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Statistical Optimization of Sertraline Hydrochloride Loaded Solid Lipid Nanoparticles Using Box-Behnken Design

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Abstract : Sertraline hydrochloride is an antidepressant with limited bioavailability and solid lipid nanoparticles (SLN) is one of the approaches to improve bioavailability. This study describes a box behnken experimental design to optimize the formulation of sertraline hydrochloride loaded solid lipid nanoparticles (SLN) by the probe sonication method. For optimization, a three factors and two levels box - behnken design was applied to study the effect of independent variables (factors) i.e. drug to lipid ratio (X_1), surfactant concentration (X_2) and probe sonication time (X_3) on dependent variables (responses) i.e. particles size (Y_1), entrapment efficiency (Y_2). Polynomial equations were generated on the basis of statistical analysis of data. The particle size and % EE for the 13 batches (R_1 to R_{13}) showed a wide variation of 145-201 nm and 80.5-88.8 %, respectively. The physical characteristics of sertraline hydrochloride loaded SLN were evaluated using FT-IR, differential scanning calorimetry and X-ray diffraction. The results of the optimized formulation showed an average particle size of 130.6 nm and entrapment efficiency of 85.30 %.
Keywords: Sertraline hydrochloride, Depression, Solid lipid nanoparticles, Box - Behnken design, Probe sonication.

Introduction

Solid lipid naoparticles (SLN) were introduced in 1990 as an alternative carrier system to the existing traditional carriers, such as liposomes, emulsions and polymeric nanoparticles. Solid lipid nanoparticles are composed of biocompatible or biodegradable lipid matrix that is solid at body temperature, dispersed in aqueous surfactant solution and exhibit size range in between 50-1000 nm.[1] SLN offer distinct advantages over conventional dosage forms. They are biocompatible, biodegradable and non-immunogenic. They can incorporate both hydrophilic and lipophilic drug compounds.[2]

Sertraline hydrochloride, is selective serotonin reuptake inhibitor (SSRI) recommended by the National Institute for Health and Clinical Excellence (NICE) as a first-line treatment of depression including obsessive-compulsive disorder, panic disorder and post-traumatic stress disorder.[3] The SSRIs also have good affinity for α1, α2, H1 and muscarinic receptors. Sertraline hydrochloride belongs to biopharmaceutical classification system (BCS) class II having low aqueous solubility and high permeability. The oral bioavailability of sertraline hydrochloride is poor (40%) due to extensive first pass metabolism in intestinal gut and liver.[3]

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Design of Expert (Stat-Ease Inc, Minneapolis, MN) is an effective, systematic and prominent tool to determine the effects of individual variables at all possible combinations, using minimum experimental efforts. Application of factorial design has played an important role in determining the relationship between independent variables and the responses obtained, for design and development of pharmaceutical formulations.[4]

The purpose of this study was to develop the mathematical model box – behnken design in order to reduce the adequate condition for preparation of sertraline hydrochloride loaded SLN with desired characteristic able to improve the bioavailability of drug.

Material and Methods

Material

Glycerol monostearte, tween 80 and span 80 were obtained as a gift sample from Loba Chemical Pvt. Ltd., Mumbai. Sertraline hydrochloride was obtained as gift sample from Wockhardt Research Centre Ltd. Aurangabad. All other chemicals were of reagent grade and used without further purification.

Preparation of SLN

SLN were prepared by pre-emulsion followed by probe-sonication method. Briefly the lipid phase consist of glyceryl monostearate, drug sertraline hydrochloride and lipophilic surfactant span 80 (2% w/v) heated at 70-75ºC.The aqueous phase was prepared by dissolving hydrophilic surfactant tween 80 (1% w/v) in 200 ml of distilled water at same temperature. Hot aqueous phase was added into lipid phase with continue stirring. The prepared pre-emulsion is further subjected to probe sonication (PCI analytics, Mumbai.) for 15 min.[5]

Experimental design and statistical analysis

The experimental work is completed before the optimization called the simultaneous optimization method. In this method one or more selected experimental responses are recorded for a set of experiments called as response surface methodology (RSM), carried out in a systematic way, to predict the optimum and the interaction effects. For optimization of solid lipid nanoparticles, a statistically experimental box behnken design was employed. Initial studies carried to decide the excipients and their levels in the experimental design. Three independent variables like drug to lipid ratio (X_1), Surfactant concentration (X_2), and Probe sonication time (X_3) were selected on the basis of results of trial-optimization study (Table 1). The effects of three independent variables were observed on particle size (PS) and entrapment efficiency (% EE). The response surface methodology of the Box-Behnken design (version10, Stat-Ease, Inc., Minneapolis, Minnesota, USA), using a three factors and three levels, was employed to optimize dependent variables like particle size (PS) and entrapment efficiency (EE) and arranged according to a Box Behnken experimental design (Table 2).

Table 1- Independent variables and their selected levels in box behnken design.

Independent variables	**Coded levels**		
	-1	**0**	**+1**
Drug: lipid ratio	1:3	1:5	1:7
Surfactant concentration	1%	2%	3%
Probe sonication time	10	15	20

Table 2- A box behnken experimental design layout.

Formulation Code	Coded Factor Levels (Independent variables)		
	X_1	X_2	X_3
R_1	0	1	1
R_2	1	1	0
R_3	-1	0	-1
R_4	-1	1	0
R_5	0	0	0
R_6	1	0	1
R_7	1	-1	0
R_8	-1	0	1
R_9	1	0	-1
R_{10}	0	-1	1
R_{11}	0	-1	-1
R_{12}	0	1	-1
R_{13}	-1	-1	0

Evaluation of solid lipid nanoparticles

Average particle size and zeta potential

The average particle size of optimized batch was analyzed by Horiba SZ-100 nanoparticles analyzer, at 28°C. The zeta potential of optimized batch was determined by Nano particle analyzer. Laser Doppler Micro-electrophoresis was used to measure zeta potential. The zeta potential of optimized batch recorded in figure 2.

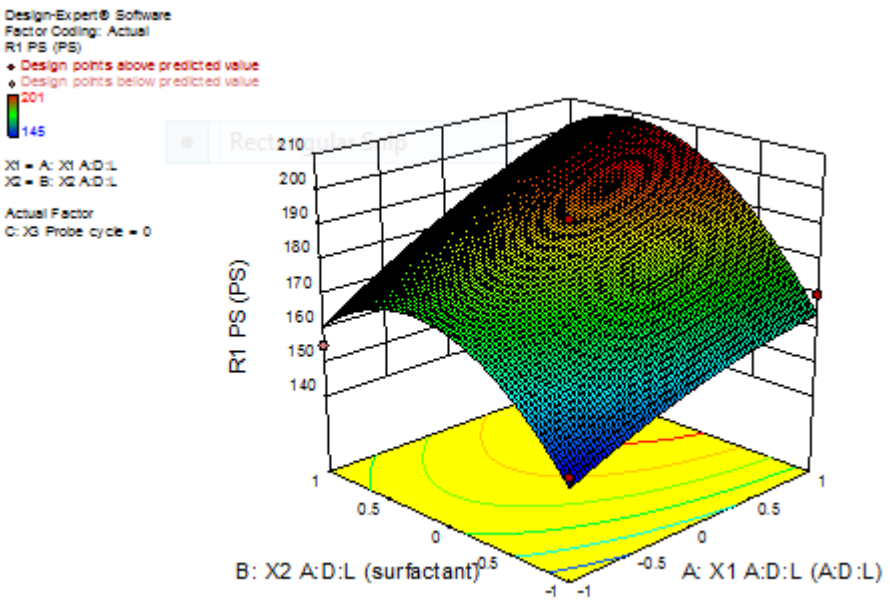

Figure 1. 3D response plot of particle size

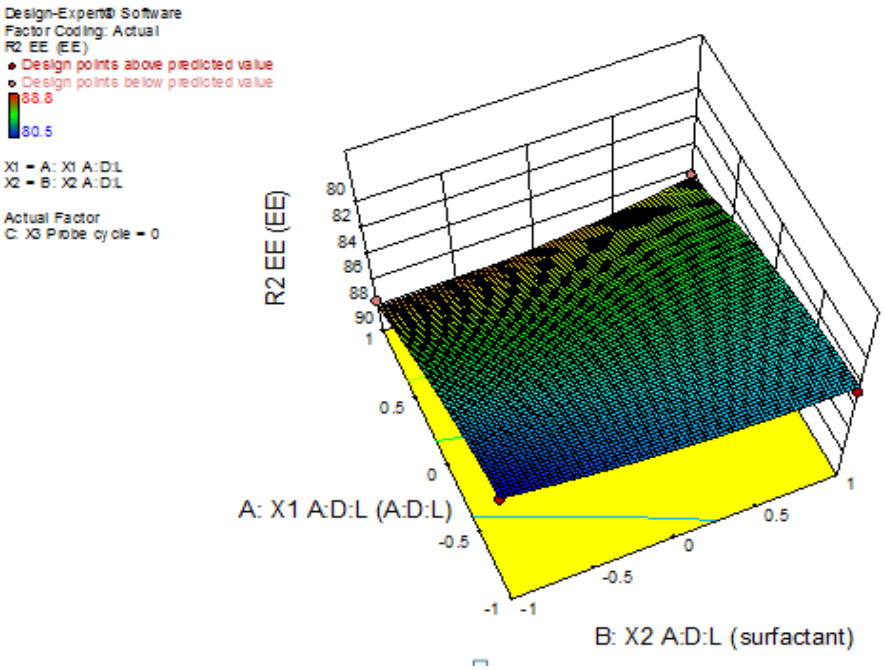

Figure 2. 3D Response surface plot of entrapment efficiency

Entrapment efficiency

The entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium of SLN dispersion by cooling centrifugation. The SLN dispersion was aggregated with the help of methanol and centrifuged in cooling centrifugation (REMI-C24 BL. Remi Elektrotechnik ltd. Vasai, India) at 10,000 rpm for 40 min. 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 (Shimadzu, V-1800, Japan). The % EE is depends on amount of lipid, concentration of surfactant, solubility of drug in lipid and process temperature.

The percent entrapment efficiency (%EE) was found using the formula

$$EE\ \% = \frac{Wt - Ws}{Wt} \times 100 \qquad (1)$$

Where, W_t is the total weight of drug used, W_s is the drug remaining in the supernatant or free drug.

Differential scanning calorimetry (DSC) study

Differential scanning calorimetric (DSC) analysis was done to confirm the drug lipid association in nanoparticulate formulations and melting point and melting enthalpies. The DSC thermogram of pure drug and optimized SLN (freeze dried) batch were recorded by using a differential scanning calorimeter (PerkinElmer 4000, UK) equipped with a computerized data station. The sample (appox. 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.

X-ray diffraction

The drug and optimized SLN (freeze dried) batch which showed the lowest particle size and the highest entrapment efficiency was subjected to X-ray crystallographic studies. 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 2θ angle of 3-30° using lynux eye detector and filtered using Ni filter.

Fourier Transmission Infrared Spectroscopy (FTIR) Studies.

A FTIR spectrophotometer (Bruker Germany, model alpha T) was used for infrared analysis of samples. About 1-2 mg of sample was mixed with dry potassium bromide and the samples were examined at

transmission mode. FTIR studies were carried out on physical mixture of drug + glyceryl monostearate, drug + tween 80 and drug + span 80 and SLN loaded with sertraline hydrochlide.

***In Vitro* Release Study.**

The *in vitro* drug release from sertraline hydrochloride loaded SLN and suspension in phosphate buffer solution (PBS) pH 7.4 and 0.1 N HCl was examined by the dialysis bag method. In brief, SLN dispersion was added to the dialysis bag (molecular weight cut off 12000) and the dialysis bag was tied to placed 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.[6]

Results and Discussion

Experimental design and statistical analysis

Glycerol monostearte, tween 80 and span 80 were selected as a lipid, lipid phase surfactant and aqueous phase surfactant respectively for further study on the basis of result obtained in the preliminary screening studies. Different batches (R_1-R_{13}) were prepared with different independent variables at different levels and the response like average particle size and entrapment efficiency were obtained.

The average particle size and entrapment efficiency for 13 batches (R_1-R_{13}) showed a wide variation in between range 145-201 nm and 80.5-88.8 % respectively in table 3. Design expert software was substituted the data of dependent and independent variable and generate the polynomial equation. The obtain data clearly indicate that the selected independent variable strongly affects on the results of response variables. Polynomial equations were generated and optimized on basis of ANOVA in the software. The models were evaluated in terms of statistically significant coefficients and R^2 values for particle size and entrapment efficiency of sertraline hydrochloride loaded SLN dispersion studied are listed in (Table 4 and 5). Nine coefficients (*a* to *i)* were calculated with k as the intercept.

$$Y= k + aX_1 + bX_2 + cX_3 + dX_1X_2 + eX_1X_3 + fX_2X_3 + gX_1^2 + hX_2^2 + iX_3^2 \quad (2)$$

The polynomial equation can be used for estimation of response variables. Composition of optimized batches and comparison of the observed responses with that of the predicted responses along with percentage error is listed in Table no 5.

Table 3: Particle size and entrapment efficiency of R_1-R_{13} batches of SLN

Formulation Code	Particle size(nm)	Entrapment efficiency (%)
R_1	186.9	83.5
R_2	199.3	86.1
R_3	167.1	82
R_4	155	82.7
R_5	191.6	84
R_6	201	88.8
R_7	170	87.5
R_8	165.9	82.7
R_9	188.9	88.8
R_{10}	145	85
R_{11}	145.5	84
R_{12}	176.6	84.9
R_{13}	145.4	80.5

Table 4 - Statistical analysis for particle size

Source	Sum of squares	Df	Mean square	F value	p-value Prob> F	Quadratic model
Model	4828.39	9	536.49	9.84	0.0429	significant
A-D:L	1978.21	1	1978.21	36.30	0.0092	
B-SC	1565.20	1	1565.20	28.72	0.0127	
C-PC	53.56	1	53.56	0.98	0.3946	
AB	97.02	1	97.02	1.78	0.2744	
AC	44.22	1	44.22	0.81	0.4341	
BC	29.16	1	29.16	0.54	0.5175	
A^2	27.60	1	27.60	0.51	0.5280	
B^2	979.41	1	979.41	17.97	0.0240	
C^2	125.17	1	125.17	2.30	0.2269	
Residual	163.50	3	54.50			
Cor Total	4991.89	12				

Table 5 - Comparison of the observed responses and predicted responses

Batch code	Composition Drug lipid ratio/surfactant conc./sonication time	Response	Predicated value	Experimental value	Percent error
VR1	-1/-1/-1	PS (nm)	130.98	130.6	0.2903
		EE (%)	85.21	85.00	0.2461
VR2	-0.9/-1/-0.9	PS (nm)	138.5	137.7	0.5772
		EE (%)	85.71	87.69	2.310
VR3	-0.8/-1/-0.8	PS (nm)	143.5	146.9	2.364
		EE (%)	86.2	88.69	2.888

Effect of independent variable on particle size:

The second-order polynomial equation (3) co-relating the response of particle size (Y_1) is given below;

$$Y_1=+191.60+15.73*A+13.99*B+2.59*C+4.92*AB+3.32*AC+2.70*BC-3.48*A^2-20.70*B^2-7.40*C^2 \quad (3)$$

The Model F-value of 9.84 implies the quadratic model is significant. There is only a 4.2% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, B2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Pred R-Squared" of 0.9672 is not as close to the "Adj R-Squared" of 0.8690 as one might normally expect; i.e. the difference is more than 0.2. "Adeq Precision" measures the signal to noise ratio (Table 4). The 3D surface plot of particle size show in figure 1.which gives the interaction of all variable factors with responses. According to the equation, when X_1 factor (D:L ratio) was increased, then PSA was increased, When X_2 factor (Surfactant concentration) increases, particle size increased, & When X_3 factor (Probe sonication time) increases, particle size decreased . Negative effect was seen on particle size when sonication time was increased. An increase in particle size from 188.90 nm (Y_1) to 201 nm (Y_2) was observed on increasing the drug to lipid ratio from 1:5 to 1:7 (Table 4).

Effect of independent factors on % entrapment efficiency

The second-order polynomial equation co-relating the response of particle size (Y_2) is given below;

$$Y_2=+84.00+2.91*A+0.025*B+0.037*C-0.90*AB-0.18*AC-0.60*BC+0.71*A^2+0.51*B^2+0.86*C^2 \quad (4)$$

The Model F-value of 21.99 implies the model is significant. There is only a 1.37% chance that an F-value this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are

significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The "Pred R-Squared" of 0.9851 is in reasonable agreement with the "Adj R-Squared" of 0.9403. The difference is less than 0.2."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 35.121 indicates an adequate signal (Table 5). This model can be used to navigate the design space. Accordding to the equation, when X_1 factor (D:L ratio) was increased, then EE was increased, When X_2 (surfatant concentration) factor increases, it shows very low effect on the EE and when X_3 factor (probe sonication time) was increased, then EE was increased Somewhat negative effect was seen when both factor was increased.Three-dimensional response surface plots for response variable is presented in Figure 2, which is very useful to study the interaction effects of the factors on the response.

The criteria for selection of suitable feasible region (from the intensive grid search) were primarily based upon the highest possible % entrapment efficiency (>80%) and particle size which are less than 150 nm (table 6). Composition of optimized batches and comparison of the observed responses with that of the predicted responses along with percentage error is listed in Table 6. On the basis of minimum percent error of particle size and entrapment efficiency the batch VR_1 selected as optimized batch for further studies.

Table 6 - statistical analysis for EE

Source	**Sum of squares**	**Df**	**Mean square**	**F value**	**p-value Prob> F**	**Quadratic model**
Model	77.69	9	8.63	21.99	0.0137	significant
A-D:L	67.86	1	67.86	172.89	0.0010	
B-SC	5.000E-003	1	5.000E-003	0.013	0.9173	
C-PC	0.011	1	0.011	0.029	0.8763	
AB	3.24	1	3.24	8.25	0.0639	
AC	0.12	1	0.12	0.31	0.6154	
BC	1.44	1	1.44	3.67	0.1513	
A^2	1.16	1	1.16	2.96	0.1840	
B^2	0.60	1	0.60	1.53	0.3042	
C^2	1.70	1	1.70	4.33	0.1288	
Residual	1.18	3	0.39			
Cor Total	78.87	12				

Particle size analysis

The z-average particle diameter of the prepared SLNs ranged from 145-201 nm (Table 3) The effect of lipid concentration on the particle size can be seen from particle size of sample R_3, R_7 and R_{13} (145.5 nm, 165.9 nm and 201 nm respectively) with low to high lipid concentration. When X_2 factor (surfactant concentration) increases, particle size increased as indicated by the coefficient *b* (+13.99). The effect of sonication time was less significant. Particle size distribution of optimized batch VR_1 was found to be 130.6 nm shown in Figure 3. The zeta potential of optimized batch was found to be -30.4 mV shown in figure 4.

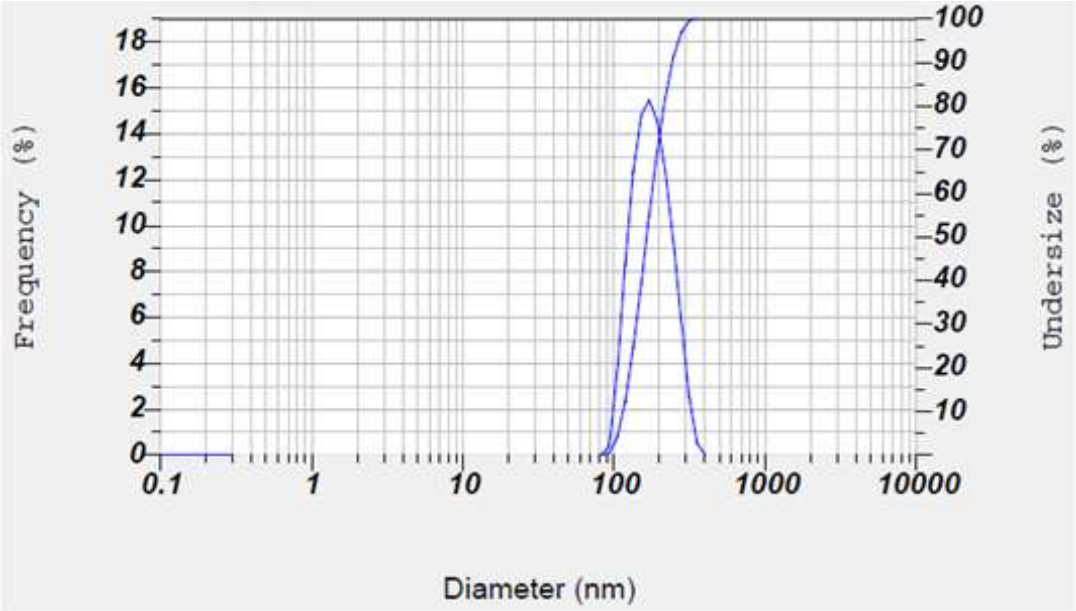

Figure 3 - Particle size distribution curve of SLN

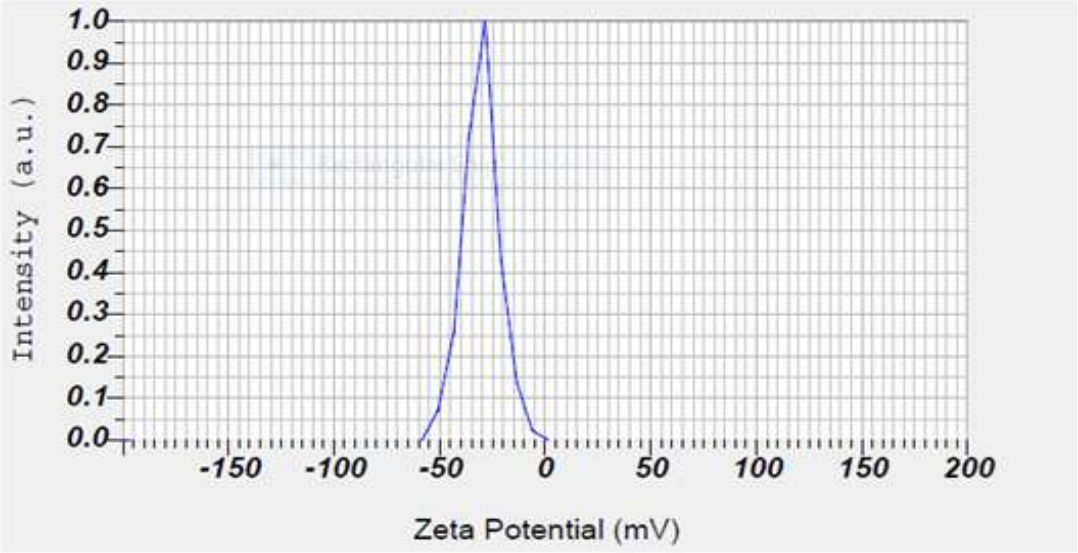

Figure 4 - Zeta potential of optimized SLN batch

Entrapment efficiency

The % entrapment efficiency of different SLN formulation is shows in table 3. The maximum amount of drug could be incorporated in the SLN dispersion. The formulation batch R_2, R_6, R_7 and R_9 shows maximum % of EE 86.1, 88.7, 87.5 and 88.8% respectively due to high concentration of lipid (1:7), while remaining batches shows less % of EE (80.2-84.3%). Both surfactant concentration and sonication time show less significant effect on entrapment efficiency. The final optimized batch shows the 85.3% EE (Table 5).

Differential Scanning Calorimetry

The DSC thermogram of sertraline hydrochloride, drug loaded SLN is shown in Fig. 5. The peak of sertraline hydrochloride is completely absent in lyophilized SLN batch. It has been reported that when the sertraline 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.

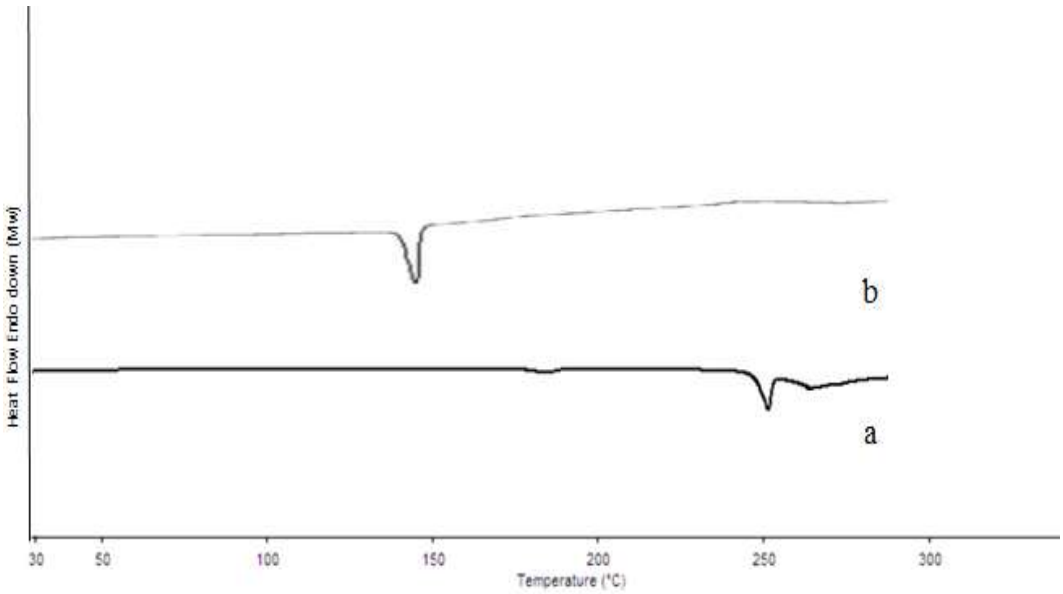

Figure 5. DSC spectra a) sertraline hydrochloride b) loaded SLN

X-ray diffraction

X-ray diffraction data listed in following Figure 5 was good in agreement with results established by DSC measurements. The diffraction pattern of the pure drug showed remarkable difference from those of the SLN, as they showed relative sharp peak than the SLN. It was clear that from Sertraline 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 sertraline hydrochloride and sertraline hydrochloride-SLN. It was confirmed that sertraline hydrochloride existed in amorphous state in the sertraline hydrochloride-SLN because of the disappeared sharp peak of sertraline hydrochloride in the diffraction pattern.

FTIR

From FTIR study, the characteristic peaks of drug such as of aromatic ring (1461-1531cm^{-1}), C-NH (2873-2983 cm^{-1}) and CH-tetra hydro naphthalene ring (2973.25 cm^{-1}) disappeared and were replaced by the peak of glycerol monostearate, tween 80. Remaining peaks are also either shifted or replaced in the IR spectrum of formulation shown in Figure 6. This established drug entrapment in lipid matrix.

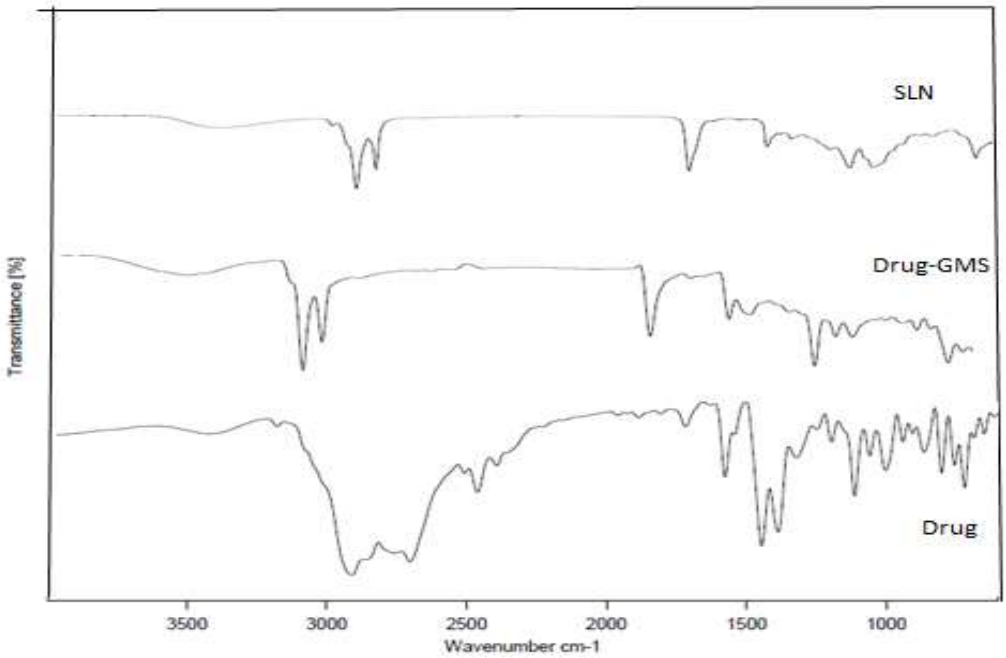

Figure 6. IR spectra sertraline hydrochloride, drug-GMS and loaded SLN

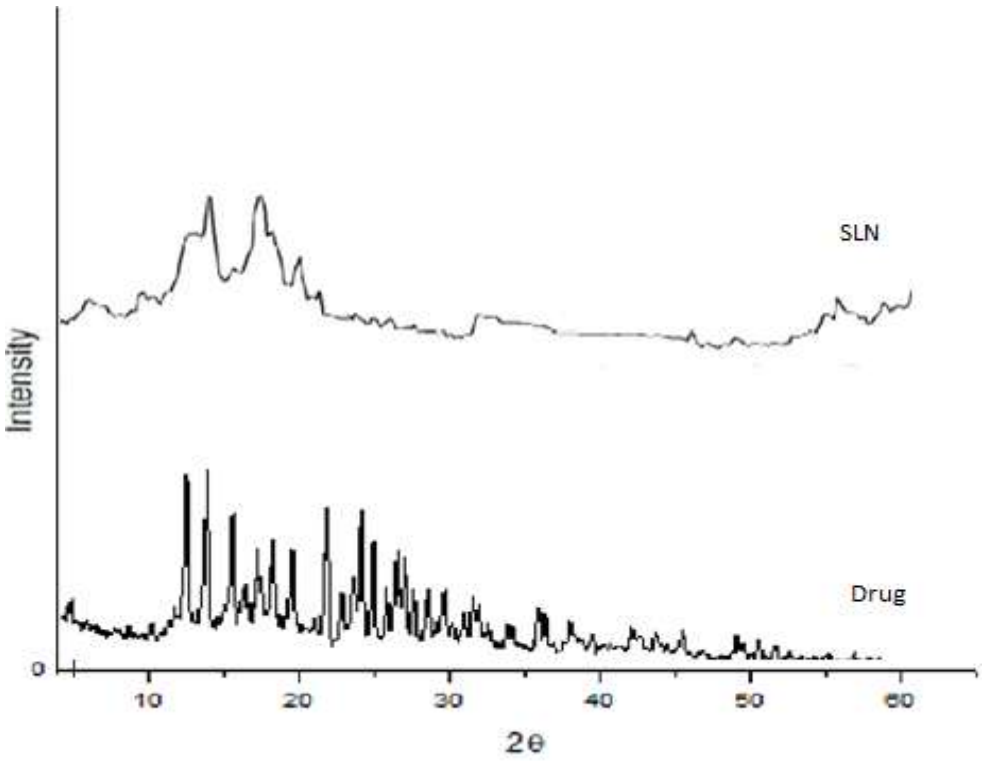

Figure 7. XRD crystallography of sertraline hydrochloride and SLN.

***In Vitro* release study**

The *in-vitro* release of sertraline hydrochloride from sertraline hydrochloride SLN dispersion and dispersion of pure Sertraline Hydrochloride was evaluated using dialysis membrane in phosphate buffer solution pH 7.4 pH and 0.1 N HCl. The total amount of cumulative release from drug suspension and SLN dispersion shown in Figure 7 and 8 for both medium. In present investigation sertraline hydrochloride loaded SLN dispersion of optimized formulation showed significantly low release of drug (89.52±0.94%) than

dispersion of pure drug (74.64±0.96%) in PB pH 7.4 and (87.15±0.81%), (65.12±0.99%) in 0.1N HCL respectively.

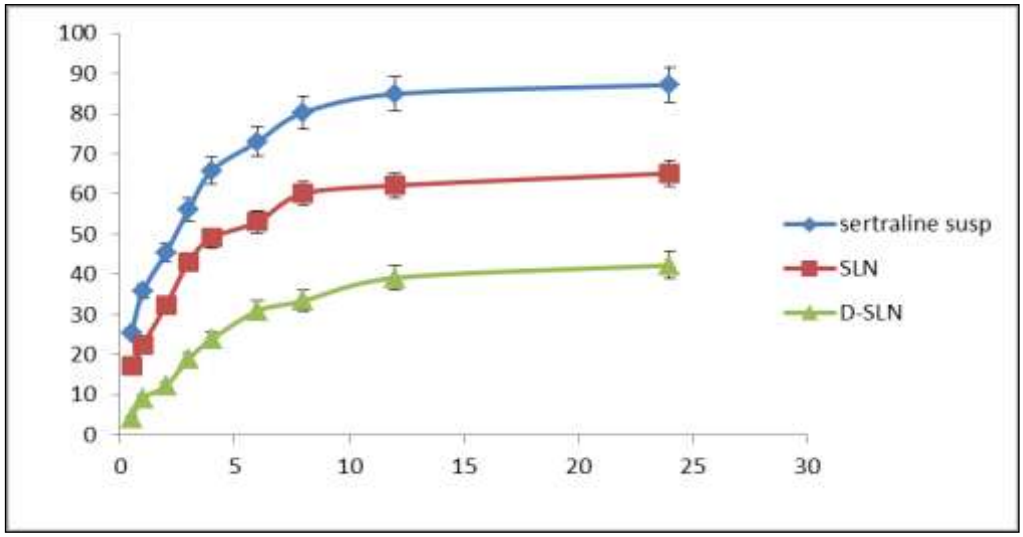

Figure 8. % cumulative release in PBS pH 7.4

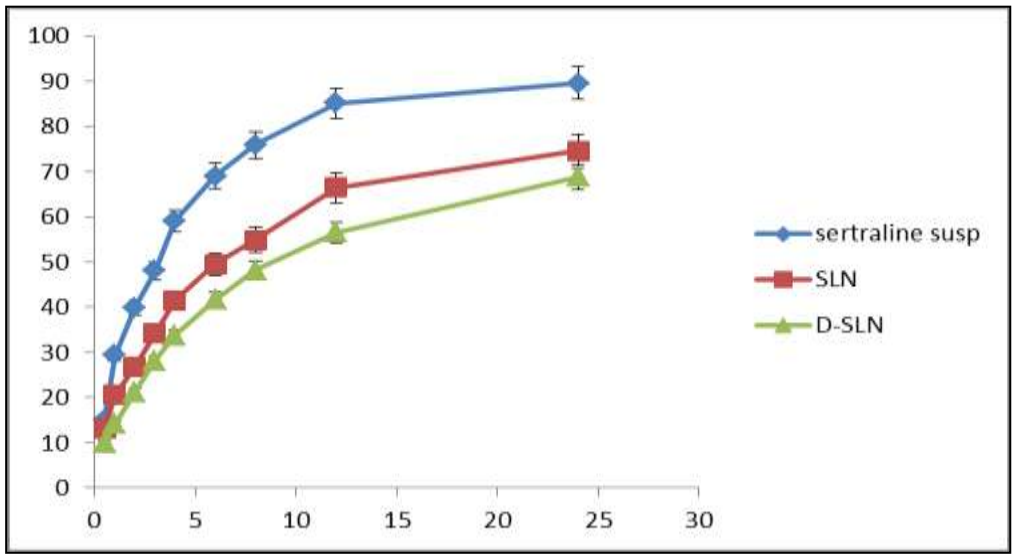

Figure 9. % cumulative release in PBS 0.1 N HCL

Conclusion

The pre-emulsion followed by probe sonication technique was used to prepare solid lipid nanoparticles of reproducible sizes in the range of 145 to 201 nm by addressing the effects of processing parameters. The application of box-behnken design proved to be a useful tool for optimization of Sertraline hydrochloride loaded SLN. Using this design one can select a suitable composition of formulation to obtain sertraline hydrochloride loaded SLN in the size range of 145 to 201 nm depending on the application of the system and successfully develop the solid lipid nanoparticles.

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