29	0.0022*		0.05	0.8339
$X_2 \square X_3$	3.0690	0.1233	0.02	0.8906		0.01	0.9164
X_1 2	1.5953	0.2470	0.04	0.8496		0.14	0.7154
X_2 2	0.0023	0.9634	0.92	0.3687		17.45	0.0042*
X_3 2	1.0101	0.3484	0.07	0.8021		2.66	0.1470

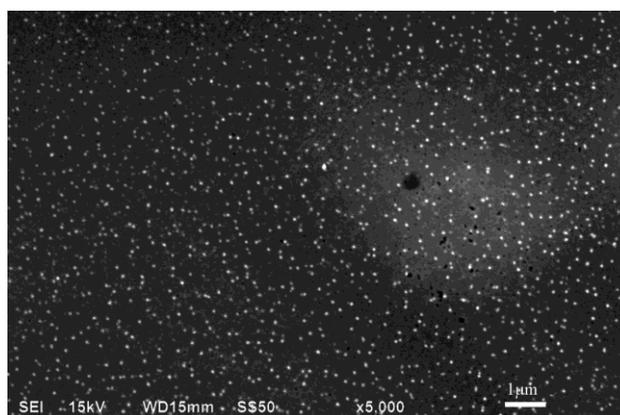


Figure 7 scanning electron micrographs of Ceftriaxone loaded lipid nanoparticles consisting of glyceryl monostearate 10%, poloxamer 8%, drug/lipid ratio 13.5% (X5000).

It was known that the particle size distribution was one of the most important characteristics for evaluation of the stability of colloidal systems. The mean particle size (\bar{z}) and the polydispersity index (PI) were 2 measures to evaluate the width of the size distribution. Figure 8 shows that the particle size distribution of Ceftriaxone -loaded SLN was 248 ± 4.8 nm; the polydispersity index of particle size was 0.277 ± 0.058 (run 3).

Zeta potential is the measure of overall charges acquired by particles in a particular medium and is considered as one of the benchmarks of stability of the colloidal system. Particles will repel each other if the systems have high positive or negative value of zeta potential, and a system having value ± 30 mV is considered a stable formulation if dispersed in a liquid as colloidal dispersion. In the present study, the zeta potential value of optimum formulation was -8.74 mV. The absolute value of zeta potential was lower than those values reported in the literature. This might be attributed to poloxamer 188, a nonionic surfactant which decreases the electrostatic repulsion between the particles and sterically stabilizes the nanoparticles by forming a coat around their surface.^[16] The negative charge of SLN may result from fatty acids released from the hydrolysis of GMS. In such a system, the hydrophilic emulsifiers were thought to align alongside each other, imparting more rigidity and strength to the emulsifier film through hydrogen bonding.^[17]

In addition, with regard to particle distribution, the polydispersity index is an indicator of the homogeneity of

the size distribution. The PI value of 0.277 indicated that the optimal composition could be used to produce a stable SLN dispersion with a relatively narrow size distribution for Ceftriaxone -loaded SNL.

In vitro drug release behavior

The drug release behavior in vitro of Ceftriaxone l- loaded SNL was investigated using a dialysis membrane in pH 7.4 ATF (37 ± 0.5). In Figure 9, free Ceftriaxone exhibited a rapid release of 90% of drug within 6 hours, whereas the release profiles of SNL indicated a biphasic pattern with a burst release during the first 8 hours, followed by a sustained release over 48 hours. The initial fast release of drug from the SNL could be explained by drug desorption from the outer surface of the SNL and the larger specific surface of the smaller particles increasing the initial drug release rate. Furthermore, SLN had been prepared so as not to remove existing free drug, but the initial burst related to free drug could not be ignored. The mechanism of release was determined by finding the R² value for each kinetic model viz First-order, Higuchi, Peppas–Korsmeyer, and Hixon–Crowell model corresponding to the release data obtained. The Peppas–Korsmeyer model was the best ($y = 0.9428x - 0.9385$, $R^2 = 0.9969$) in that the R² value of the Korsmeyer–Peppas model is much larger than other kinetic models. Thus it can be said that the drug release follows Peppas–Korsmeyer model mechanism. The n value is 0.9428 which is above 0.89, so that the release can be characterized by super case I transport,^[18] which means the drug release rate is dominated by polymer disentanglement and erosion.

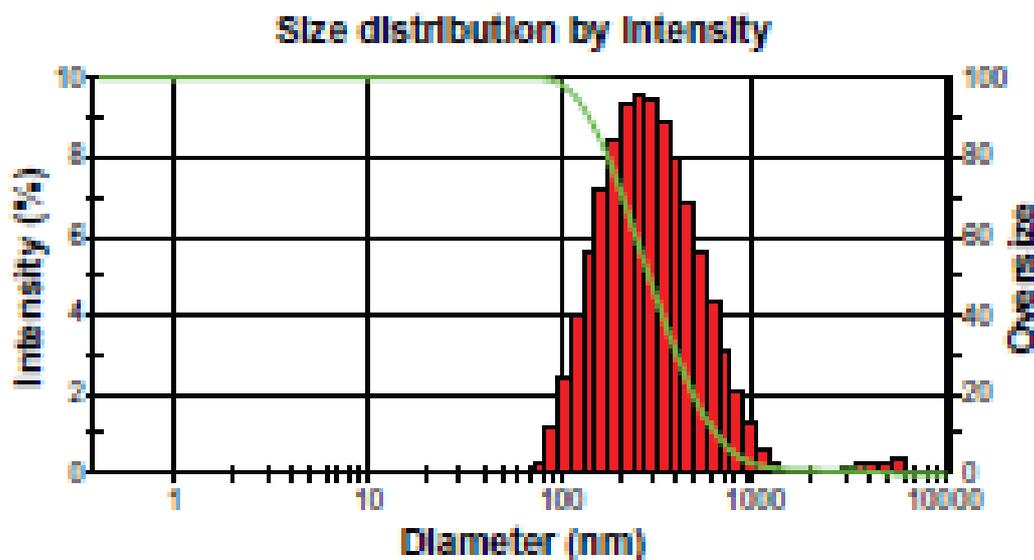


Figure 8 Particle distribution of Ceftriaxone -loaded solid lipid nanoparticles.

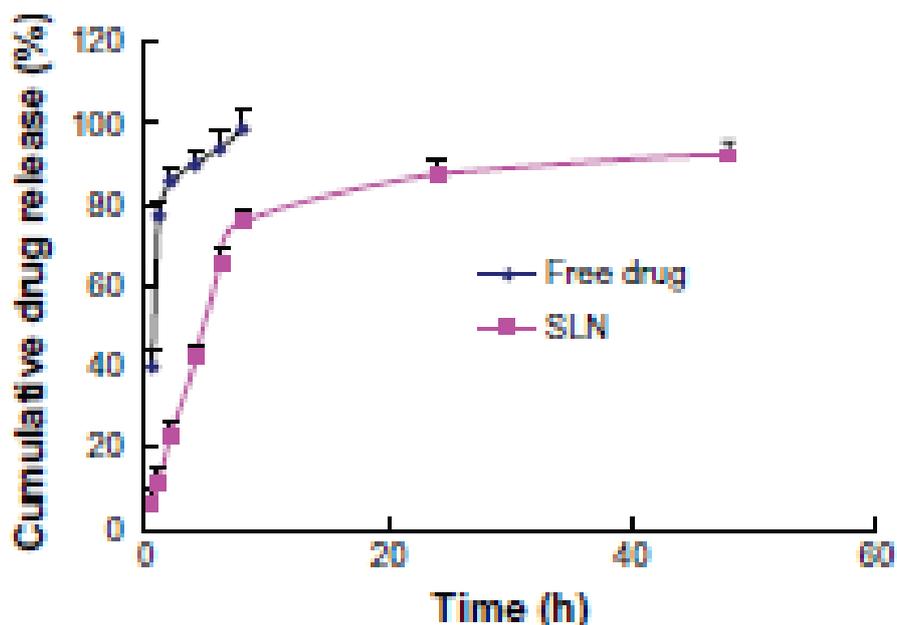


Figure 9 In vitro drug release profile of Ceftriaxone from solid lipid nanoparticles (SLN) and free drug (pH 7.4 artificial tear fluid was used as dialysis medium).

CONCLUSION

The purpose of the present study was to optimize a solid lipid nanoparticle (SLN) of Ceftriaxone by investigating the relationship between design factors and experimental data using response surface methodology. A Box-Behnken design was constructed using solid lipid (X1), surfactant (X2), and drug/lipid ratio (X3) level as independent factors. SLN was successfully prepared by a modified method of melt-emulsion ultrasonication and low temperature- solidification technique using glyceryl monostearate as the solid lipid, and poloxamer 188 as the surfactant. The dependent variables were entrapment efficiency (EE), drug loading (DL), and turbidity. Properties of SLN such as the morphology, particle size, zeta potential, EE, DL, and drug release behavior were investigated, respectively. As a result, the nanoparticle designed showed nearly spherical particles with a mean particle size of 248 nm. The polydispersity index of particle size was 0.277 ± 0.058 and zeta potential was -8.74 mV. The EE (%) and DL (%) could reach up to $83.29\% \pm 1.23\%$ and $10.11\% \pm 2.02\%$, respectively. In vitro release studies showed a burst release at the initial stage followed by a prolonged release of Ceftriaxone from SLN up to 48 hours. The release kinetics of the optimized formulation best fitted the Peppas–Korsmeyer model. These results indicated that the Ceftriaxone - loaded SLN could potentially be exploited as a delivery system with improved drug entrapment efficiency and controlled drug release.

REFERENCES

- Lucks, J. and R. Müller, Medication vehicles made of solid lipid particles (solid lipid nanospheres SLN). EP0000605497, 1991.
- Mehnert, W. and K. Mäder, Solid lipid nanoparticles: production, characterization and applications. *Advanced drug delivery reviews*, 2001; 47(2): 165-196.
- Mehnert, W. and K. Mader, Solid lipid nanoparticles: production, characterization and applications. *Adv Drug Deliv Rev*, 2001; 47(2-3): 165- 96.
- Muller, R.H., K. Mader, and S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. *Eur J Pharm Biopharm*, 2000; 50(1): 161-77.
- Muller, R.H., M. Radtke, and S.A. Wissing, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev*, 2002; 54 Suppl 1: S131-55.
- WWW.Drug bank online.com
- Yang C, Zhao X, Hu H, et al. Preparation, Optimization and Characteristic of Huperzine A Loaded Nanostructured Lipid Carriers. *Chem Pharm Bull*, 2010; 58(5): 656–661.
- Bottari F, Di Colo G, Nannipieri E, Saettone M, Serafini M. Influence of drug concentration on in vitro release of salicylic acid from ointment bases. *J Pharm Sci.*, 1974; 63(11): 1779–1783.
- Myers R, Montgomery D, Anderson-Cook C. *Response surface methodology: process and product optimization using designed experiments*. New York: John Wiley & Sons, 2009.
- Woitiski C, Veiga F, Ribeiro A, Neufeld R. Design for optimization of nanoparticles integrating biomaterials for orally dosed insulin. *Eur J Pharm Biopharm*, 2009; 73(1): 25–33.
- Rahman Z, Zidan A, Habib M, Khan M. Understanding the quality of protein loaded PLGA nanoparticles variability by Plackett-Burman design. *Int J Pharm*, 2010; 389(1–2): 186–194.

12. Shah M, Pathak K. Development and Statistical Optimization of Solid Lipid Nanoparticles of Simvastatin by Using 2³ Full-Factorial Design. *AAPS Pharm Sci Tech.*, 2010: 1–8.
13. Yang Y, Chung T, Bai X, Chan W. Effect of preparation conditions on morphology and release profiles of biodegradable polymeric micro-spheres containing protein fabricated by double-emulsion method. *Chem Eng Sci.*, 2000; 55(12): 2223–2236.
14. Schubert M, Müller-Goymann C. Solvent injection as a new approach for manufacturing lipid nanoparticles-evaluation of the method and process parameters. *Eur J Pharm Biopharm*, 2003; 55(1): 125–131.
15. Leroux J, Allémann E, Doelker E, Gurny R. New approach for the preparation of nanoparticles by an emulsification-diffusion method. *Eur J Pharm Biopharm*, 1995; 41(1): 14–18.
16. Schwarz C, Mehnert W, Lucks J, Müller R. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. *J Control Release*, 1994; 30(1): 83–96.
17. Gullapalli R, Sheth B. Influence of an optimized non-ionic emulsifier blend on properties of oil-in-water emulsions. *Eur J Pharm Biopharm*, 1999; 48(3): 233–238.
18. Peppas NA, Sahlin JJ. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *Int J Pharm*, 1989; 57(2): 169–172.