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STATISTICAL OPTIMIZATION OF CHLORPROMAZINE HYDROCHLORIDE - LOADED SOLID LIPID NANOPARTICLES USING 3^2 FULL FACTORIAL DESIGN

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ABSTRACT

Chlorpromazine hydrochloride is an atypical anti-psychotic drug with extensive first-pass metabolism and limited oral bioavailability and solid lipid nanoparticles (SLN) is one of the approaches to improve its bioavailability. This study introduces a 3^2 full factorial design used for optimization of chlorpromazine hydrochloride loaded solid lipid nanoparticles prepared by probe sonication method. The independent two variables Drug: Lipid ratio, probe sonication time at three levels arranged in a 3^2 design to study effect on dependent variables particle size and entrapment efficiency. From the statistical analysis of data polynomial equations were generated. The particle size and %EE for the 9 batches (R1 to R9) showed a wide variation of 130-152 nm and 76-87%, respectively. Physical characterization of chlorpromazine hydrochloride - SLN done by differential scanning calorimeter, X- ray diffraction, Infrared spectrophotometry and particle size analyzer. Particle size, zeta potential and entrapment efficiency of optimized formulation was found to be 141.69 nm, -30.0 mV and 86.74% respectively. The *in vitro* drug release study of chlorpromazine hydrochloride solid lipid nanoparticles using dissolution apparatus shows controlled release of chlorpromazine than dispersion of pure drug.

KEYWORDS: Chlorpromazine hydrochloride, atypical antipsychotic, Solid Lipid Nanoparticles, 3^2 full factorial design, probe sonication.

INTRODUCTION

Oral route is the most preferred route for drug administration due to greater ease of administration, negligible pain, high patient compliance and no needle based injuries. However, newer formulations and dosage forms are unable to deliver via oral drug delivery systems due to low drug solubility, poor GI absorption, and metabolism related issues, continuous fluctuation drug plasma levels and variability due to food effects which may compromise the conventional dosage delivery system.[1]

Recently, Solid lipid nanoparticles (SLNs) have gained increased attention because of their unique structure and properties, such as good biocompatibility, protection for the incorporated compound against degradation, good stability, good tolerability, improved therapeutic effect, lower cytotoxicity, controlled release of drugs and best production scalability. Solid lipid nanoparticles (SLNs) are promising carriers for oral delivery of lipophilic as well as hydrophilic drug candidates. SLN gives targeted delivery and enhanced oral bioavailability. When encapsulated in lipid-base vehicles poorly water soluble drugs shows enhanced bioavailability.[2]

SLN were derived from oil-in-water emulsions by replacing the liquid lipid (oil) by a solid lipid, that is, a lipid being solid at room as well as at body temperature. Because of their solid matrix, drug release from SLN can be modulated which could be used to optimize the plasma drug concentration profile. SLN enhance lymphatic transport of the drugs, reduce the hepatic first-pass metabolism and improve bioavailability because intestinal lymph vessels drain directly into the thoracic duct, further into the venous blood, thus by passing the portal circulation A variety of matrix materials used for the fabrication of SLN are hard fat or cetylpalmitate, fatty acid and glycerides (monoglycerides, diglycerides and triglycerides). SLN fabricated using lipids of less ordered crystal lattices favour successful drug inclusion compared with those prepared using highly ordered crystal lipids. This is owing to the crystallinity and polymorphic behavior of lipids. More complex lipids (triglycerides) form less perfect crystals with many imperfections. These imperfections offer space to accommodate the drug. Accordingly, crystallinity and polymorphic behavior are the most essential key issues which have a strong influence on the drug incorporation and release rate. Therefore, degree of crystallinity is to be considered in selection of the lipids for formulation of SLN.[1,3]

Single lipid matrix has perfect crystal lattice which is responsible for drug expulsion and consequently for less entrapment efficiency (EE) and physical instability. On other side, binary lipid matrix can create deformation in crystal order of lipids and avoid the drug expulsion. Up to now, the use of binary lipid matrix in fabrication of SLN has not received due attention by the researchers. So as to improve the EE and physical stability of SLN, an attempt was made to disturb the crystal lattice (crystal order) of the glyceryl monostearate (GMS) by the addition of trace amount of Compritol 888 ATO.[3]

SLNs were loaded with an antipsychotic drug chlorpromazine hydrochloride. The drug has low oral bioavailability with extensive first-pass metabolism. With a view to improve the bioavailability, the drug-loaded binary SLN were prepared. So, in this work we designed and evaluated chlorpromazine hydrochloride -loaded binary SLNs to improve the oral bioavailability. Chlorpromazine hydrochloride -SLNs were prepared by high speed homogenization followed by probe sonication method. Optimization of SLNs batches were carried out using 3^2 design. Freeze dried optimized batch then evaluated by particle size, EE, zeta potential, XRD, DSC and SEM. In vitro drug release studies were performed using drug suspension and optimized batch of SLN.

MATERIAL AND METHOD

Material

Chlorpromazine hydrochloride was kindly gifted by GlaxoSmithKline pharmaceutical company. Glyceryl monostearate (GMS), Compritol ATO 888, Span 80, Tween 80 were provided by Loba Chemie Pvt. Ltd., Mumbai, India. Borneol were purchased from Yarrow chemie Pvt.ltd. Mumbai India. All other chemicals were of reagent grade and used without further purification.

Method

Preparation of SLN[4,5]

Solid lipid nanoparticles were prepared by probe sonication followed by high speed homogenization method. Accurately weighed lipids {GMS and Compritol (70:30)} was added in beaker, which is held in water bath at 80°C. When lipid was melted then drug was added in molten lipid and mixed it and then lipophilic surfactant (span 80) were added in melted solid lipid. The aqueous surfactant solution was prepared separately in a beaker. Both mixtures were heated in controlled temperature water bath (at 5°C higher than melting point of lipid to avoid solidification of lipid). The aqueous surfactant solution was added in lipidic

mixture at same temperature. The mixture was homogenized using high speed homogenizer at 4000 rpm for 10 min. Then the pre-emulsion was subjected to probe sonication and different cycles (5, 10, and 15). Final product obtained was cooled at room temperature to get the solid lipid nanoparticles.

Experimental design and Statistical analysis[6,7]

Most formulation studies involve variation of one factor at a time, keeping other factors constant. Factorial design enables all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. In this study, a 3^2 full factorial experimental design was introduced to optimize the formulation of nanoparticles. Initial studies were undertaken to decide on the excipients and their levels in the experimental design. The choice of binary mixture of lipid was done on the basis of solubility of chlorpromazine hydrochloride in the various combination of binary mixture of lipid at various ratios. Aqueous phase surfactant and lipid phase surfactant were selected on the basis of stability of dispersion prepared by using different surfactants. In order to optimize the preparation of formulations, the drug: lipid ratio (X1) and sonication time (X2) were chosen as independent variables. These two factors that might affect the nanoparticle formulation and three levels of each factor were selected (Table 1) and arranged according to a 3^2 full factorial experimental table (Table 2).

Evaluation of Solid lipid nanoparticles

Particle size analysis

The mean particle size and particle size distribution (Polydispersity index) of selected formulation was determined by Horiba SZ-100 nanoparticle analyzer, at 28°C. The mean diameter of each batch is recorded in Table 3.

Zeta Potential Measurement

The zeta potential of selected formulation was measured by Nano particle analyzer. Laser Doppler Micro-electrophoresis was used to measure zeta potential. An electric field was applied to a solution of molecules or a dispersion of particles, which then move with a velocity related to their zeta potential. This velocity was measured using laser interferometry technique which enables the calculation of electrophoretic mobility, and from this, the zeta potential and zeta potential distribution. The zeta potential of optimized batch show in Fig 4.

Entrapment efficiency

The entrapment efficiency of prepared SLN was calculated by centrifugation method. About 5 mL of dispersion of solid lipid nanoparticle was taken in centrifuge tube and further it was centrifuged in cooling centrifuge (REMI-C24 BL. Remi Elektrotechnik ltd. Vasai, India) at 15,000 rpm for 40 mins. After centrifugation the supernatant was removed and diluted with appropriate solvent. The concentration of drug (free drug) in supernatant layer was determined by using UV-VIS Spectrophotometry.

The entrapment efficiency (EE) is calculated by using Eqn (i)

$$\% \text{ EE} = \frac{\mathbf{W}\ initial\ drug - \mathbf{W}\ free\ drug}{\mathbf{W}\ initial\ drug} \times 100 \qquad \ldots\ldots\ldots (i)$$

Where,

W *initial* drug = Weight of initial drug added into the formulation.

W *free* drug = Weight of free drug into the formulation

Differential Scanning Calorimetry

The DSC thermogram of SLN was recorded by using a differential scanning calorimeter (PerkinElmer 4000, UK) equipped with a computerized data station. The sample (approx. 1mg) was weighed and heated in a closed pierced aluminum pan at a scanning rate of 10°C/min between 30- 300°C and 20 mL/min of nitrogen flow. DSC measurements were carried out on pure chlorpromazine hydrochloride, chlorpromazine hydrochloride -loaded SLN, and Surface modified SLN.

X-ray diffraction

SLN were studied for X-ray diffraction. The powder X ray diffraction patterns was recorded using an X-ray diffractometer (Bruker D8 advance) with 2.2 KW copper as an anode material and dermic X-ray tube as a source. The sample was analyzed using the 2□□angle of 3-30° using lynux eye detector and filtered using Ni filter. X-ray diffraction measurements were carried out on pure chlorpromazine hydrochloride, chlorpromazine hydrochloride - loaded SLN, and Surface modified SLN.

FTIR Studies

FTIR studies were carried out on pure chlorpromazine hydrochloride, chlorpromazine hydrochloride with GMS and Compritol, chlorpromazine hydrochloride with Span 80 and chlorpromazine hydrochloride with tween 80 by using Bruker Germany (model alpha T).

***In vitro* release of chlorpromazine hydrochloride from SLNs**

The in vitro drug release from Chlorpromazine hydrochloride loaded SLN and suspension in PBS pH 7.4 and 0.1 N HCl was examined by the dialysis bag method (D 5 8000, Lab India. dissolution apparatus). In brief, SLN dispersion was added to the dialysis bag (molecular weight cut off 12000) and the dialysis bag was tied to place into 900 mL dissolution medium (PBS pH 7.4 and 0.1 N HCl) with stirring rate of 50 rpm at 37°C. Then 10 mL of dissolution medium was withdrawn at the different time points for 24 hours (0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hour) and fresh release medium to equal volume was added quickly to maintain the sink condition. The samples were analyzed by UV-VIS Spectrophotometry. Each experiment was performed in triplicate.

RESULT AND DISCUSSION

Experimental design and statistical analysis

The objective of this study was to prepare solid lipid nanoparticles of chlorpromazine hydrochloride by pre-emulsion probe sonication method and to optimize the effects of formulation variables on response parameters. Based on preliminary studies, binary mixture of GMS and compritol 888 ATO (70:30), Span 80 and Tween 80 were chosen as lipid, lipid phase surfactant and aqueous phase surfactant respectively. Drug: binary lipid ratio and sonication time were selected as variables and entrapment efficiency and particle size as response parameters. A 3^2 full factorial design was selected as it helps study the effect on response parameters by changing both variables simultaneously with a minimum number of experimental runs.

The particle size and EE for the 9 batches (B1 to B9) showed a wide variation 130-152 nm and 76- 87%, respectively (Table III). The data clearly indicated strong dependence of response variables on the selected independent variables.

In order to quantify the effect of formulation variables on the response parameters, it was necessary to construct a mathematical model which would help in predicting values of response parameters at any selected values of formulation variables within the boundaries of

the design. It may be the case that the levels of formulation variables which are intermediate between the selected levels yield optimum formulation. Design Expert 10.0 software was used to generate a mathematical model for each response parameter and the subsequent statistical analysis.

The coefficients of the polynomial equations generated using Design expert 10 for particle size and %EE of DTH-loaded SLN dispersion studied are listed in (Table IV) along with the values of r^2. Five coefficients (a to e) were calculated with k as the intercept.

$$Y = k + aX_1 + bX_2 + cX_1X_2 + dX_1^2 + eX_2^2 \quad (1)$$

The equation was used to obtain estimates of the responses at various factor combinations.

For particle size response, the Model F-value of 15.58 implies the model is significant. There is only a 2.34% chance that a "Model F-Value" this large could occur due to noise. P value were found to be 0.0234, with a value less than 0.0500 indicating model terms are significant.

The "Predicted R-Squared" of 0.6506 is in reasonable agreement with the "Adjusted R-Squared" of 0.9011."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 12.295 indicates an adequate signal thus the proposed model can be used to navigate the design space.

For **%**EE response, the Model F-value of 20.95 implies the model is significant. There is only a 1.54% chance that a "Model F-Value" this large could occur due to noise. P value were found to be 0.0154, with a value less than 0.0500 indicating model terms are significant.

The "Predicted R-Squared" of 0.7872 is in reasonable agreement with the "Adjusted R-Squared" of 0.9257."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 11.338 indicates an adequate signal. Thus the proposed model can be used to navigate the design space.

Since the values of r^2 are relatively high for both the responses, i.e., 0.9011 for particle size and 0.9257 for %EE, the polynomial equations form an excellent fit to the experimental data and are highly statistically valid.

Composition of optimized batches and comparison of the observed responses with that of the predicted responses along with percentage error is listed in Table.

Three-dimensional response surface plots for each response parameter were constructed to study the effects of both formulation variables simultaneously along with the behavior of the system and it was shown in fig and fig.

Evaluation of Solid Lipid Nanoparticles

Particle Size Analysis

The particle size determined using Horiba SZ-100 nanoparticles analyzer, at 28°C showed size ranging from 130 – 151 nm (Table 3). The effect of lipid concentration on the particle size can be seen from particle size of sample B7, B5 and B3 (131.5 nm, 141.4 nm and 150.9 nm respectively) with low to high lipid concentrations (1:3, 1:5 and1:7) as lipid concentration increases particle size increases. In case of sonication time Figure 1 shows that as time of sonication increases particle size decreases. So, the sonication time shows a negative influence on particle size.

Entrapment efficiency

A high amount of drug was incorporated in nanoparticle dispersion. The %EE of different formulations prepared (Table 3) indicates the positive influence of lipid content on drug entrapment. The formulations B_3, B_6 and B_9 which have lipids at high level but surfactant at low middle and high levels, respectively showed % entrapment of 85.3, 86.3 and 86.50, respectively. This indicates overwhelming influence of lipid on entrapment, irrespective of surfactant content. Formulations B_1, B_4, which had lipid content at low level and sonication at low and medium level, respectively show less % EE (< 80%). The partitioning of drug between lipid and water phases during pre-emulsion formation affects drug entrapment in nanoparticles. This in turn depends on the amount of lipid, solubility of drug in lipid, process temperature and surfactant concentration. Therefore, the positive influence of lipid content on entrapment is explained.

Differential Scanning Calorimetry

The DSC thermogram of chlorpromazine hydrochloride drug loaded SLN is shown in Figure 4. The peak of chlorpromazine hydrochloride is completely absent in lyophilized SLN batch. It has been reported that when the chlorpromazine hydrochloride does not show its endothermic peak in the SLN, it is said to be in the amorphous state. Hence, it could be concluded that the drug is present in the amorphous phase and may have been homogeneously dispersed in the lipid nanoparticles.

X-ray diffraction

X-ray diffraction data listed in following Fig. 5 was good in agreement with results established by DSC measurements. The diffraction pattern of the bulk matrix showed remarkable difference from those of the SLN, as they showed relative sharp peak than the SLN. It was clear that from chlorpromazine hydrochloride-SLN, the less ordered crystals were majority and the amorphous state would contribute to the higher drug loading capacity as seen previously. There is a significant difference between the diffraction patterns of chlorpromazine hydrochloride and chlorpromazine hydrochloride-SLN. It was confirmed that chlorpromazine hydrochloride existed in amorphous state in the chlorpromazine hydrochloride -SLN because of the disappeared sharp peak of chlorpromazine hydrochloride in the diffraction pattern.

FTIR

From FTIR study, the characteristic peaks of drug such as of Amine (3389.97), Alcohol (3351.85), Sulfide (750.40) disappear and replace by the peak of GMS Remaining peaks also either shifted or replaced in the IR spectrum of formulation shown in Fig.6 This established drug entrapment in lipid matrix.

***In vitro* release of chlorpromazine hydrochloride from SLNs**

The in vitro drug release from plain drug suspension and SLN is plotted against different time points for 24 hours. The drug release was studied in PBS 7.4 pH and 0.1 N HCl. The total % cumulative release from drug suspension and SLN shown in Table 6 and Fig.8 for two medium (PBS pH 7.4 and 0.1 N HCl).

Table 1: Independent variables and their selected levels for formulation of solid lipid nanoparticle.

Independent variables	**Coded levels**			**Dependent variables**
Drug : Lipid Ratio (X_1)	**-1**	**0**	**+1**	Particle size **(Y_1)**
	1:3	**1:5**	**1:7**	
No. of probe cycles (X_2)	**5**	**10**	**15**	Entrapment efficiency **(Y_2)**

Table 2: A 3^2 Experimental Design Layout.

Formulation code	Coded level	
B1	-1	-1
B2	0	-1
B3	+1	-1
B4	-1	0
B5	0	0
B6	+1	0
B7	-1	+1
B8	0	+1
B9	+1	+1

Table 3: Particle size and entrapment efficiency of B1-B9 batches of SLN.

Formulation Code	Particle size (nm)	Entrapment efficiency (%)
B1	144.5	76
B2	148.2	80.5
B3	150.9	85.3
B4	138.4	78.3
B5	141.4	79.5
B6	147.6	87.33
B7	131.5	77.77
B8	139.6	81.30
B9	140.2	86.50

Table 4: Values of the coefficients for the polynomial equations and r2 for various response variables of the chlorpromazine hydrochloride -SLN.

Coefficient code	Polynomial coefficient values for response variables	
	Particle Size	Entrapment efficiency
k	+143.06	+80.75
a	+4.05	+4.51
b	-5.38	+0.63
c	+0.57	-0.14
d	-0.88	+1.43
e	+0.017	-0.48
r^2	0.9629	0.9722

Table 5: Comparison of experimental results with predicted responses.

Batch No.	Lipid Conc./ Sonication time	Practical particle size (µm)	Theoretical particle size (µm)	% Error	Practical % EE	Theoretical % EE	% Error
B1	490/15	140.58	140.49	0.064	84.9	84.65	0.35
B2	560/15	140.99	140.82	0.120	86.1	85.31	0.92
B3	630/15	142.10	141.14	0.680	86.1	85.99	0.15
B4	700/15	141.69	141.63	0.042	86.74	86.70	0.046

Table 6: % cumulative release.

Time (Hr)	% CR in PBS pH 7.4		% CR in 0.1 N HCl	
	chlorpromazine hydrochloride Suspension	SLN	chlorpromazine hydrochloride Suspension	SLN
0.5	21.61	13.58	22.82	14.78
1	30.77	19.75	33.6	20.25
2	38.26	29.21	42.32	32.36
3	45.91	37.57	53.23	41.64
4	55.25	45.21	62.65	53.34
6	69.23	54.51	75.38	63.57
8	78.58	63.21	81.02	69.07
12	85.56	71.23	85.1	73.03
24	87.87	78.95	89.21	76.22

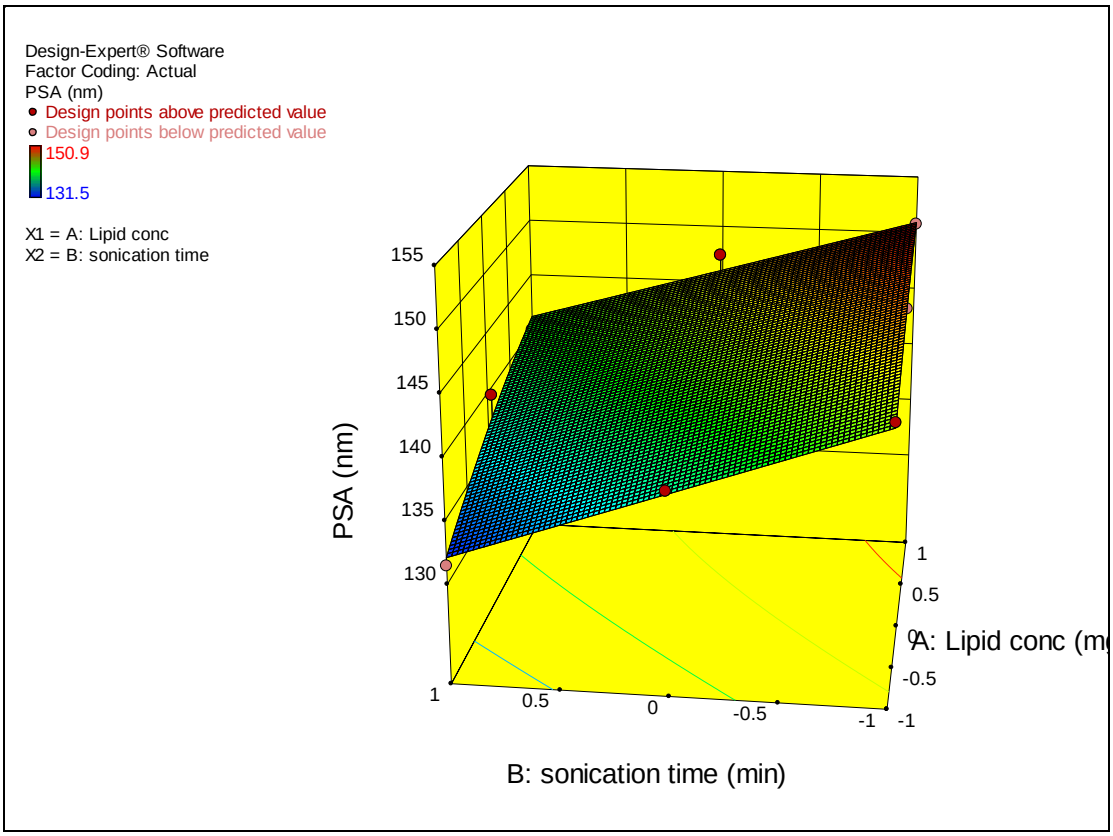

Figure 1: Three- dimensional response surface plots for particle size.

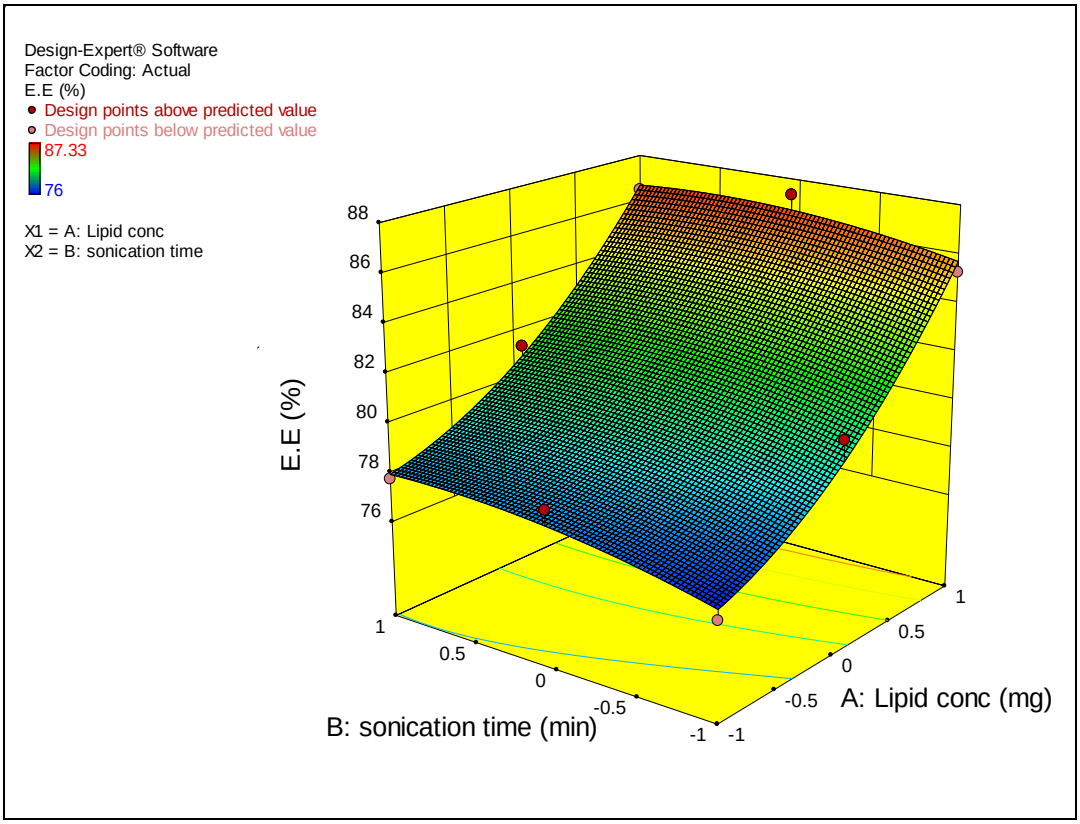

Figure 2: Three- dimensional response surface plots for entrapment efficiency.

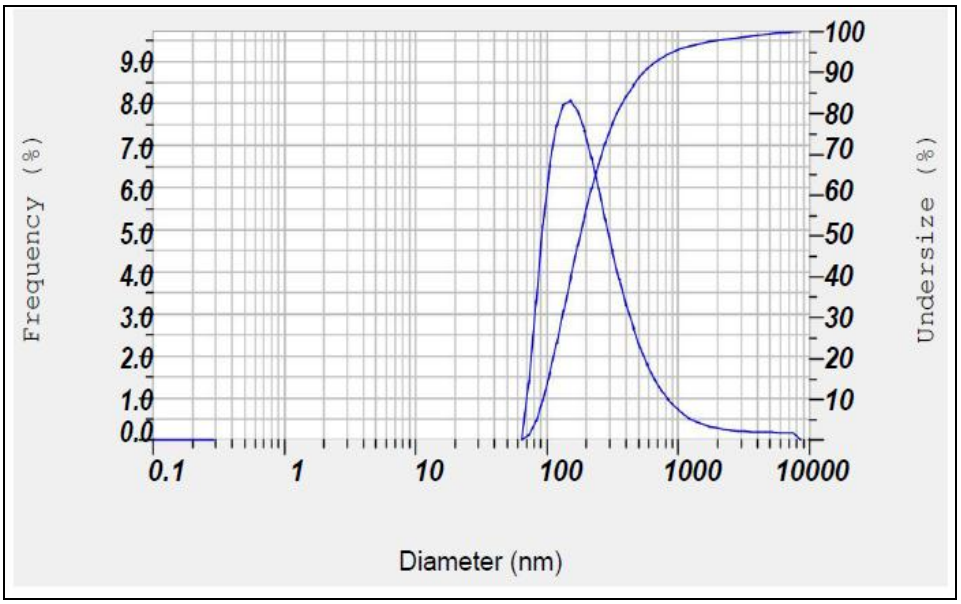

Figure 3: Particle size of optimized batch 'B4'.

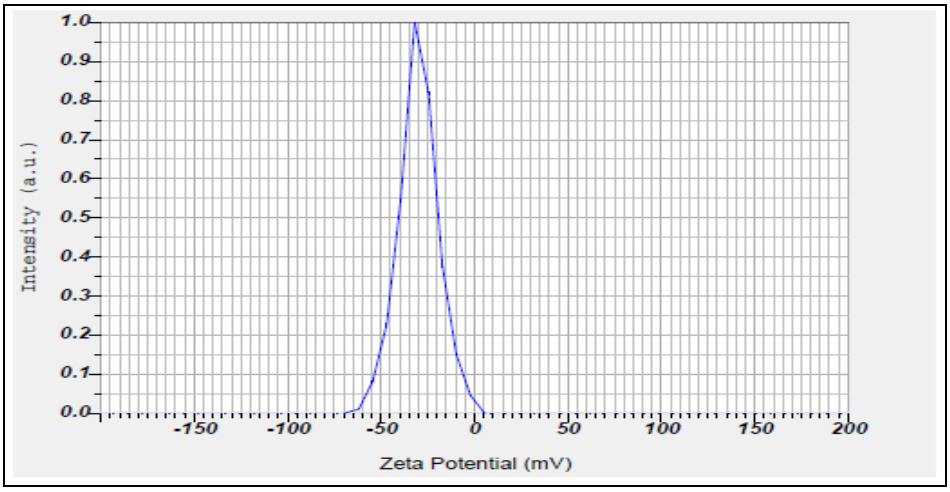

Figure 4: Zeta potential of optimized batch 'B4'.

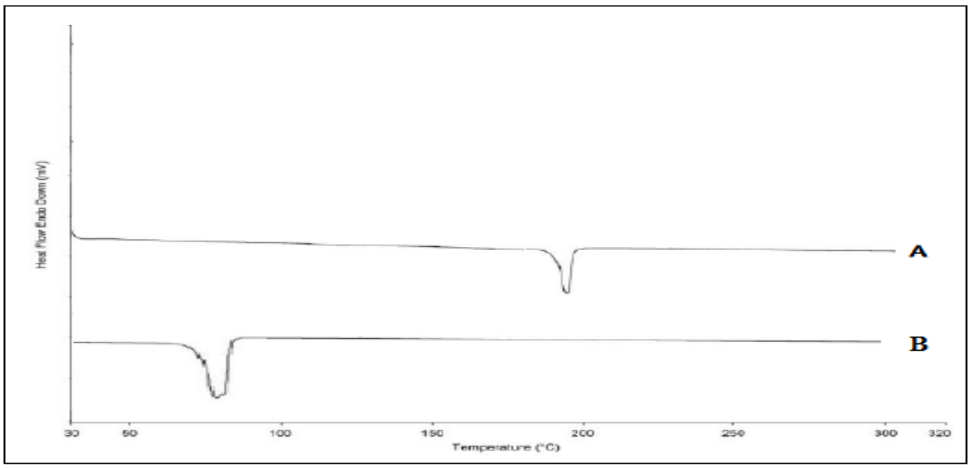

Figure 5 . DSC thermogram of chlorpromazine hydrochloride (A), chlorpromazine hydrochloride -SLN (B)

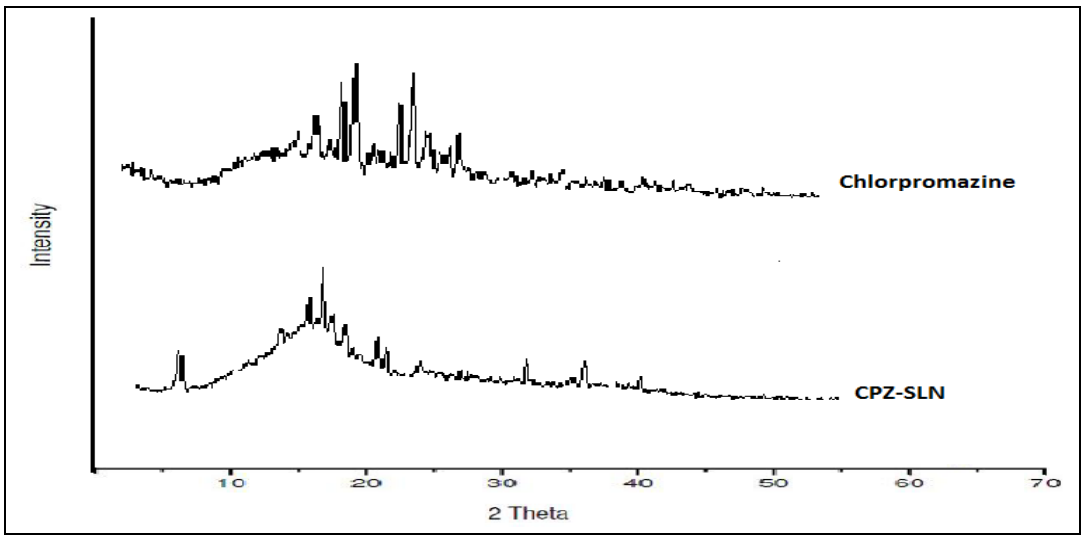

Figure 6: XRD of Chlorpromazine hydrochloride Drug and chlorpromazine hydrochloride- SLN.

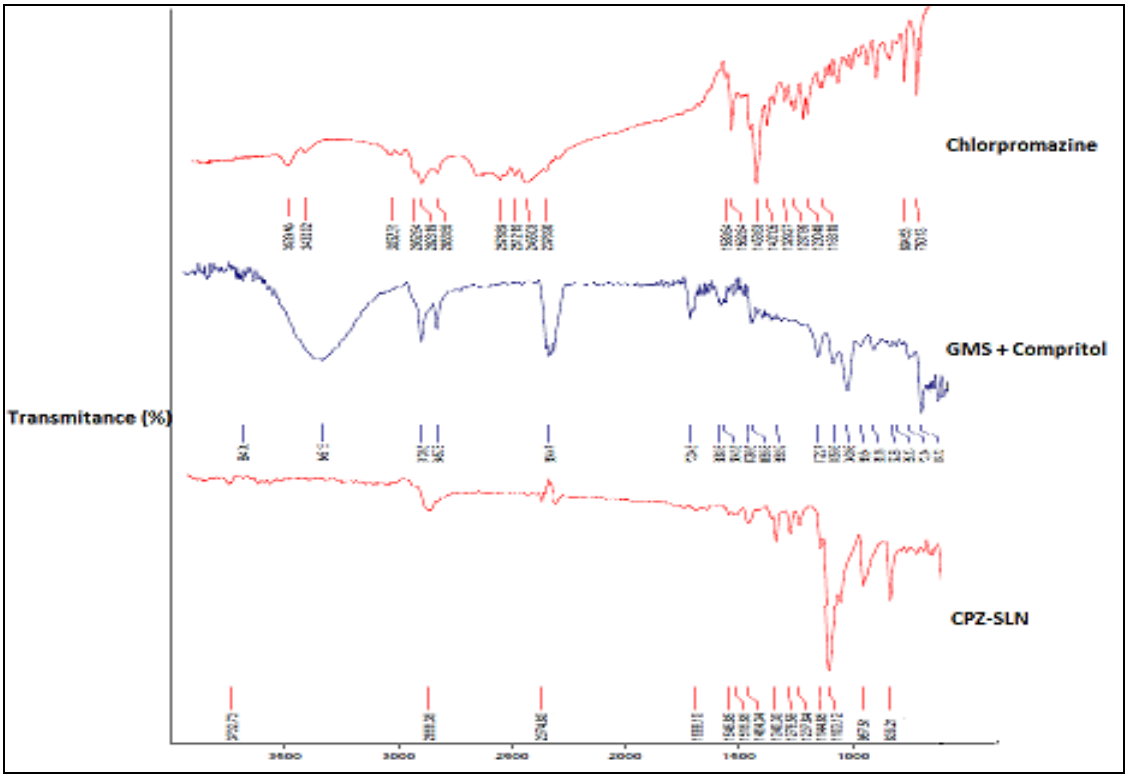

Figure 7: IR Spectra of Chlorpromazine hydrochloride Drug, GMS:Compritol (70:30) and chlorpromazine hydrochloride – SLN.

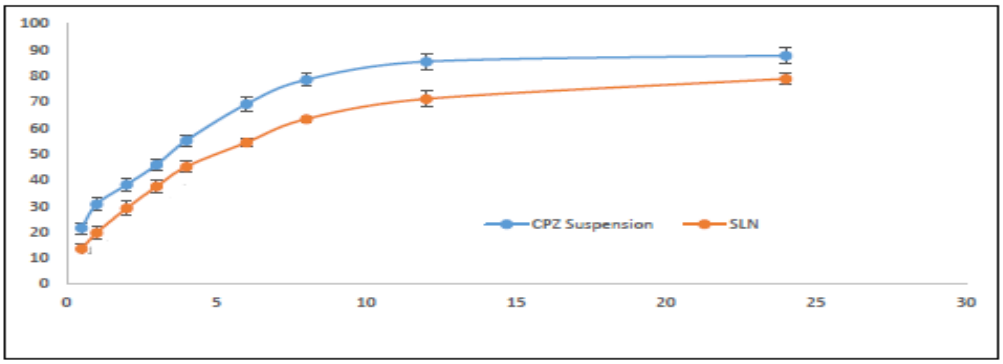

Figure 8.1 % cumulative release in phosphate buffer pH 7.4

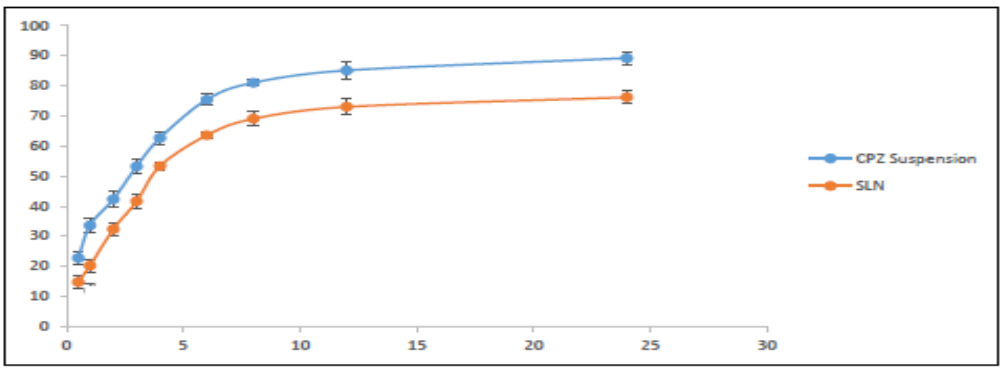

Figure 8.2 % cumulative release in 0.1 N HCL

Figure 8: *In vitro* % cumulative release of drug.

CONCLUSION

The pre-emulsion followed by probe sonication technique was used to prepare solid lipid nanoparticles of reproducible sizes in the range of 130 to 152 nm by addressing the effects of processing parameters. The application of 3^2 factorial design proved to be a useful tool for optimization of chlorpromazine hydrochloride-loaded SLN. Using the factorial design one can select a suitable composition of formulation to obtain chlorpromazine hydrochloride-loaded SLN in the size range of 130 to 152 nm depending on the application of the system.

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