INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

FORMULATION AND EVALUATION OF EZETIMIBE LOADED SOLID LIPID NANOPARTICLES

Mayuri Desai, Divya Shah, Jayant Sarolia, Pranav Shah*, Jaimini Gandhi

Department of Pharmaceutics, Maliba Pharmacy College, Uka Tarsadia University, Gopal Vidyanagar Campus, Surat, Gujarat, India.

ARTICLE INFO

Article history
Received 10/08/2017
Available online
12/09/2017

Keywords
Solid Lipid Nanoparticles,
Ezetimibe,
High Speed Homogenization,
3^2 Full-Factorial Design,
Hypercholesterolemia,
In Vitro Drug Release,
Glyceryl Monostearate,
Poloxamer 188,
Hyperlipidemia,
Sustained Drug Release.

ABSTRACT

Ezetimibe is an anti hyperlipidemic drug which has poor aqueous solubility (0.00846 gm/L) and low bioavailability (35%). The SLNs were prepared using high speed homogenization technique. Glyceryl monostearate (GMS) and Poloxamer 188 were employed as lipid carrier and surfactant respectively. A two factor, three level (3^2) full factorial design was applied to study the effect of independent variables i.e. amount of GMS (X 1) and amount of Poloxamer 188 (X 2) on dependent variables i.e. Particle size (Y 1), % Entrapment efficiency (Y2) and % Cumulative drug release at 24hour (Y3). Particle size, Poly dispersity index (PDI), % Entrapment efficiency (%EE), zeta potential, drug content, in vitro drug release and particles morphology were evaluated for SLNs. Contour plots and response surface plots showed visual representation of relationship between the experimental responses (dependent variables) and the set of input (independent) variables. The optimized batch (B10) contained 500 mg of GMS and 750 mg of Poloxamer 188. Batch B10 exhibited particle size of 38.91±2.23 nm; Polydispersity index (PDI) of 0.221±0.091; zeta potential of -0.623 mV; % EE of 78.1±0.916% and % CDR at 24 hour of102.61±0.927%. The drug release experiments exhibited an initial rapid release experiments exhibited an initial rapid release followed by sustained release extended up to 24 hour. Differential scanning calorimetry (DSC) studies hoed that there was no chemical interaction between drug and lipid. The developed formulation may be adsorbed via the lymphatic route thereby avoiding hepatic first pass metabolism. This may lead to improvement in bioavailability, reduction dose and dose related side effect, etc.

Corresponding author
Pranav Shah
Department of Pharmaceutics,
Maliba Pharmacy College, Uka Tarsadia University,
Gopal Vidyanagar Campus, Surat, Gujarat, India-394350
+919979296582
pranav.shah@utu.ac.in

*Please cite this article in press as **Pranav Shah** et al. Formulation and Evaluation of Ezetimibe Loaded Solid Lipid Nanoparticles. Indo American Journal of Pharmaceutical Research.2017:7(08).*

INTRODUCTION

Ezetimibe is an antihyperlipidemic drug which is belongs to the cholesterol absorption inhibitor category. Ezetimibe indicated as adjunctive therapy to the diet for the reduction of elevated total cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (Apo B), and non-high density lipoprotein cholesterol (non HDL-C) in patients with primary (heterozygous familial and non-familial) hyperlipidemia. Ezetimibe is classified as a class II drug based on the biopharmaceutical classification system, because of its low water solubility and high permeability. They often show low oral bioavailability because of their slow and limited drug release in the alkaline environment of intestine, application of the strategies that improve the dissolution and/or apparent solubility of this poorly water-soluble drug of particular importance to increase its oral bioavailability.(1,2)

Hypercholesterolemia (Hyperlipidemia) is a condition characterized by very high levels of cholesterol in the blood. Cholesterol is a waxy, fat-like substance that is produced in the body and obtained from foods that come from animals. Too much cholesterol, however, increase a person's risk of developing heart disease. (3) People with hypercholesterolemia have a high risk of developing a form of heart disease called coronary artery disease. This condition occurs when excess cholesterol in the bloodstream is deposited in the walls of blood vessels, particularly in the arteries that supply blood to the heart (coronary arteries).
Solid lipid nanoparticles (SLNs) are attractive submicron colloidal carriers (10-1000 nm) for hydrophilic as well as lipophilic drugs. The drugs are entrapped in biocompatible lipid core and surfactant at the outer shell (Figure 1. Diagram of solid lipid nanoparticle). SLNs can be employed to improve the bioavailability and to obtain sustained at the outer shell. SLNs can be employed to improve the bioavailability and to obtain sustained release of the drug. They provide advantages like lack of acute and chronic toxicity of the carrier, good tolerability and biodegradability as well as scalability to large production. In addition, the protected against chemical/enzymatic degradation. Hence, SLNs are considered to be better alternative than polymeric nanoparticles, [55] liposomes, microemulsion, nanoemulsion and self-emulsifying drug delivery systems.

The present research work was aimed to develop SLNs of Ezetimibe to improve its oral bioavailability and give sustained release of drug. Glyceryl Monostearate (GMS) was selected as the solid lipid matrix for encapsulation of Ezetimibe in SLNs formulation prepared by High Speed homogenization technique. In this study the SLNs formulation was optimized by using 2-factor, 3- level 3^2 full factorial design. After selecting the critical variables (independent variable) that is amount of lipid (GMS) and amount of surfactant (Poloxamer 188) affecting particle size and entrapment efficiency, the response surface methodology of the 3^2 full factorial designs are one of the most efficient designs to study the quadratic response surfaces and the second surfaces and the second order polynominal model. The optimized formulation was evaluated in terms of parameters like particle size, PDI, zeta potential, % EE, drug content and drug release.

MATERIALS AND METHODS

Ezetimibe was obtained as a gift sample from IPCA Laboratories, Mumbai, India. GMS and Poloxamer 188 (Pluronic F 68) were purchased from Gattefosse, Mumbai, India and Balaji Drugs, Surat, India respectively. All the other reagents and solvents used were of analytical grade. Design Expert 10.0.2 software was used to optimize the formulation.

Preliminary trials

Preliminary trials were conducted to screen lipid and surfactant as well as to fix the stirring speed and stirring time. The effect on these factors on particle size and entrapment efficiency was used as criteria for screening. GMS and Poloxamer 188 were selected and stirring time were fixed 500 rpm and 15 min respectively.(13)

Preparation of Ezetimibe SLNs

The design matrix was built by the statistical software package, design-expert (version 10.0.2) and Table 1 shows the factors and their respective levels. Table 2 indicates the quantitative formula of the batches. SLNs were prepared by using high speed homogenization technique. GMS was the melted lipid phase. Poloxamer 188 was dissolved in hot 80 ml of distilled water. The lipid phase maintained at a temperature of 70˚C, was added drop wise to the hot aqueous hot aqueous surfactant solution under stirring at 500 rpm for 15 min. with a mechanical stirrer. This resulted into formation of an emulsion which was the subsequently homogenized in a high speed homogenizer (IKA T25

Digital S22 Homogenizer, India) for 15 minutes at 15000 rpm and maintained temperature at 80˚C. Later the mixture was cooled to room temperature yielding SLNs.

Optimization of factorial design

A 2-factor, 3-level design was used to statistically optimize the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation. Two factors, amount of GMS (A) (X1) and amount of Poloxamer 188 (B) (X 2) used were varied and their levels low medium and high medium were coded as -1, 0 and +1 respectively. The particle size (nm) (Y 1), %EE (Y2) and % cumulative drug release (Y 3) were taken as the response variables. In this design, experimental trials were performed at all 9 possible combinations. All other formulation variables and processing variables were kept invariant throughout the study.

$$Y = b0+b1A+b2B+b12AB+b11\ A2+b22B2 \qquad \text{Equation 2.1}$$

Where Y is the measured response associated with each factor level combination; B 0 is an intercept; b1 and b2 are regression coefficients computed from the observed experimental values of Y. A and B are the coded levels of independent variables. The terms AB represent the interaction and A^2 and B^2 represent quadratic terms.

Differential Scanning Calorimetry (DSC)

The thermogram of Ezetimibe, GMS and nanoparticles were obtained using Schimadzu DSC- 60 (Schimadzu Instruments, Japan) differential scanning calorimeter. 10 mg samples were placed in aluminium pans and heated from 25˚C to 300˚C at a scanning rate of 10˚C/min under nitrogen flow rate of 20 ml/min. An empty aluminium pan was used as reference. The instrument was calibrated with an Indium standard.(14)

Drug Content

1 ml SLNs dispersion was taken into 100 ml volumetric flask and volume was made up with methanol. It was sonicated for 5 min in bath sonicator. Solution was filtered through cellulose whatman filter paper (0.45µ) and filtrate was analysed at spectrophotometrically 233 nm. (15)

Particle size, Polydispersity index and Zeta potential

Freeze dried nanoparticles were dispersed in double distilled water. Particle size and Zeta potential was measured using a Malvern Zetasizer 3000 (Malvern Instruments, UK).

The measurement of particle size was based on photon correlation spectroscopy. Polydispersity index was studied to determine the narrowness of the particle size distribution. Zeta potential was studied to determine the surface charge of SLNs. The zeta potential was determined using electrophoretic light scattering (ELS) at 25˚C with electric field strength of 23 V/cm using Zetasizer nano ZS.

Percentage entrapment efficiency (%EE)

Entrapment efficiency is defined as the ration of amount of entrapped drug to the amount of total drug used for preparation of nanoparticles. 2 ml of the SLNs dispersion was placed in centrifuge (Remi Instrument Ltd., Mumbai, India) at 10000 rpm for 30 min at 4˚C. Supernatant was suitably diluted with methanol and analysed spectrophotometrically at 233 nm.

$$EE\ (\%) = \frac{\text{Wt.of drug used in formulation} - \text{Wt.of unbound drug in supernatent}}{\text{Wt.of drug used in formulation}} * 100$$

In vitro drug release study

The dialysis membrane (Himedia, molecular cut off 12,000 to 14,000D) technique was used to characterize the prepared nanosuspension using modified diffusion cell with 2.2 cm diameter and 55.95 cm^2 surface area. The cell consisted of two chambers, the donor and the receptor. To one end of the open cylinder which acted as donor compartment, dialysis membrane-150 was placed. SLNs were placed in the donor compartment. Receptor Compartment consisted of 50 ml of Acetate buffer pH 4.5(16) and was agitated continuously using magnetic stirrer. Temperature was maintained at 37±1°C throughout the study. Sample of 5 ml were withdrawn at predetermined time intervals (0.5,1, 2, 3, 4, 5, 12, 24 hours) and replaced by an equal volume of diffusion medium. Samples were analysed using UV-visible spectrophotometer at 232 nm.

Counter plots and surface response plots

Contour plots and surface response plots are diagrammatic representation of the values of the response. They are helpful in explaining the relationship between independent and dependent variables. Response surface methodology (RSM) shows how an experimental response and a set of input variables are related. RSM sets a mathematical trend in the experimental design for determining the optimum level of experimental factors required for a given response. The reduced models were used to plot two dimension contour plots and three dimension RSM at the values of A and B between -1 and +1 at predetermined value of particle size, %EE and % cumulative drug release.

RESULTS AND DISCUSSION

Formulation optimization of Ezetimibe loaded SLNs

The present work was focused on the formulation development of Ezetimibe loaded Solid lipid nanoparticles for oral delivery. Based on the preliminary batches, GMS and Poloxamer 188 were selected as lipid and surfactant respectively whereas stirring time were fixed at 500 rpm and 30 min respectively. Preliminary studies decided the levels at which factors will be studied. Ezetimibe loaded nanoparticles were prepared nanoparticles were prepared by high speed homogenization technique.

The effect of formulation variables namely amount of GMS (X1) and Poloxamer 188 (X2) was studied using 3^2 factorial design. The particle size, %EE and %cumulative drug release (%CDR) for the 9 batches (B1 to B9) showed a variation and were found in range of 51.45 to 294.2 nm, 77.89% to 86.05% and 90.16 to 101.86%. The data clearly indicated the dependence of response variables on the selected independent variables.

Batches	Practicle size (nm)	Zeta potential (Mv) %	Entrapment efficiency (Mean±SD)	(n=3) Drug content (%)	(Mean±SD), (n=3)
B1	113.7	0.044	0.195	84.30±0.381	98.21±1.402
B2	256.9	0.48	-8.15	85.24±0.323	99.78±1.642
B3	294.2	0.506	-21.9	86.05±0.455	100.13±1.486
B4	86.41	0.127	-0.327	80.09±0.510	100.58±0.877
B5	149.1	0.078	-15.5	81.67±0.434	98.51±0.969
B6	188.9	0.433	-11.2	85.22±0.425	99.05±1.502
B7	51.45	0.264	-0.941	77.89±0.420	101.11±0.663
B8	90.86	0.184	-0.213	80.97±0.440	99.81±2.591
B9	106.6	0.112	-1.6	83.18±0.421	98.96±1.805

Drug Content

The drug content of all batches of SLNs is tabulated in Table 3. The drug content was found in the range of 98.21% to 101.11% indicating that the Ezetimibe was uniformly distributed in nanoparticle dispersion and there was no loss of the material during the preparation.(17)

Data analysis of Y 1 (Particle size)

The particle size and PDI results of all the nine batches of Ezetimibe loaded SLNs are tabulated in Table 3. The particle sizes of batches B1-B9 were found in the range of 51.45 to 294.2 nm and the PDI was in the range of 0.044 to 0.506. That results indicated a profound effect of amount of GMS and Poloxamer 188 on the particle size. The response (Y 1) obtained to various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation.

$$Y1=+158.41+56.38A-69.31B-31.34AB-25.41A^2+10.81B^2 \qquad \text{Equation 2.2}$$

The above equation shows wide range of coefficient value. The model coefficients estimated by regression for particle size are shown in Table 4. The regression coefficients having P values 0.05 are highly significant. The terms A, B and AB were found to be significant. The terms A^2 & B^2 having P value 0.05 were insignificant in contributing to prediction of particle size. The reduced equation can now be written as

$$Y1=+158.41+56.38A-69.31B-31.34AB \qquad \text{Equation 2.3}$$

The two independent variables A (amount of GMS) & B (amount of Poloxamer 188) as well as the interaction term (AB) were found to be significant (P 0.05) in affecting Y 1 (particle size). The positive co-efficient value for independent variable A (+56.38) indicated positive effect on dependent variable Y1. As amount of lipid increases, the particle size increase. While negative coefficient for independent variable B (-69.31) which indicates as concentration of Surfactant increase particle siz decrease and interaction term AB (-31.34) indicated negative effect on dependent variable Y 1. The P value is 0.05 for all the response factors indicating that the models are significant.

Batches B1 to B3 contained increasing amounts of GMS whereas amount of surfactant was constant (250 mg). The particle sizes of the batches B1, B2 & B3 were 113.7 nm, 256.9 nm and 294.2 nm respectively. An increase in particle size depends on the amount of lipid increased. This could probably be explained by the increase in aggregation of particles as the amount of GMS is increased.

A similar trend of particle size was observed in batches B4 to B6 and batches B7 to B9.

The particle sizes of the batches B1, B4 and B7 were 113.7, 86.41 and 51.45 nm respectively.

A decrease in particle size was observed on increasing the amount of surfactant. A similar trend was observed in batches B2, B5, B8 and in B3, B6, and B7.

The PDI is an important parameter that governs the physical stability of SLNs dispersion and should be as low as possible for the long term stability of SLNs dispersion. The PDI defined as dispersion homogeneity, has the range of 0 to 1. Values close to 1 indicate heterogeneity and those less than 0.6 indicate homogeneity. The PDI value of all formulation was found in the range of 0.044 to 0.516 which was less than 0.6, indicating their homogeneity. Results indicated that all the formulation had a narrow particles size distribution.

Zeta potential provides information related to the storage stability of colloidal dispersions. In general, the greater the zeta potential value of a nanoparticulate system, the better the colloidal suspension stability due to repulsion effect between charged nanoparticles. The zeta potential values ranged between -24 to -40 mV. The surfactant concentration affected the charge on the particle. As the concentration of surfactant increased there was decrease in the zeta potential value. This is because the surfactant is non- ionic and increasing its concentration lowers the total charge on the particle.

Zeta potential values in the ± 15 mV to ± 50 mV are common for well stabilized nanoparticles. Hence, it was concluded that the nanoparticles would remain stable.(19)

Data analysis of Y 2 (%EE)

% EE of SLNs was determined using ultracentrifugation method. The % EE varied from 77.89±0.420 to 86.05±0.455. The results clearly indicated that Y2 is strongly affected by the amount of lipid and amount of surfactant selected for the study. The response Y 2 obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation.

$$Y2=+82.22+2.03A-2.26B+0.89AB+0.16A^2+0.61B^2 \quad \text{Equation 2.4}$$

The above equation shows wide range of coefficient values. The model coefficients estimated by multiple linear regression for %EE are shown in Table 4. The regression coefficients having P value 0.05 are highly significant. The terms AB, A^2 and B^2 having P value 0.05 were insignificant in contributing to prediction of %EE. The reduced equation can now be written as

$$Y2 =+82.22+2.03A-2.26B \quad \text{Equation 2.5}$$

The two independent variables A (amount of GMS) & B (amount of Poloxamer 188) were found to be significant (P 0.05) in affecting Y 2. The positive co efficient value for independent variable A (+2.03) indicated positive effect on dependent variable Y 2 .i.e. Amount of lipid increase will tend to increase %EE. The negative co efficient value for independent variable B (-2.26) indicated negative effect on dependent variable Y 2. Increase in surfactant concentration will tend to decrease the % Entrapment efficiency. Means as increase amount of surfactant, will tend to increase the solubility of drug in aqueous phase and drug is not available for encapsulate in lipid. In multiple regression analysis, the coefficient value of amount of lipid (A) of response Y 1 (Particle size) was +56.36 and the coefficient value of amount of lipid (A) of response Y2 (% EE) was +2.03 which indicate the effect of amount of lipid (A) on response Y1 (Particle size) was higher than compare to Y2 (%EE). Same as the coefficient value of amount of surfactant (B) of response Y 1 (Particle size) was -69.31 and the coefficient value of amount of surfactant (B) of response Y 2 (%EE) was -2.26 which indicate the effect of amount of surfactant (B) on response Y 1 (Particle size) was higher than compare to Y 2 (%EE).

Batches B1, B2 and B3 contained 500 mg, 850 mg and 1200 mg of GMS respectively whereas amount of surfactant was constant (250 mg). The %EE of the batches B1, B2 and B3 were 84.30%, 85.24% and 86.05% respectively. Increase in amount of lipid led to increase in %EE, which could be explained by more amount of the lipid available for Ezetimibe to dissolve. Similar trend was observed in batches B4 to B6 and B7 to B9. Our results were in agreement with findings of Yadav K et al.(20)

Data analysis of Y 3 (% Cumulative drug release)

In vitro drug release study of the Ezetimibe loaded SLNs was studied using modified dialysis method. The results (Figure 2) showed sustained release behaviour in acetate buffer pH 4.5. The response Y3 obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation.

$$Y3 =+93.99-3.31A+2.81B-0.57AB+0.17A^2 +1.42B^2 \quad \text{Equation 2.6}$$

The regression coefficients having P value 0.05 are highly significant. The terms AB, A^2 and B^2 having P value 0.05 were insignificant in contributing to prediction of % CDR at 24 hour. The reduced equation can now be written as:

$$Y3 =+93.99-3.31A+2.81B \quad \text{Equation 2.7}$$

The two independent variables A (amount of GMS) & B (amount of Poloxamer 188) were found to be significant (P 0.05) in affecting Y 3. The negative co efficient value for independent variable A (-3.31) indicated negative effect on dependent variable Y 3.i.e. Amount of lipid increase will tend decrease in %CDR at 24 hour. The positive co efficient value for independent variable B (+2.81) indicated positive effect on dependent variable Y 3. Increase in amount of surfactant will tend to increase the % CDR at 24 hour. Almost all batches showed the initial rapid drug release within two hours followed by 85% drug release up to 24 h and beyond. The observed value for % CDR at 24 hours for all 9 batches B1-B9 varied from 90.16 to 101.86%. The % CDR at 24 hour of the batches B1, B2 & B3 were 95.87%, 90.08% and 90.06% respectively. A decrease in % CDR at 24 h was observed on increasing the amount of lipid from 500 mg, 850 mg and 1200 mg. A similar trend of % CDR at 24 hour was observed in batches B4 to B6 and batches B7 to B9. The amount of lipid was in increasing order whereas the surfactant amount was constant at 500 mg and 750 mg respectively.(21)

Contour plots and response surface analysis

Two-dimensional contour plots and 3-D response surface plots for variables Y 1 (particle size) are shown in Figure 3and Figure 4 respectively. Similarly, two dimensional contour plots and 3-D response surface plots for variables Y2 (%EE) are shown in Figure 5 and Figure 6 respectively. Two dimensional contour plots and 3-D response surface plots for variables Y 3 (%CDR at 24 hour) 7 and 8 respectively. In Figure 3 and Figure 4 the contour plot and 3-D response surface plot were developed for the particle size. Figure 3 and Figure 4 reveal a decline in particle size (51.45 nm) with an increase in amount of surfactant. Increase in amount of lipid led to an increase in particle size (294.2 nm). The lowest particle size was reported with the highest amount of surfactant and the lowest amount of lipid.

In Figure 5 and Figure 6 the contour plot and 3-D response surface plot were developed for the % Entrapment efficiency. Figure 5 and Figure 6 reveal an increase in entrapment efficiency (86.05%) with an increase in amount of lipid. Increase in amount of surfactant led to decrease in %Entrapment efficiency (77.89%).The highest entrapment efficiency was obtained with the highest amount of lipid.

In Figure 7 and Figure 8, the contour plot and 3-D response surface plot were developed for the % cumulative drug release at 24 hours (%CDR). %CDR increased from 90.16 to 101.86% with the increasing amount of surfactant (101.86%) and % CDR decreased to 90.16% with increase in amount of lipid.

The optimum formulation was selected based on the criteria of attaining the constraints of variables response as shown in the Table 5. Upon 'trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with amount of lipid 500mg and amount of Poloxamer 188 750mg were found to fulfil the maximum requisite of an optimum formulation because of minimum particle size, with good % Entrapment efficiency and Optimum % cumulative drug release at 24 hours.

The zeta potential of optimized batch was found to be in the range (+30mv to -30mv) that indicate the formulation is stable. The zeta potential value of optimum formulation (batch B10) was −0.623 mV. This might be attributed to Poloxamer 188, a non-ionic surfactant which decreases the electrostatic repulsion between the particles and stearically stabilizes the nanoparticles by forming a coat around their surface. The negative charge of SLN may result from fatty acids released from the hydrolysis of GMS.[23]

DSC Studies

DSC thermogram of GMS showed the endothermic melting peak at 63.25^{o} C. DSC thermogram of physical mixture of drug and lipid (1:1) showed two distinct endothermic melting peaks for drug and lipid at 165.87°C and 58.48°C respectively. The intensity of melting peak of drug was reduced in physical mixture which may be due to dilution effect. These results agreed with that of A.R.Gardouh et al. who studied the DSC analysis of GMS and found that melting endotherm of it was at 60.39°C. He also studied the DSC analysis of physical mixture of GMS and various model drug and found that the DSC thermogram of physical mixture showed the characteristic peaks of both GMS at 56°C with melting and degradation peaks of model drug. This indicates that there was no significant change in content of Ezetimibe in presence of GMS.

DSC thermogram of GMS showed the endothermic melting peak at 63.25^{o} C. DSC thermogram of physical mixture of drug and lipid (1:1) showed two distinct endothermic melting peaks for drug and lipid at 165.87°C and 58.48°C respectively. The intensity of melting peak of drug was reduced in physical mixture which may be due to dilution effect. These results agreed with that of A.R.Gardouh et al. who studied the DSC analysis of GMS and found that melting endotherm of it was at 60.39°C. He also studied the DSC analysis of physical mixture of GMS and various model drug and found that the DSC thermogram of physical mixture showed the characteristic peaks of both GMS at 56°C with melting and degradation peaks of model drug. This indicates that there was no significant change in content of Ezetimibe in presence of GMS.

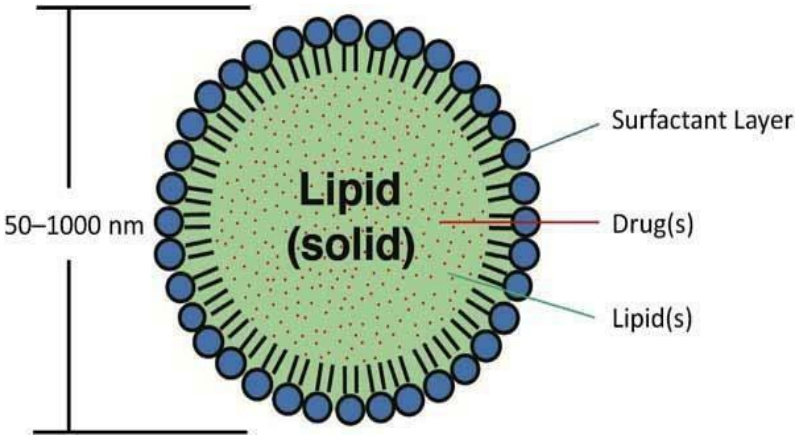

Figure 1. Diagram of solid lipid nanoparticle.

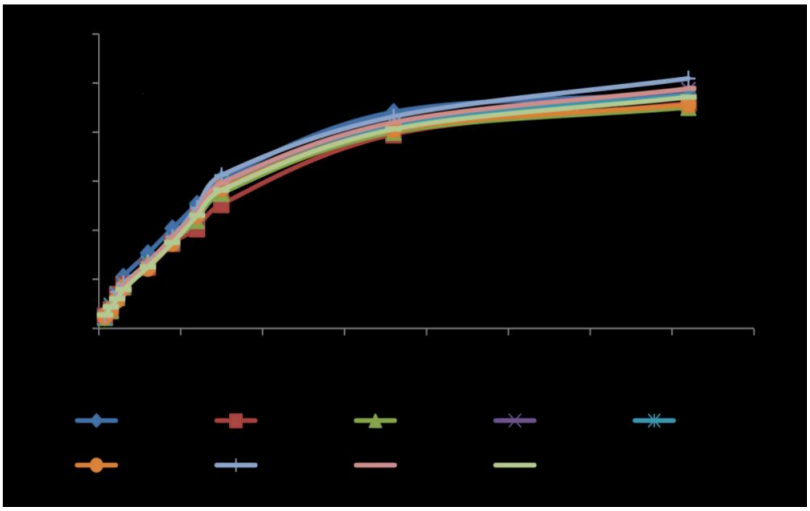

Figure 2. Drug release profile of batches B1-B9.

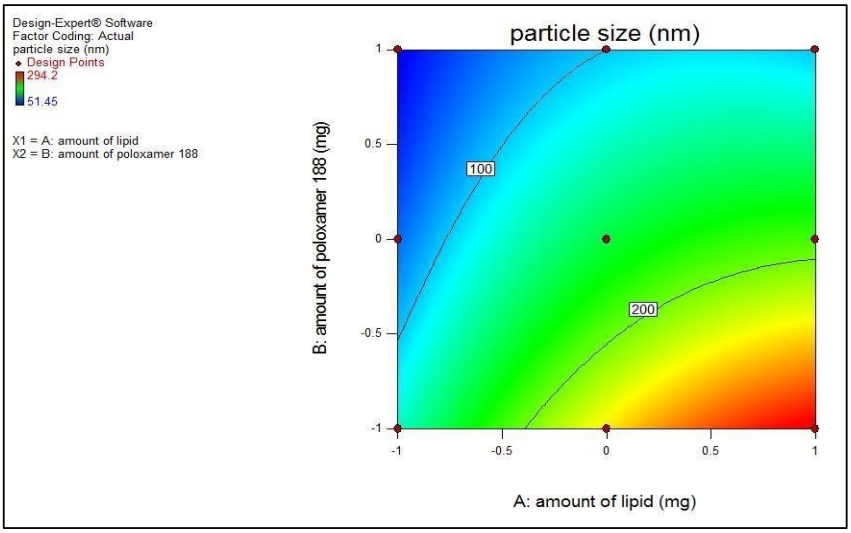

Figure 3. Contour plot for Y 1 (Particle size).

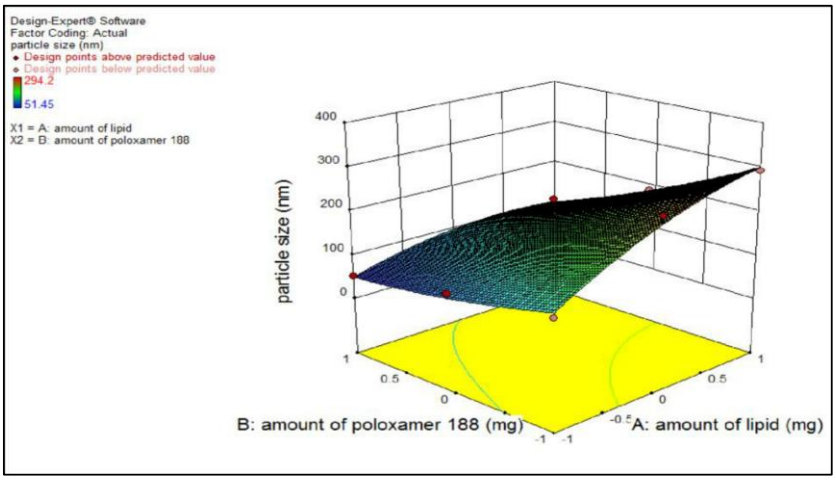

Figure 4. Response surface plot for Y 1 (Particle size).

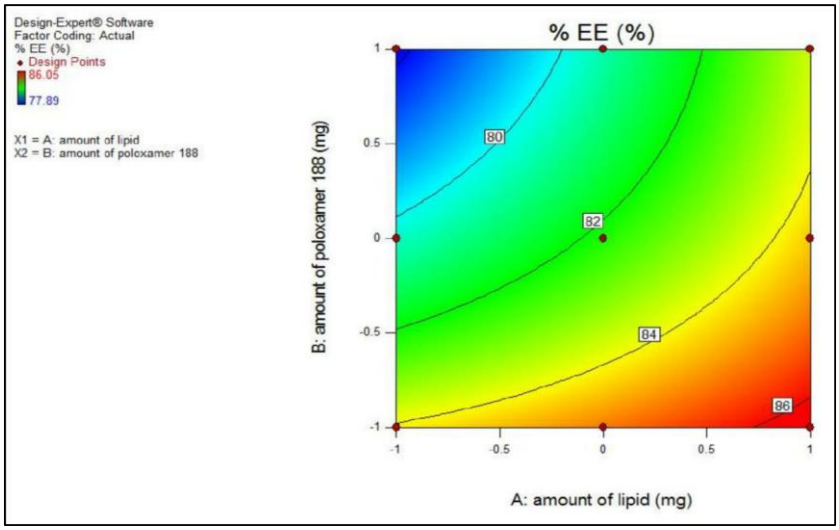

Figure 5. Contour plot for Y 2 (%EE).

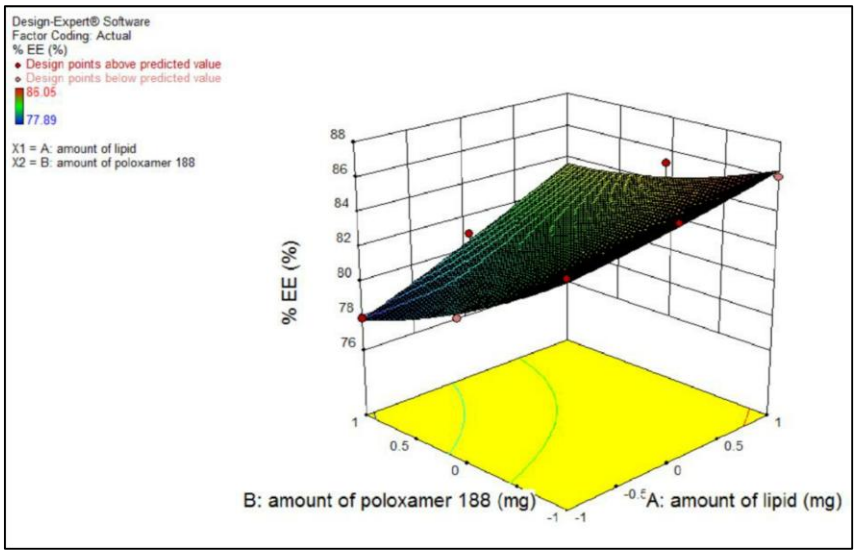

Figure 6. Response surface plot for Y 2 (%EE).

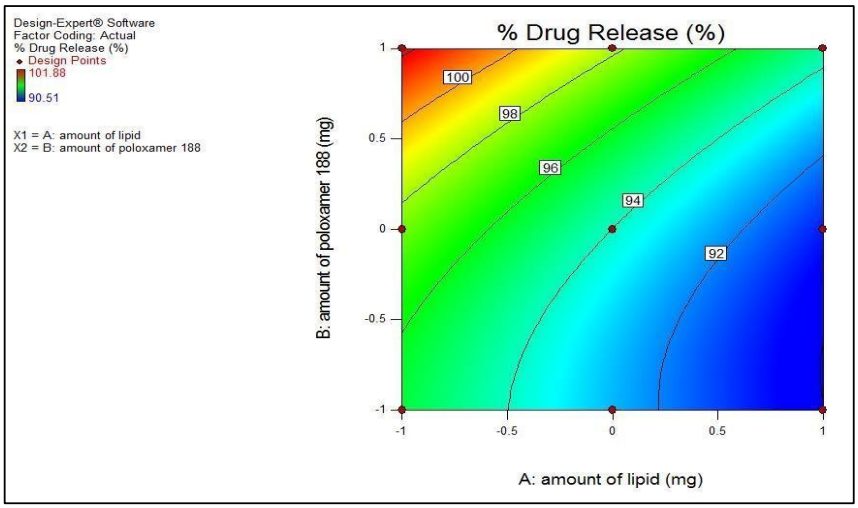

Figure 7. Contour plot for Y 3 (%CDR).

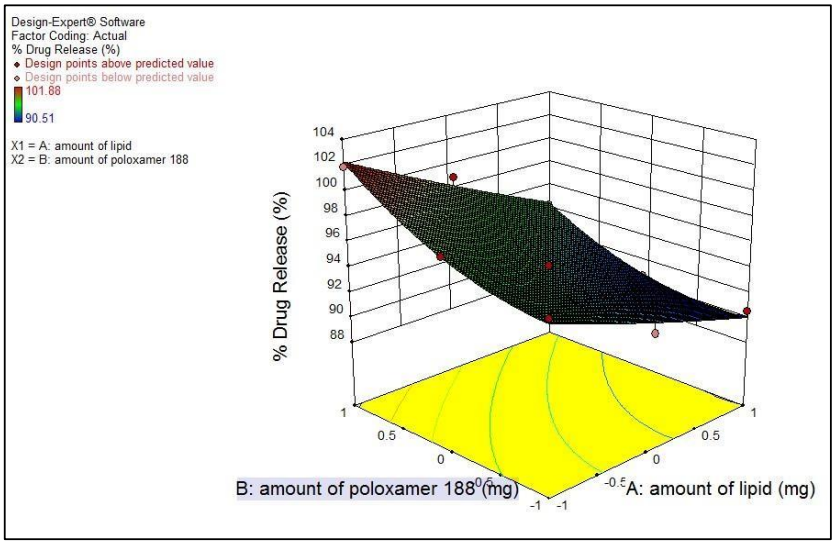

Figure 8. Response surface plot for Y 3 (%CDR).

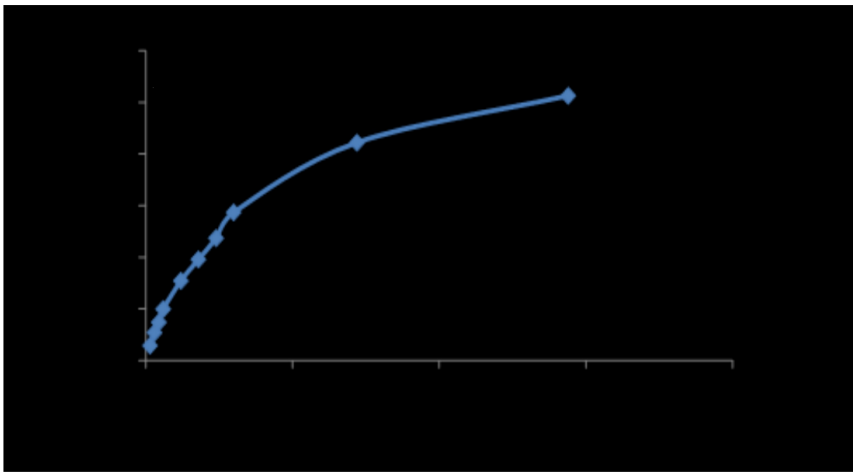

Figure 9. Drug release profile of optimized batch (B10).

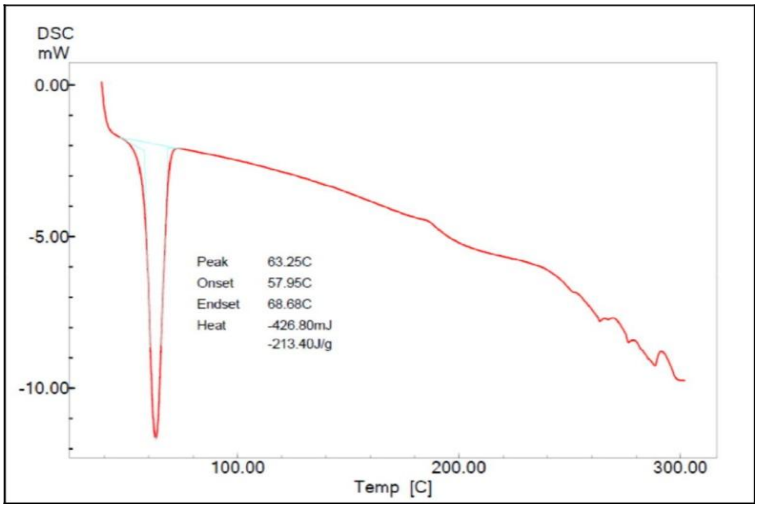

Figure 10. Overlay plot.

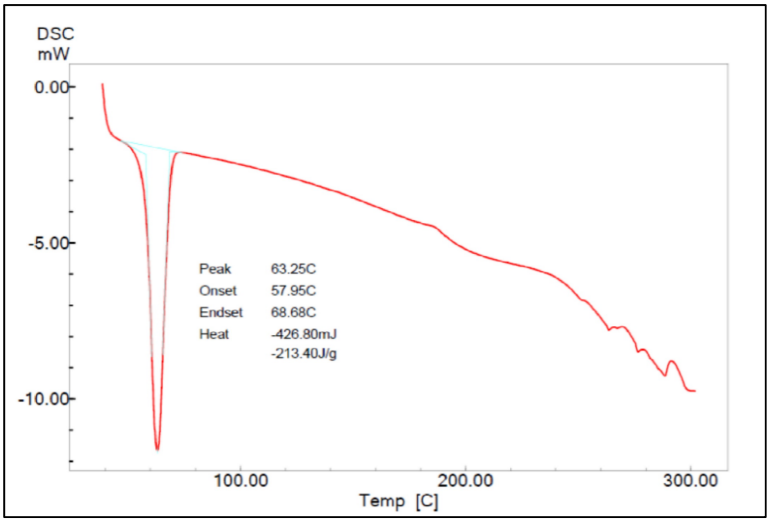

Figure 11. DSC thermogram of GMS.

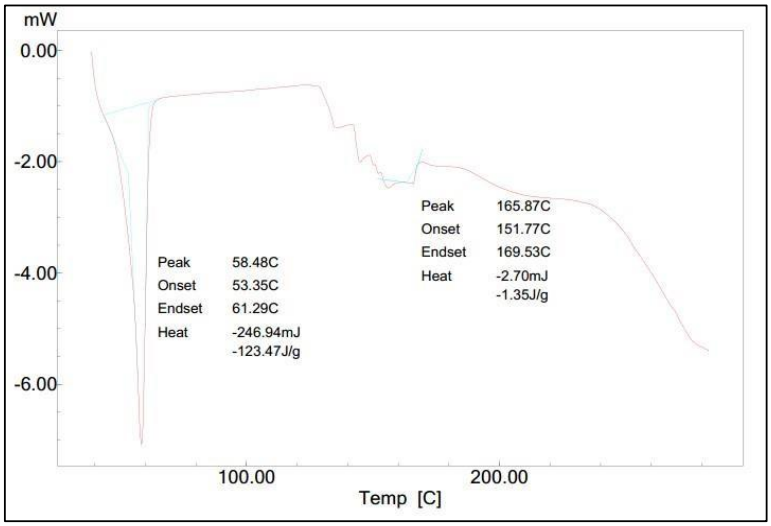

Figure 12. DSC thermogram of Physical mixture (Drug: Lipid) (1:1).

Table 1. Execution of 32 full factorial designs.

Batch	B1	B2	B3	B4	B5	B6	B7	B8	B9
Level of factor A	-1	0	+1	-1	0	+1	-1	0	+1
Level of factor B	-1	-1	-1	0	0	0	+1	+1	+1

Table 2. Formulation of SLNs Batches.

Ingredients	Batches								
	B1	B2	B3	B4	B5	B6	B7	B8	B9
Ezetimibe (mg)	10								
Glyceryl Monostearate (mg)	500	850	1200	500	850	1200	500	850	1200
Poloxamer 188 (mg)	250	250	250	500	500	500	750	750	750
distilled water (ml)	50								

Table 3. Evaluation of batch B1-B9.

Dependent variable	Particle size (Y_1)		% EE (Y_2)		% CDR (Y_3)	
	P value	Coefficient	P value	Coefficient	P value	Coefficient
Intercept	-	+158.41	-	+82.22	-	+93.99
A	0.0037	+56.36	0.0064	+2.03	0.0019	-3.31
B	0.0020	-69.31	0.0047	-2.26	0.0030	+2.81
AB	0.0331	-31.34	0.0931	+0.89	0.2406	-0.57
A^2	0.1205	-25.41	0.7739	+0.16	0.7712	+0.17
B^2	0.4273	+10.81	0.3199	+0.61	0.0817	+1.42

Table 4. Summary of results of multiple regression analysis for response Y1, Y2 and Y3.

Batches	Particle size (nm)	PDI	Zeta potential (mV)	% Entrapment efficiency (Mean±SD), (n=3)	Drug content (%) (Mean±SD), (n=3)
B1	113.7	0.044	-0.195	84.30±0.381	98.21±1.402
B2	256.9	0.48	-8.15	85.24±0.323	99.78±1.642
B3	294.2	0.506	-21.9	86.05±0.455	100.13±1.486
B4	86.41	0.127	-0.327	80.09±0.510	100.58±0.877
B5	149.1	0.078	-15.5	81.67±0.434	98.51±0.969
B6	188.9	0.433	-11.2	85.22±0.425	99.05±1.502
B7	51.45	0.264	-0.941	77.89±0.420	101.11±0.663
B8	90.86	0.184	-0.213	80.97±0.440	99.81±2.591
B9	106.6	0.112	-1.6	83.18±0.421	98.96±1.805

Table 5. In vitro drug release profile of batch (n=3) (B1-B9).

Time (min)	B1	B2	B3	B4	B5	B6	B7	B8	B9
0	0	0	0	0	0	0	0	0	0
15	4.35 ± 0.876	4.29 ± 0.506	4.59 ± 0.755	4.94 ± 0.695	4.29 ± 0.207	4.92 ± 0.326	5.24 ± 0.794	4.94 ± 0.358	5.48 ± 0.896
30	7.85 ± 1.699	7.63 ± 1.344	7.34 ± 0.979	9.07 ± 1.213	9.21 ± 0.860	7.67 ± 0.130	9.55 ± 0.848	8.39 ± 0.891	8.71 ± 0.359
45	13.80 ± 1.520	12.46 ± 1.312	12.67 ± 0.951	13.29 ± 1.067	13.75 ± 1.035	11.66 ± 0.876	14.83 ± 0.195	13.01 ± 0.632	11.90 ± 0.415
60	21.02 ± 2.530	16.66 ± 1.121	17.01 ± 1.355	17.19 ± 2.411	17.98 ± 1.925	16.51 ± 0.996	18.22 ± 0.314	17.49 ± 1.078	15.67 ± 1.042
120	30.64 ± 3.891	24.50 ± 0.800	26.69 ± 3.458	25.80 ± 2.199	26.39 ± 2.043	24.61 ± 1.350	26.88 ± 0.635	26.79 ± 1.538	25.09 ± 1.923
180	40.81 ± 4.922	35.71 ± 3.046	35.32 ± 3.908	35.25 ± 1.624	35.79 ± 2.471	34.80 ± 1.824	37.32 ± 2.086	37.14 ± 2.366	34.81 ± 2.652
240	50.77 ± 6.955	45.23 ± 3.947	44.06 ± 4.250	45.71 ± 1.694	46.96 ± 3.243	46.06 ± 1.401	49.06 ± 1.179	47.5 ± 2.975	45.89 ± 1.615
300	60.98 ± 8.141	55.83 ± 4.663	55.12 ± 3.975	57.56 ± 1.567	59.15 ± 5.752	57.06 ± 2.796	62.46 ± 0.492	59.35 ± 4.306	56.36 ± 2.415
720	88.25 ± 3.240	79.01 ± 1.649	80.04 ± 2.961	83.26 ± 1.079	83.14 ± 2.301	81.03 ± 1.792	86.31 ± 0.873	84.03 ± 2.059	81.19 ± 0.865
1440	95.87 ± 1.615	90.08 ± 1.513	90.16 ± 0.990	97.17 ± 0.315	95.15 ± 0.873	91.27 ± 1.031	101.86 ± 1.135	97.72 ± 1.007	94.25 ± 0.959

Table 6. Formula for optimized batch (B10).

Ingredients	Quantity
Ezetimibe	10 mg
Glyceryl monostearate	500 mg
Poloxamer 188	750 mg
Distilled water	50 ml

Table 7. Evaluation parameter for optimized batch (B10).

Evaluation Parameters	Results
Particle size	38.91±2.23 nm
% Entrapment efficiency	78.1±0.916 %(n=3)
Drug content	100.78±1.200 % (n=3)
PDI value	0.221±0.091
Zeta potential	-0.623 mV

Table 8. In vitro diffusion study for optimized batch (B10).

Time (min)	% Cumulative drug release
15	5.87±1.551
30	10.89±1.768
45	14.89±1.656
60	20.03±0.891
120	30.97±2.253
180	39.23±2.944
240	47.48±3.850
300	57.46±5.258
720	84.44±3.450
1440	102.61±0.927

Table 9. Results of optimized batch (B10 for response variables)

Response	Experimental Value	Predicted Value
Particle size (Y 1)	38.91 nm	48.49 nm
%EE (Y 2)	78.1±0.916%	77.82%
% CDR (Y 3)	102.61±0.927%	102.27%

CONCLUSION

The SLNs of Ezetimibe were successfully formulated using GMS as carrier lipid and Poloxamer 188 as a surfactant using High speed homogenization technique. The optimization of amount of lipid and amount of surfactant in the SLNs formulation was carried out using 3^2 full factorial designs. The developed SLNs exhibited controlled drug release up to a period of 24 h. The developed formulation was found to be stable with no significant change in particle size, and drug content. The SLNs due to their size and lipophilic characteristics may be useful in avoiding the first pass metabolism. The Ezetimibe SLNs may provide a better bioavailability, reduction in dose, dosing frequency, dose related side effects and better control of the disease.

REFFERENCE

1. Patel J, Sheehan V, Gurk-Turner C, editors. Ezetimibe (Zetia): a new type of lipid-lowering agent. Baylor University Medical Center Proceedings; 2003: Baylor University Medical Center.
2. Suchy D, Labuzek K, Stadnicki A, Okopień B. Ezetimibe–a new approach in hypercholesterolemia management. Pharmacological Reports. 2011;63(6):1335-48.
3. Tripathi KD. Essential of Medical Pharmacology. 6 ed: Jaypee Brothers Medical Publishers; 2008. pp 618-19.
4. Masters SB, Trevor AJ. Basic & clinical pharmacology. Katzung BG, editor. McGraw-Hill Medical; 2016.
5. Bouhairie VE, Goldberg AC. Familial Hypercholesterolemia. Cardiology clinics. 2015;33(2):169-79.
6. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Cardiovascular and renal disorders. Pharmacotherapy a pathophysiologic approach 7th edition. New York, McGraw- Hill. 2008.
7. Kreuter J. Encyclopedia of Nanoscience and Nanotechnology: American Scientific Publishers; 2004. pp 166.
8. Weiss J, MAIER C, Leuenberger B, Novotny M, Tedeschi C, Kessler A. Solid lipid nanoparticles (i). Google Patents; 2014.

9. Singh SK, Naini V. Encyclopedia of Pharmaceutical Technology, Swarrick J, editor. North Carolina, USA: Informa Healthcare 2007. pp 1993-2003.
10. Zur Mühlen A, Schwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery–drug release and release mechanism. European journal of pharmaceutics and biopharmaceutics. 1998;45(2):149-55.
11. Jenning V, Gysler A, Schäfer-Korting M, Gohla SH. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. European Journal of Pharmaceutics and Biopharmaceutics. 2000;49(3):211-8.
12. Müller R, Radtke M, Wissing S. Nanostructured lipid matrices for improved microencapsulation of drugs. International journal of pharmaceutics. 2002;242(1):121-8.
13. Dhoranwala K, Shah P, Shah S. Formulation Optimization of Rosuvastatin Calcium-Loaded Solid Lipid Nanoparticles by 32 Full-Factorial Design. NanoWorld J. 2015;1(4):110-9.
14. Yasir M, Sara U. Preparation and optimization of haloperidol loaded solid lipid nanoparticles by Box–Behnken design. Journal of Pharmacy Research. 2013;7(6):551-8.
15. Kondawar M, Kamble K, Maharshi K, Khandare M. UV Spectrophotometric estimation of Ezetimibe and Fenofibrate in Bulk drug and Dosage form using Simultaneous Equation Method. International Journal of ChemTech Research. 2011;2011:3.
16. The United States Pharmacopeia U. The National Formulary, NF 25 (USP30-NF25). The dissolution procedure: development and validation. Mack Printing Rockville; 2007.
17. Srivalli KMR, Mishra B. Preparation and pharmacodynamic assessment of ezetimibe nanocrystals: Effect of P-gp inhibitory stabilizer on particle size and oral absorption. Colloids and Surfaces B: Biointerfaces. 2015;135:756-64.
18. Thode K, Müller RH, Kresse M. Two time window and multiangle photon correlation spectroscopy size and zeta potential analysis Highly sensitive rapid assay for dispersion stability. Journal of pharmaceutical sciences. 2000;89(10):1317-24.
19. Das S, Ng WK, Tan RB. Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? European Journal of Pharmaceutical Sciences. 2012;47(1):139-51.
20. Yadav KS, Sawant KK. Formulation optimization of etoposide loaded PLGA nanoparticles by double factorial design and their evaluation. Current drug delivery. 2010;7(1):51-64.
21. Cohen S, Yoshioka T, Lucarelli M, Hwang LH, Langer R. Controlled delivery systems for proteins based on poly (lactic/glycolic acid) microspheres. Pharmaceutical research. 1991;8(6):713-20.
22. Gardouh AR, Gad S, Ghonaim HM, Ghorab MM. Design and characterization of glyceryl monostearate solid lipid nanoparticles prepared by high shear homogenization. British Journal of Pharmaceutical Research. 2013;3(3):326.
23. Martins S, Tho I, Souto E, Ferreira D, Brandl M. Multivariate design for the evaluation of lipid and surfactant composition effect for optimisation of lipid nanoparticles. European Journal of Pharmaceutical Sciences. 2012;45(5):613-23.