

Research Article

Design and Characterization of Dolutegravir Sodium Loaded Solid Lipid Nano Particles

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Abstract

Dolutegravir loaded SLN were prepared by using hot homogenization technique followed by ultra-sonication method. A 3² full factorial design has been employed to produce. The statistical optimization reduced the number of experiments that were carried out for obtaining formulations with desired properties. The derived polynomial equations, response and contour plots helped in predicting the values of selected independent variables for preparation of optimum SLN with desired properties. Solid lipid nanoparticles showed minimum particles size, optimum zeta potential and higher %EE. This experimental design revealed that Phospholipon 90H based formulation showed better result compared to Sphingomyelin, Soya Phosphatidyl Ethanolamine based formulations which could be due to maximum carbon chain length and high phase transition temperature. The drug excipients characterization parameters reveal that there is no drug-excipients interaction.

Keywords: Dolutegravir; SLN; Statistical optimization

Introduction

For decades, various pharmaceutical dosage forms such as tablets, capsules, liquids, suppositories, creams, ointments, injections, aerosols, etc. have been used as drug delivery systems for treatments of acute and chronic diseases. Colloidal drug delivery systems namely oil-in-water emulsions, liposomes, micelles, micro particles and nanoparticles opened a new frontier for targeting drugs and pharmaceuticals. Nanoparticles are solid colloidal particles in which the active principles are dissolved, entrapped, and or to which the active principle is adsorbed or attached [1-5].

Nanoparticles offer several advantages in drug delivery owing to their small particle size, large surface area and the capability of changing their surface properties. In general, nanoparticles can be used to target the delivery of drugs, to sustain its effect, to improve bioavailability, to solubi-

lize it for intravascular delivery and to improve its stability against enzymatic degradation.

Optimization using Response Surface Methodology (RSM) is a powerful, efficient and systematic tool that shortens the time required for the development of pharmaceutical dosage forms and improves research and development of work. RSM, where all the factors are studied in all possible combinations, are considered to be the most efficient in estimating the influence of individual variables and their interactions using minimum experiments. The application of RSM in pharmaceutical formulation development has played a key role in understanding the relationship between the independent variables and the responses to them. The independent variables are controllable, whereas responses are dependent.

Hence, in the present investigation Dolutegravir was selected as the drug of choice as it was having the required characteristics for the design of the SLNs. There were very few reports on the use of statistical approach in the development and optimization of SLNs hence the statistical approach like full factorial design was utilized to obtain optimized formulation with less number of experiments [6-10].

Materials

Dolutegravir was obtained gift sample from Glenmark Pharmaceuticals, Sphingomyelin, Soya Phosphatidyl Ethanolamine, Phospholipon 90H, Tween 20, Span 20, PEG 200, PEG 400, Propylene glycol were obtained gift sample from Merck, Capmul MCM, Captex 200, Abitec Group, Labrafac LipophileWL1349, Labrasol, Labrafil gift sample from Gattefosse, France.

Methods

Appearance and melting point

The organoleptic characteristics like colour, odour and texture were observed by sensory organs. The melting point was determined using capillary fusion method where a small amount of drug was filled in a capillary sealed from one side and kept inverted. The temperature at which drug started liquefy was recorded and compared with literature value and shown in Table 1.

Table 1: Formulation codes in 3² factorial designs

Formulation	Levels	
	Poloxamer 407 (mg)	Cremophor EL (mg)
F1 (-1,-1)	100	100
F2 (-1,0)	100	150
F3 (-1,+1)	100	200
F4 (0,-1)	150	100
F5 (0,0)	150	150
F6 (0,+1)	150	200
F7 (+1,-1)	200	100
F8 (+1,0)	200	150
F9 (+1,+1)	200	200

Determination of absorption maxima (λ_{max}) of Doulttegravir using UV spectrophotometer

1000 $\mu\text{g/ml}$ of stock solution of Doulttegravir was prepared by dissolving 50 mg of drug in 50 ml of Dimethyl formamide and diluting 1 ml of above solution to 10 ml with ethanol. The 10 $\mu\text{g/ml}$ of drug concentrations were then prepared and scanned using UV spectrophotometer to determine the λ_{max} of drug. Observed λ_{max} of Doulttegravir was at 259 nm.

Solubility study of the drug

The solubility study of Doulttegravir was carried out in various solvents. Accurately weighed 20 mg of drug was added to screw capped vials containing 10 ml of solvent. The vials were kept in a water bath shaker at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ and shaken for 24 h. The mixtures were then filtered through millipore filter membrane of pore size 0.45 μm , diluted and drug was analyzed using UV spectrometer.

Preparation of calibration curve of doultegravir using UV-spectrophotometric method

The calibration curve of Doulttegravir was prepared using phosphate buffer pH 6.8. Accurately weighed 50 mg of drug was dissolved in 50 ml ethanol to obtain concentration of 1 mg/ml. The 1 ml of prepared solution was further diluted 10 times to obtain stock solution of 100 $\mu\text{g/ml}$. From the secondary stock solution 2.5 ml, 2 ml, 1.5 ml, 1 ml and 0.5 ml were taken separately and diluted to 10 ml separately with pH 6.8 buffer to get 25 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$, 15 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ concentrations respectively.

Selection of oils, surfactants and cosurfactants

The lipids, surfactants and co-surfactants were selected based on solubility of the drug. The study was carried out by taking 2 ml of selected lipid (Corn oil, Olive oil, Soyabean Oil, Peanut oil, Sesame oil, Labrafac Lipophilew11349, Capmul MCM, Ethyl oleate, Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H)/surfactant

(Span 80, Tween 80, Tween 20, Span 20, Labrasol, Cremophor EL, Labrafil)/cosurfactant (Poloxamer 407, PEG 200, PEG 400, Propylene Glycol) in glass vial containing excess amount of drug. The mixtures were mixed manually for 30 minutes in order to facilitate proper mixing of drug with the vehicles. The vials were sonicated for 2 h and kept in water bath for 48 h for equilibration. The vials were centrifuged at 3000 rpm for 20 minutes, followed by filtration. The filtrate was suitably diluted with ethanol and drug dissolved in various vehicles was analysed by UV spectrophotometer at 259 nm [11-13].

Preparation of solid lipid nanoparticles

Doulttegravir loaded SLN were prepared with 3 different Phospholipids namely Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H by hot homogenization technique followed by ultrasonication method. These 3 were selected since they had better solubility when compared to other lipids.

Doulttegravir, Phospholipids and Cremophor EL were heated above the melting temperature of lipid around 56°C - 68°C and mixed rapidly with glass rod in hot molten condition. Poloxamer 407 dissolved in water heated to equal temperature and was added to the molten lipid phase and homogenization was carried out. Hot homogenization was carried out for 3 minutes at 5000 rpm in order to get coarse emulsion. Finally the obtained pre-emulsion was subjected to ultrasonication.

Optimization of ultrasonication time

Ultrasonication was carried out with the help of ultrasonic homogenizer. Ultrasonication was carried out for different time intervals 5 minutes, 10 minutes, 15 minutes, 20 minutes and 25 minutes. With the help of Zetasizer NanoZS average particle size of obtained SLN was measured [14].

Factorial design

A 3² randomized full factorial design was used in this study and 2 factors were evaluated, each at 3 levels, experimental trials were performed at all 9 possible combinations. Amount of Poloxamer 407 (X_1) and Cremophor EL (X_2) were selected as 2 independent variables which were varied at 3 levels, low level (-1), medium level (0), high level (+1).

Amount of drug simvastatin (50 mg), Phospholipids (400 mg) concentrations and dispersion medium water 20 ml were kept constant. Particle size (Y_1), zeta potential (Y_2) and entrapment efficiency (Y_3) were selected as dependent variables. Values of variables and formulation codes are shown in the Tables 1 and 2. Design-Expert® 12 software trial version was used for the generation and evaluation of statistical experimental design.

Table 2: Variables in 3² factorial designs

Independent variable	Low (mg)	Medium (mg)	High (mg)
X_1 : Poloxamer 407	100	150	200

X ₂ : Cremophor EL	100	150	200
Dependent variable	-	-	-
Y ₁ Particle size (nm)	-	-	-
Y ₂ Zeta Potential (Mv)	-	-	-
Y ₃ Entrapment Efficiency (%)	-	-	-

Optimization of surfactant and co-surfactant

The optimization of surfactant and co-surfactant was done by using 3² factorial designs. Poloxamer 407 was chosen as independent factor (X₁) and taken at 3 different concentrations 100 mg, 150 mg and 200 mg. Cremophor EL was selected as independent factor (X₂) and taken at 3 different concentrations 100 mg, 150 mg and 200 mg. amount of drug 50 mg, Phospholipids 400 mg and the final volume of SLN 20 ml was kept constant. The particle size (Y₁) zeta potential (Y₂) and % entrapment efficiency (Y₃) were selected as 3 dependent factors as shown in Table 3.

Table 3: Compositions of Dolutagravir loaded SLN

Compositions		
F6 (0,+1)	150	200
F7 (+1,-1)	200	100
F8 (+1,0)	200	150
F9 (+1,+1)	200	200

Determination of particle size distribution, Polydispersity Index (PDI), and zeta potential of SLN

The particle size distribution, polydispersity index, and zeta potential of Dolutagravir loaded SLN were measured using a Malvern Zetasizer. About 100 µL of the prepared SLN dispersion was diluted to 5 mL with double distilled water and analyzed with Zetasizer. Photon correlation spectroscopy is the most widely used technique for measurement of particle size and zeta potential. The principle of dynamic light scattering at a scattering angle of 90° is used to measure particle size.

Determination of percentage entrapment efficiency (%EE)

The percentage of drug entrapped in the lipid is determined by measuring the concentration of the drug in the aqueous phase by ultrafiltration method using centrisart devices. Centrisart consist of filter membrane (Molecular weight cut off 20,000 daltons) at the base of sample recovery chamber. About 1 ml of undiluted sample is placed in the outer chamber on the top of the sample holder. The unit is centrifuged at 3,500 rpm for 15 minutes-20 minutes. The solid lipid nanoparticles along with the encapsulated drug remain in the outer chamber and the aqueous phase is moved into the sample recovery chamber through membrane.

$$(\%) EE = [(C_d - C) / C_d] * 100$$

Where, C_d is the concentration of total drug and C is the concentration of untrapped drug.

Statistical analysis of the data and optimization

Response surface modelling and evaluation of the quality of fit of the model for the current study were performed employing Design Expert® 12 software trial version. Polynomial models including linear, interaction and quadratic terms were generated for all the response variables using Multiple Linear Regression Analysis (MLRA).

A second order polynomial equation that describes the effect of independent factors on the response is expressed in the following forms:

$$\text{Linear model: } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2$$

$$\text{2FI (interaction) model: } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2$$

$$\text{Quadratic model: } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

Where, Y is the dependent variable, β_0 is the arithmetic mean response of the 9 runs, and β_1 and β_2 are the estimated coefficients for the factors X₁ and X₂ respectively. The main effect (X₁ and X₂) represents the average result of changing one factor at a time from its low to high value. The interaction term (X₁X₂) shows how the response changes when 2 factors were changed simultaneously. The polynomial terms (X₁X₁, X₂X₂) are included to investigate nonlinearity. The equation enables the study of the effects of each factor and their interaction over the considered responses.

The polynomial equation was used to draw conclusions after considering the magnitude of coefficients and the mathematical sign it carries, positive or negative. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect. The best fitting mathematical model was selected based on the comparisons of statistical parameters which include the Coefficient of Variation (CV), the coefficient of determination (R²), adjusted coefficient of determination (adjusted R²) and the Predicted Residual Sum of Square (PRESS), provided by Design Expert software. Among them, PRESS indicates how well the model fits the data and for the chosen model it should be small relative to the other models under consideration. Level of significance was considered at p<0.05. Mathematical relationships in the form of polynomial equations are generated using Multiple Linear Regression Analysis (MLRA) and used to find out the relative influence of each factor on the response.

Analysis Of Variance (ANOVA) for the responses was performed to identify significant effect of factors on responses and the model parameters were obtained. The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. These plots are very useful to study of the effects of 2 factors on the response at one time and predict the responses of dependent variables at the intermediate levels of independent variables. Subsequently, a numerical optimization technique by the desirability and graphical optimization technique by the overlay plot approach were used to generate the new formulation with the desired responses. An optimized formulation was developed by setting constraints (goals) on the dependent and independent variables.

Compatibility study

Interaction between the drug, oil, surfactant and cosurfactant were studied by FT-IR. The blank KBr pellets were prepared, onto which oil, surfactant and cosurfactant were dropped individually and it was pressed with another blank KBr pellet using hydraulic press. The pure drug was mixed with KBr in the ratio of 1:3 and punched in a hydraulic press at 5 ton-6 ton load. The prepared pellets were scanned from 4000 cm^{-1} to 400 cm^{-1} using FT-IR spectrophotometer (FT-IR 8400 S, Shimadzu). The FT-IR spectra of the physical mixture were compared with the spectra of pure drug, phospholipid, surfactant and cosurfactant.

Differential Scanning Calorimetry (DSC): DSC was performed using a Differential scanning calorimeter (DSC 220C, Seiko, Japan) at a heating rate of 100°C/min from 30°C to 300°C in nitrogen atmosphere.

In vitro drug release studies from SLN

The *in vitro* release studies of Dolutagravir loaded solid lipid nanoparticles were carried out by using modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm with molecular weight cut off 10,000 daltons was used. Membrane was soaked in double distilled water for 12 hours before mounting in Franz diffusion cell. Doluta-

gravir loaded 2 mL of SLN dispersion equivalent to 4 mg was applied to the donor compartment. And the receptor compartment was filled with 12 ml of dialysis medium of 6.8 phosphate buffer. Samples (100 μL) were withdrawn from receiver compartment through side tube at regular time intervals and the same was replaced with fresh dialysis medium maintained at same temperature. In the similar way pure drug equivalent to 4 mg was also added to the 2 ml of distilled water and release studies were performed for comparison.

Release kinetics

The analysis of drug release kinetics and mechanism from a pharmaceutical dosage form is an important but complicated process.

The order of drug release from SLNs was described by using zero order or first order kinetics. The mechanism of drug release was studied by using Higuchi diffusion model and Hixon-Crowell erosion model. Korsmeyer-Peppas support the drug release mechanism for further judgment. The respective equations for these models are shown in below Table 4. According to Korsmeyer-Peppas equation, the release exponent 'n' value is used to characterize different release mechanisms for a dosage form with cylindrical shape and summarized in Table 5.

Table 4: Appearance and melting point

Drug	Parameter	Reported	Observed
Dolutegravir	Appearance	White to light yellowish white	White to light yellowish white
	Odour	None	None
	Melting point	190-193°C	192°C

Table 5: Solubility of drug in various solvents

Solvent	Dolutegravir
	Solubility ($\mu\text{g/ml}$)
Distilled water	2.69 \pm 2.3
0.1 N HCl	32.5 \pm 0.33
Phosphate buffer pH 6.8	550.87 \pm 10.4
Ethanol	1879.38 \pm 32.6
Methanol	1250.4 \pm 9.4

Results and Discussion

Appearance and melting point

From the above data it was observed that the appeared and observed results were same.

Solubility of drug in various solvents

It was found from the above solubility studies that Dolutegravir has highest solubility in Ethanol.

Determination of λ_{max}

From the above, it was found that λ_{max} of Dolutegravir at 259 nm (Figure 1). Standard plot that was plotted helps in understanding the wavelength at which drug absorbs. The Table 6 details the relationship between concentration and absorbance at 259 nm. Figure 2 explains the standard plot of Dolutegravir in pH 6.8 buffer and Table 7 shows the

Statistical parameters for standard curves of Dolutegravir in pH 6.8 buffer.

Table 6: Calibration curve of Dolutegravir in pH6.8 buffer

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
0	0
5	0.196 \pm 0.21
10	0.401 \pm 0.96
15	0.603 \pm 0.43
20	0.801 \pm 0.55
25	0.999 \pm 0.37
Data is expressed as mean \pm SD (n=3)	

Table 7: Statistical parameters for standard curves of Dolutegravir in pH 6.8 buffer

S. No	Parameter	pH 6.8 buffer
1.	λ_{max} (nm)	259
2.	Linearity range ($\mu\text{g/ml}$)	0-25
3.	Regression equation	y=0.0406x-0.0023
4.	Intercept	0.0023
5.	Slope	0.0406
6.	Correlation coefficient (R^2)	0.999

Optimization of ultrasonication time on particle size

Average particle size of Sphingomyelin, Soya Phosphati-

dyl Ethanolamine and Phospholipon 90H based SLN were prepared with ultrasonication time of 5 minutes, 10 minutes, 15 minutes, 20 minutes and 25 minutes and are shown in Table 8 and Figure 3. The particle size was affected by

the sonication time. As the sonication time increased the particle size was decreased up to 20 minutes and further decrease in particle size was not observed at 25 minutes.

Table 8: Effect of sonication time on particle size (nm) (n=3)

Sonication time (min)	Sphingomyelin SLN		Soya Phosphatidyl Ethanolamine SLN		Phospholipon 90H SLN	Effect
	Size (nm)	PDI	Size (nm)	PDI	Size (nm)	PDI
5	178 ± 0.6	0.381 ± 0.1	193 ± 2.1	0.212 ± 0.2	159 ± 0.3	0.341 ± 0.7
10	133 ± 1.6	0.316 ± 0.1	127 ± 0.9	0.198 ± 0.5	131 ± 1.9	0.276 ± 0.8
15	102 ± 1.3	0.296 ± 0.3	107 ± 1.5	0.229 ± 0.8	103 ± 0.7	0.182 ± 0.2
20	99 ± 0.9	0.424 ± 0.2	98 ± 0.3	0.139 ± 0.4	95 ± 0.2	0.441 ± 0.4
25	98 ± 1.0	0.464 ± 0.4	98 ± 1.3	0.275 ± 0.6	96 ± 0.3	0.217 ± 0.1

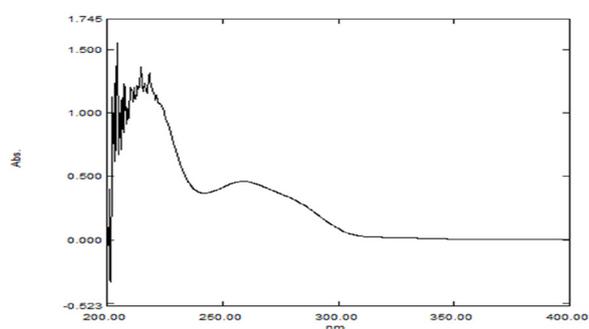


Figure 1: UV Spectra of Doultegravir at 259 nm

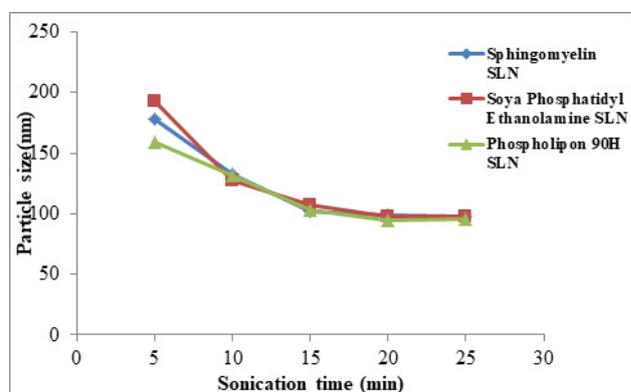


Figure 3: Relationship between Sonication time (min) Vs Particle Size (nm)

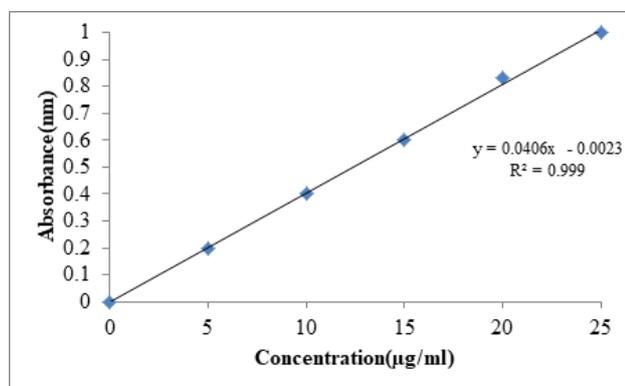


Figure 2: Standard plot of Doultegravir in pH 6.8 buffer

Table 9: Characterization of SLN prepared with Sphingomyelin

Batch	% Drug content	Size (nm)	Zeta potential (mV)	PDI	%EE
SNF1	99.1 ± 0.1	157.8 ± 0.7	-25.1 ± 0.1	0.227 ± 0.6	58.3 ± 1.2
SNF2	96.0 ± 0.1	110.8 ± 1.8	-22.9 ± 0.6	0.175 ± 0.2	48.6 ± 1.7
SNF3	101.7 ± 0.2	125.6 ± 1.2	-45.1 ± 4.1	0.254 ± 0.1	76.1 ± 1.1
SNF4	99.2 ± 0.1	210.6 ± 1.7	-38.3 ± 0.3	0.287 ± 0.2	53.2 ± 0.4
SNF5	100.0 ± 0.2	101.7 ± 1.3	-31.3 ± 0.1	0.201 ± 0.1	74.4 ± 0.9
SNF6	98.2 ± 0.0	102.7 ± 1.0	-35.3 ± 1.2	0.445 ± 0.5	79.1 ± 1.2
SNF7	100.2 ± 0.1	104.2 ± 1.0	-37.7 ± 0.4	0.222 ± 0.0	65.6 ± 0.8
SNF8	99.1 ± 0.0	108.8 ± 0.2	-36.7 ± 1.8	0.210 ± 0.2	54.2 ± 0.3
SNF9	101.0 ± 0.0	98.9 ± 0.6	-31.6 ± 1.1	0.212 ± 0.1	95.1 ± 0.1

Table 10: Characterization of SLN prepared with Soya Phosphatidyl Ethanolamine

Batch	% Drug content	Size (nm)	Zeta potential (mV)	PDI	% EE
SPF1	100.7 ± 0.1	213.5 ± 0.1	-18.3 ± 1.1	0.180 ± 0.2	53.3 ± 1.0
SPF2	98.2 ± 0.1	133.9 ± 0.1	-23.9 ± 0.7	0.327 ± 0.2	51.2 ± 1.0
SPF3	99.0 ± 0.1	105.3 ± 0.2	-34.2 ± 3.2	0.337 ± 0.1	71.4 ± 1.2
SPF4	98.2 ± 0.2	165.1 ± 0.2	-26.5 ± 1.2	0.262 ± 0.0	55.2 ± 1.0

Percent drug content (%)

The percentage drug content of the SLN formulations varied between 94.70% ± 0.2% to 101.7% ± 0.2% respectively as shown in Tables 9-11. Hence all the formulations were within the standard limits (90% to 110%). This indicated uniform distribution of drug in each SLN formulation and there was no wastage of the drug during preparation of SLN.

SPF5	101.0 ± 0.2	113.3 ± 0.1	-19.3 ± 0.1	0.242 ± 0.1	69.3 ± 1.0
SPF6	98.2 ± 0.2	103.9 ± 0.1	-23.1 ± 0.2	0.310 ± 0.4	75.6 ± 1.0
SPF7	94.7 ± 0.2	139.9 ± 0.2	-48.7 ± 0.4	0.377 ± 0.2	61.2 ± 0.1
SPF8	100.3 ± 0.2	116.6 ± 0.2	-45.5 ± 1.2	0.269 ± 0.6	52.3 ± 0.2
SPF9	100.7 ± 0.1	93.1 ± 0.2	-30.6 ± 1.2	0.180 ± 0.2	95.4 ± 1.1

Table 11: Characterization of SLN prepared with Phospholipon 90H

Batch	% Drug content	Size (nm)	Zeta potential (mV)	PDI	% EE
SHF1	98.0 ± 0.1	194.7 ± 0.2	-23.3 ± 0.3	0.321 ± 0.2	62.5 ± 1.2
SHF2	99.0 ± 0.2	208.7 ± 0.2	-20.4 ± 0.5	0.361 ± 0.2	54.9 ± 0.1
SHF3	100.7 ± 0.2	214.6 ± 0.2	-44.9 ± 3.2	0.274 ± 0.1	80.1 ± 1.2
SHF4	99.2 ± 0.1	137.4 ± 0.1	-24.4 ± 0.4	0.282 ± 0.2	56.3 ± 0.1
SHF5	100.1 ± 0.9	214.5 ± 0.1	-39.4 ± 1.1	0.307 ± 0.1	78.3 ± 1.1
SHF6	100.1 ± 0.1	171.5 ± 0.1	-35.4 ± 0.2	0.324 ± 0.1	82.5 ± 2.3
SHF7	97.1 ± 0.2	165.3 ± 0.1	-36.8 ± 1.9	0.198 ± 0.2	67.2 ± 0.2
SHF8	98.1 ± 0.1	131.8 ± 0.2	-38.6 ± 2.4	0.301 ± 0.3	58.3 ± 0.1
SHF9	99.0 ± 0.2	96.4 ± 0.2	-30.5 ± 1.1	0.277 ± 0.2	97.4 ± 2.0

Particle size distribution (nm), zeta potential (mV) and Polydispersity Index (PDI)

The mean particle size was in the range of 98.9 nm ± 0.6 nm to 210.6 nm ± 1.7 nm, 93.1 nm ± 0.2 nm to 213.5 nm ± 0.1 nm and 96.4 nm ± 0.2 nm to 214.5 nm ± 0.1 nm for Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H based formulations respectively as shown in Figure 4 after 20 minutes time of ultrasonication time. The Polydispersity Index (PDI) was in the range of 0.175 ± 0.2 to 0.445 ± 0.5. Zeta potential values of SLN ranged from -22.9 mV ± 0.6 mV to -45.1 mV ± 4.1 mV, -18.3 mV ± 1.1 mV to -48.7 mV ± 0.4 mV, -20.4 mV ± 0.5 mV to -44.9 mV ± 3.2 mV for Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H based formulations respectively. The zeta potential value was found to be > ± 30 mV for almost all the formulations prepared. For any liquid dosage form surface charge is essential for its stability. Zeta potential value > ± 30 mV is essential for effective stability and to inhibit aggregation of particles. As the poloxamer 407 concentration increased particle size was decreased. In 3 formulations optimum size was obtained at 200 mg of poloxamer 407 concentrations. The low polydispersity index in all the formulations indicated the homogeneity of the particle size. The formulations showed negative zeta potential since solid lipid nanoparticles have negative charge on their surface.

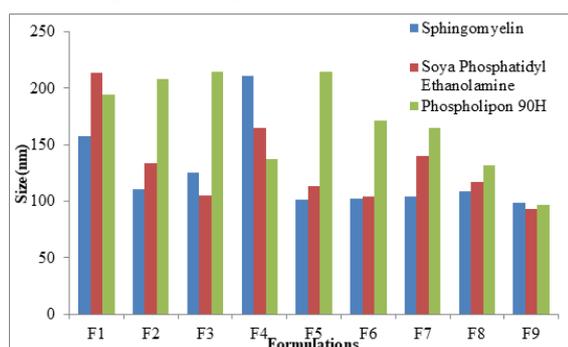


Figure 4: Comparison of particle size of SLNs prepared by different Phospholipids

Determination of percent Entrapment Efficiency (%EE)

The percent entrapment efficiency of SLN was determined after separating entrapped and entrapped drug by ultra-filtration. The percent entrapment efficiency varied from 48.6% to 97.4% for all the formulations as shown in Figure 5. Highest entrapment efficiency of 97.4% was observed for Phospholipon 90H based SLN. The lowest entrapment efficiency was observed when the independent variables poloxamer 407 (X_1) and Cremophor EL (X_2) were at 100 mg and 150 mg concentrations for all the SLN formulations prepared with 3 different phospholipids.

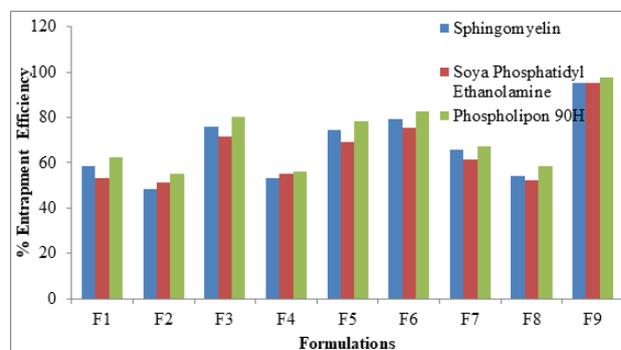


Figure 5: Comparative % EE of SLN formulations

The highest entrapment efficiency was observed when the independent variables poloxamer 407 (X_1) and Cremophor EL (X_2) were at higher level (200 mg) concentrations for all the SLN formulations prepared with 3 different phospholipids. There is no difference in entrapment efficiency, among 3 phospholipids. This could be due to maximum carbon chain length in the 3 phospholipids.

Statistical analysis of the data and optimization

In the current study 3 Phospholipids namely Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H were selected for the preparation of SLN using fixed concentration of 400 mg. From the preliminary study, it was found that poloxamer 407 and Cremophor EL has strong effect on physico-chemical properties of solid lipid

nanoparticles such as particle size, entrapment efficiency, stability and *in vitro* behavior. Amount of poloxamer 407 (X_1) and amount of Cremophor EL (X_2) were selected as independent factors for preparation of solid lipid nanopar-

ticles respectively. Particle size, zeta potential and % entrapment efficiency were considered as the 3 dependent factors the observed responses were shown in Tables 12-14 respectively.

Table 12: Observed responses of Doultgravir loaded Sphingomyelin based SLN

Batch	Poloxamer 407	Cremophor EL	Size (nm)	Zeta potential (mV)	% EE
SNF1	-1	-1	157.8 ± 0.7	-25.1 ± 0.1	58.3 ± 1.2
SNF2	-1	0	110.8 ± 1.8	-22.9 ± 0.6	48.6 ± 1.7
SNF3	-1	1	125.6 ± 1.2	-45.1 ± 4.1	76.1 ± 1.1
SNF4	0	-1	210.6 ± 1.7	-38.3 ± 0.3	53.2 ± 0.4
SNF5	0	0	101.7 ± 1.3	-31.3 ± 0.1	74.4 ± 0.9
SNF6	0	1	102.7 ± 1.0	-35.3 ± 1.2	79.1 ± 1.2
SNF7	1	-1	104.2 ± 1.0	-37.7 ± 0.4	65.6 ± 0.8
SNF8	1	0	108.8 ± 0.2	-36.7 ± 1.8	54.2 ± 0.3
SNF9	1	1	98.9 ± 0.6	-31.6 ± 1.1	95.1 ± 0.1

Table 13: Observed responses of Doultgravir loaded Soya Phosphatidyl Ethanolamine based SLN

Batch	Poloxamer 407	Cremophor EL	Size (nm)	Zeta potential (mV)	%EE
SPF1	-1	-1	213.5 ± 0.1	-18.3 ± 1.1	53.3 ± 1.0
SPF2	-1	0	133.9 ± 0.1	-23.9 ± 0.7	51.2 ± 1.0
SPF3	-1	1	105.3 ± 0.2	-34.2 ± 3.2	71.4 ± 1.2
SPF4	0	-1	165.1 ± 0.2	-26.5 ± 1.2	55.2 ± 1.0
SPF5	0	0	113.3 ± 0.1	-19.3 ± 0.1	69.3 ± 1.0
SPF6	0	1	103.9 ± 0.1	-23.1 ± 0.2	75.6 ± 1.0
SPF7	1	-1	139.9 ± 0.2	-48.7 ± 0.4	61.2 ± 0.1
SPF8	1	0	116.6 ± 0.2	-45.5 ± 1.2	52.3 ± 0.2
SPF9	1	1	93.1 ± 0.2	-30.6 ± 1.2	95.4 ± 1.1

Table 14: Observed responses of Doultgravir loaded Soya Phospholipon 90H based SLN

Batch	Poloxamer 407	Cremophor EL	Size (nm)	Zeta potential (mV)	% EE
SHF1	-1	-1	194.7 ± 0.2	-23.3 ± 0.3	62.5 ± 1.2
SHF2	-1	0	208.7 ± 0.2	-20.4 ± 0.5	54.9 ± 0.1
SHF3	-1	1	214.6 ± 0.2	-44.9 ± 3.2	80.1 ± 1.2
SHF4	0	-1	137.4 ± 0.1	-24.4 ± 0.4	56.3 ± 0.1
SHF5	0	0	214.5 ± 0.1	-39.4 ± 1.1	78.3 ± 1.1
SHF6	0	1	171.5 ± 0.1	-35.4 ± 0.2	82.5 ± 2.3
SHF7	1	-1	165.3 ± 0.1	-36.8 ± 1.9	67.2 ± 0.2
SHF8	1	0	131.8 ± 0.2	-38.6 ± 2.4	58.3 ± 0.1
SHF9	1	1	96.4 ± 0.2	-30.5 ± 1.1	97.4 ± 2.0

All the responses observed for 9 runs (3 different bases of solid lipid nanoparticles) were simultaneously fitted to linear, interaction and quadratic models using Design Expert software trial version 12. The comparative values of R^2 , adjusted R^2 , predicted R^2 , PRESS, s.d, % CV at significant p values ($P < 0.05$). A suitable polynomial model for describing the data was selected based on coefficient of determination (R^2) and PRESS values. The 3 responses Y_1 , Y_2 , Y_3 for the 3 different bases of SLN were independent, for SLN prepared with Sphingomyelin, Soya Phosphatidyl Ethanolamine, the responses Y_1 , Y_2 and Y_3 followed linear model whereas Phospholipon 90H based SLN response Y_1 and Y_3 followed linear model and Y_2 followed quadratic model. The fitted polynomial equations relating the responses are given in Tables 15-17. Model parame-

ters obtained from Analysis Of Variance (ANOVA) for the responses Y_1 - Y_3 of 3 different bases of SLN are shown in Tables 18-20. These parameters were used to construct the models that describe the effect of the independent variables on the responses (Y_1 - Y_3) (Figures 6-11).

From the ANOVA data, the F value of 3 different bases of SLN i.e. Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H based formulations were as followed for Sphingomyelin all 3 responses of F value were 2.76, 3.55 and 5.43, for Soya Phosphatidyl Ethanolamine the F values were 41.70, 24.70 and 6.53 and for Phospholipon 90H the F values were 2.76, 3.55 and 5.43 respectively. The P value less than 0.05 for the response factors indicated that the models are significant.

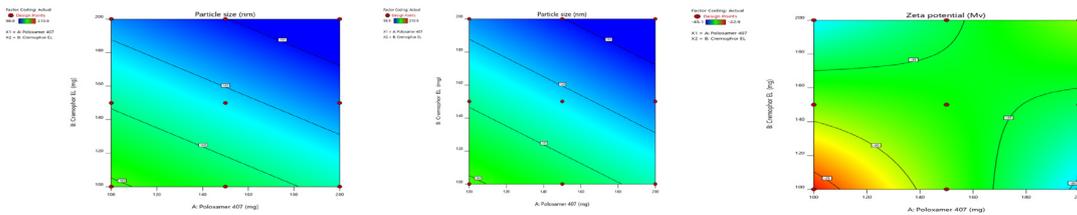


Figure 6: Contour plots for the effects of poloxamer 407 (X_1) and Cremophor EL (X_2) on particle size (Y_1), zeta potential (Y_2) and % entrapment efficiency (Y_3) in Spingomyelin based SLN

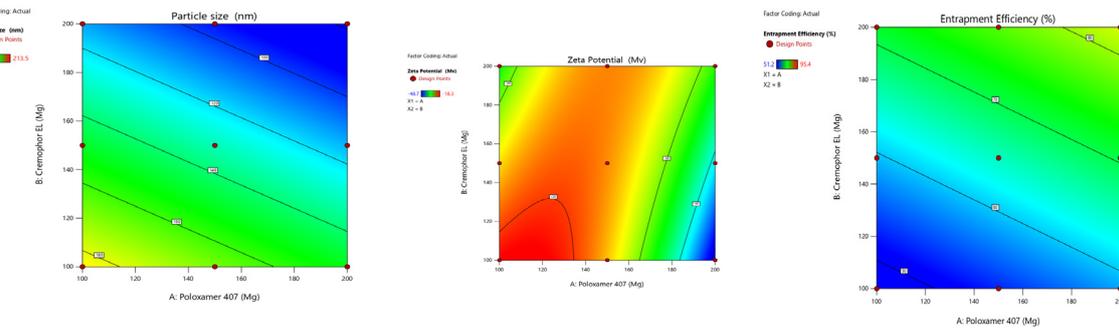


Figure 7: Contour plots for the effects of poloxamer 407 (X_1) and Cremophor EL (X_2) on particle size (Y_1), zeta potential (Y_2) and % entrapment efficiency (Y_3) in Soya Phosphatidyl Ethanolamine based SLN

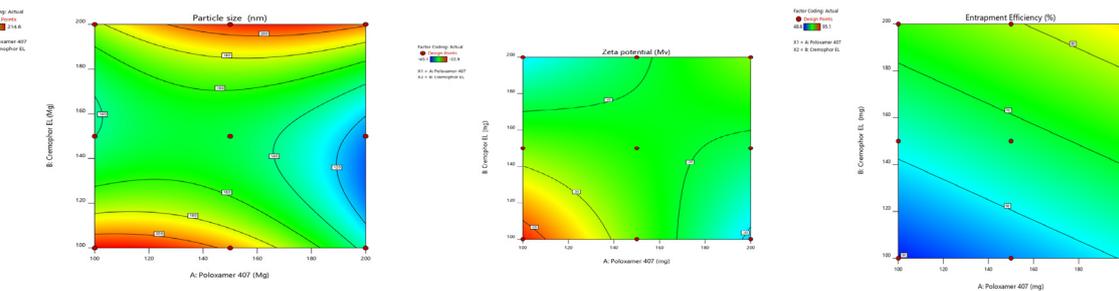


Figure 8: Contour plots for the effects of poloxamer 407 (X_1) and Cremophor EL (X_2) on particle size (Y_1), zeta potential (Y_2) and % entrapment efficiency (Y_3) in Phospholipon 90H based SLN

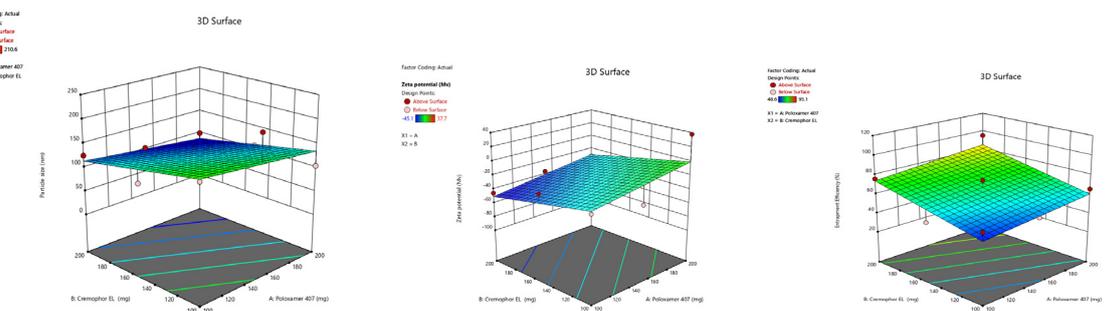


Figure 9: Response surface plots for the effects of poloxamer 407 (X_1) and Cremophor EL (X_2) on particle size (Y_1), zeta potential (Y_2) and % entrapment efficiency (Y_3) in Spingomyelin based SLN

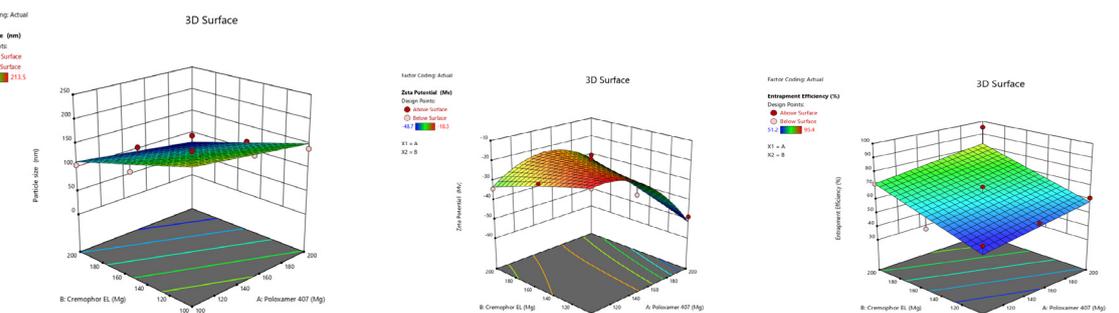


Figure 10: Response surface plots for the effects of poloxamer 407 (X_1) and Cremophor EL (X_2) on particle size (Y_1), zeta potential (Y_2) and % entrapment efficiency (Y_3) in Soya Phosphatidyl Ethanolamine based SLN

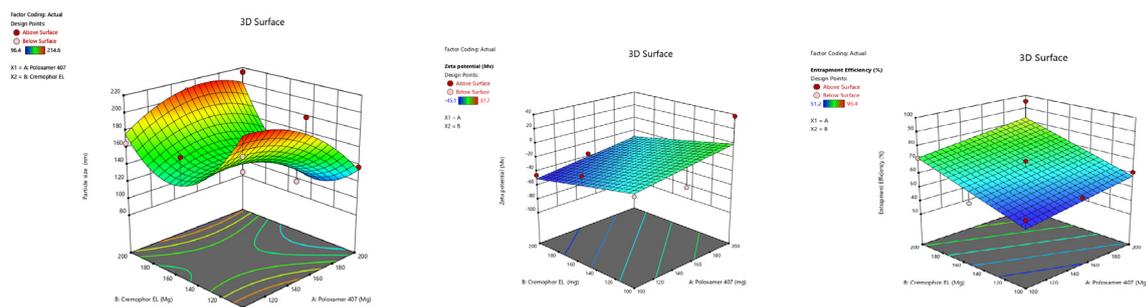


Figure 11: Response surface plots for the effects of poloxamer 407 (X_1) and Cremophor EL (X_2) on particle size (Y_1), zeta potential (Y_2) and % entrapment efficiency (Y_3) in Phospholipon 90H based SLN

Table 15: Regression analysis summary of Sphingomyelin based SLN for responses Y_1 , Y_2 and Y_3

Model R^2	R^2	Adjusted R^2	Predicted R^2	PRESS	s.d.	F-value	p-value	Remarks
Response Y_1 (Particle size(nm))=+118.77-13.75 × A-24.20 × B								
Linear	0.38	0.2422	-0.1647	14247	29.03	2.76	0.1164	-
Interactive	0.395	0.1681	-0.6553	20249	30.4	5	0.0816	Suggested
Quadratic	0.64	0.3406	-2.3531	41017	27.08	2.05	0.2101	-
Response Y_2 (Zeta Potential mV)=-33.31-2.15 × A-1.87 × B+6.52 × AB								
Linear	0.127	-0.0673	-1.183	837.4	6.1	0.65	0.5433	-
Interactive	0.57	0.4097	-0.5535	595.9	4.54	460	<0.001	Suggested
Quadratic	0.741	0.5261	-1.6651	1022.4	4.07	1.98	0.2183	-
Response Y_3 Entrapment efficiency (%)=+15.57+0.10667 × A+0.24667 × B								
Linear	0.547	0.4459	0.1473	1690	9.99	5.43	0.0285	Suggested
Interactive	0.687	0.14	-35.966	73263	12.45	0.057	0.9453	-
Quadratic	0.678	0.4103	-1.1821	21	4324.67	1.06	0.4036	-
SS: Sum of Squares, Df: Degrees of freedom, MS: Mean sum of Squares, SD: Standard deviation, F: Fischer's ratio								

Table 16: Regression analysis summary of Soya Phosphatidyl Ethanolamine based SLN for responses Y_1 , Y_2 and Y_3

Model R^2	R^2	Adjusted R^2	Predicted R^2	PRESS	s.d.	F-value	p-value	Remarks
Response Y_1 (Particle size(nm))=+114.83-17.22 × A-36.06 × B+15.44 × AB+7.45 × A²+16.95 × B²								
Linear	0.7882	0.7411	0.5133	5915	16.9	16.7	0.0009	Suggested
Interactive	0.9964	0.9901	0.5769	5142.5	3.3	13.5	0.0166	-
Quadratic	0.972	0.9487	0.7239	3355.5	7.5	11.3	0.0092	-
Response Y_2 (Zeta Potential mV)=-21.0-8.07 × A+0.95 × B+8.50 × AB-11.50 × A²-1.55 × B²								
Linear	0.3357	0.1881	-0.4729	1736.6	9.3	2.2	0.1587	-
Interactive	0.9743	0.9293	-1.9638	3494.5	2.7	4.6	0.0625	-
Quadratic	0.9537	0.9151	0.618	450.46	3.02	24.1	0.0014	Suggested
Response Y_3 Entrapment efficiency (%)=+66.17+5.50 × A+12.00 × B								
Linear	0.5921	0.5015	0.2009	1411	8.95	6.53	0.0177	Suggested
Interactive	0.7809	0.3976	-24.623	45242.1	9.83	0.45	0.6665	-
Quadratic	0.7317	0.5081	-0.9619	3464	8.89	1.15	0.3765	-
SS: Sum of squares, Df: Degrees of freedom, MS: Mean sum of squares, S.D.: Standard deviation, F: Fischer's ratio								

Table 17: Regression analysis summary of Phospholipon 90H based SLN for responses Y_1 , Y_2 and Y_3

Model R^2	R^2	Adjusted R^2	Predicted R^2	PRESS	s.d.	F-value	p-value	Remarks
Response Y_1 (Particle size(nm))=+205.33-37.26 × A-2.64 × B-22.14 × AB-16.03 × A²-31.48 × B²								
Linear	0.45199	0.3301	-0.0286	19054.8	33.5	3.7	0.0668	Suggested
Interactive	0.8773	0.6626	-13.654	20440.5	32	1	0.4369	-
Quadratic	0.8144	0.6597	-0.4775	27370.5	23.9	4.1	0.0739	-
Response Y_2 (Zeta Potential mV)=-34.41-2.88 × A-4.45 × B+6.97 × AB								
Linear	0.2437	0.0756	-0.6428	1137.4	7.6	1.4	0.2846	Suggested
Interactive	0.7987	0.4464	-23.09	16684.3	5.9	1.7	0.2873	-

Quadratic	0.6245	0.3115	-2.1611	2188.6	6.5	0.8	0.4932	-
Response Y₃ Entrapment efficiency (%) = +72.09 + 4.23 × A + 12.33 × B								
Linear	0.5837	0.4912	0.1982	1401.4	8.9	6.3	0.0194	Suggested
Interactive	0.7123	0.2088	-33.133	59656.8	11.2	0.084	0.9214	-
Quadratic	0.7003	0.4505	-1.0525	3587.2	9.3	0.94	0.4416	-

Table 18: Model parameters (ANOVA) for the dependent responses of Sphingomyelin based SLN

Source	SS	df	MS	p-value	Significance
Response Y₁					
Model	4648.2	2	2324.1	0.1164	NS
X ₁	1134.3	1	1134.3	0.2758	NS
X ₂	3513.8	1	3513.8	0.715	NS
Residual	7584.3	9	842.7	-	-
Total	12232.5	11	-	-	-
Response Y₂					
Model	218.9	3	72.9	0.0676	NS
X ₁	27.7	1	27.7	0.2792	NS
X ₂	20.9	1	20.9	0.3431	NS
X ₁ X ₂	170.3	1	170.3	0.0206	NS
Residual	170.3	1	20.5	-	-
Total	164.6	8	-	-	-
Response Y₃					
Model	1083.3	2	541.6	0.0285	S
X ₁	170.6	1	171.6	0.2235	NS
X ₂	912.6	1	912.6	0.0144	S
Residual	898.5	9	99.8	-	-
Total	1981.8	11	-	-	-
SS: Sum of squares; Df: Degrees of freedom; MS: Mean sum of squares					

Table 19: Model parameters (ANOVA) for the dependent responses of Soya Phosphatidyl Ethanolamine based SLN

Source	SS	df	MS	p-value	Significance
Response Y₁					
Model	11813	5	2363	0.0001	S
X ₁	1779.5	1	1780	0.0014	S
X ₁ ²	7799.7	1	7800	<0.0001	S
X ₂	147.8	1	147.8	0.1573	NS
X ₂ ²	765.8	1	765.8	0.0104	-
X ₁ X ₂	953.8	1	953.8	0.0063	S
Residual	339.9	3	56.6	0.0001	S
Total	12153	11	-	0.0014	S
Response Y₂					
Model	1124.4	5	224.8	24.7	4.3
X ₁	390.4	1	390.4	42.8	5.9
X ₁ ²	5.4	1	5.4	0.59	5.9
X ₂	352.6	1	352.6	38.7	5.9
X ₂ ²	6.4	1	6.4	0.72	5.9
X ₁ X ₂	289	1	289	31.7	5.9
Residual	54.6	6	9.1	-	-
Total	1179.1	11	-	-	-
Response Y₃					
Model	1045.5	2	522.7	6.5	4.2
X ₁	181.5	1	181.5	2.2	5.1
X ₂	864	1	864	10.8	5.1

Residual	720.1	9	80.02	-	-
Total	1765.6	11	-	-	-

SS: Sum of squares; Df: Degrees of freedom; MS: Mean sum of squares

Table 20: Model parameters (ANOVA) for the dependent responses of Phospholipon 90H based SLN

Source	SS	df	MS	p-value	Significance
Response Y_1					
Model	4648.2	2	2324.1	0.1164	NS
X_1	1134.3	1	1134.3	0.2758	NS
X_2	3513.8	1	3513.8	0.715	NS
Residual	7584.3	9	842.7	-	-
Total	12232.5	11	-	-	-
Response Y_2					
Model	218.9	3	72.9	0.0676	NS
X_1	27.7	1	27.7	0.2792	S
X_2	20.9	1	20.9	0.3431	S
Residual	170.3	1	170.3	0.0206	S
Total	164.6	8	20.5	-	-
Response Y_3					
Model	1083.3	2	541.6	0.0285	S
X_1	170.6	1	171.6	0.2235	NS
X_2	912.6	1	912.6	0.0144	S
Residual	898.5	9	99.8	-	-
Total	1981.8	11	-	-	-

SS: Sum of squares; Df: Degrees of freedom; MS: Mean sum of Squares

For the Sphingomyelin based SLN, the response observation for particle size X_1 , X_2 , were found to be non-significant terms, the response observation for zeta potential X_1 , X_2 , X_1X_2 were found to be non-significant terms response observation for % EE X_1 was found to be non-significant term and X_2 was found to be significant term.

For the Soya Phosphatidyl Ethanolamine based SLN, the response observation for particle size X_1 , X_2 , X_2^2 , X_1X_2 were found to be significant terms, X_1^2 found to be non significant term. The response observation for zeta potential X_1 , X_1^2 , X_1X_2 were found to be significant terms. X_2 , X_2^2 , were found to be non significant terms response observation for % EE X_1 was found to be non-significant term and X_2 was found to be significant term.

For the Phospholipon 90H based SLN, the response observation for particle size X_1 , X_2 , were found to be non-significant terms, the response observation for zetapotential X_1 , X_2 , X_1X_2 were found to be significant terms response observation for % EE X_1 was found to be non-significant term and X_2 was found to be significant term. The 3 different bases of SLN "Pred R-Squared" value of all 3 responses are not in reasonable agreement with the "Adj R-Square" value.

Optimization

To optimize 3 responses with different targets, a multi-criteria decision approach, like a numerical optimization technique by the desirability function and graphical optimization technique by the overlay plot were used. The op-

timized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). Optimum formulation was selected based on the criteria of minimum particle size (96.04 nm \pm 0.2 nm), higher zeta potential (-30.5 mV \pm 1.1 mV) and maximum % EE (97.4% \pm 0.2%). Various feasibility and grid searches were executed to establish the optimum formulation by plotting desirability function response plot and overlay plot, where one solution was found with a desirability of 1.0. The recommended poloxamer 407 and Cremophor EL were calculated by the Design Expert 12 software trial version.

Optimization was carried out by both numerical optimization and graphical optimization techniques. The 3 different bases of SLN desirability and overlay plots are shown respectively in Figures 12-14. The desirability function was found to be higher (0.806, 0.837 and 0.891, which are near to 1) for the optimized formula indicating the suitability of the formulations. The optimized SLN contain 200 mg of poloxamer 407 and 200 mg of Cremophor EL for all the 3 different phospholipids. Predicted model formulations were found matching with the optimized SNF9, SPF9 and SHF9 formulations.

From the above Table 21, the optimized SLN contain 200 mg of poloxamer 407 and 200 mg of Cremophor EL for Sphingomyelin, Soya Phosphatidyl Ethanolamine, and Phospholipon 90H based SLN formulations. 50 mg of Doultagravir and 400 mg of phospholipids were kept constant in all the formulations. The prepared optimized

formulations were found to be of good quality fulfilling all the requirements of nanoparticles.

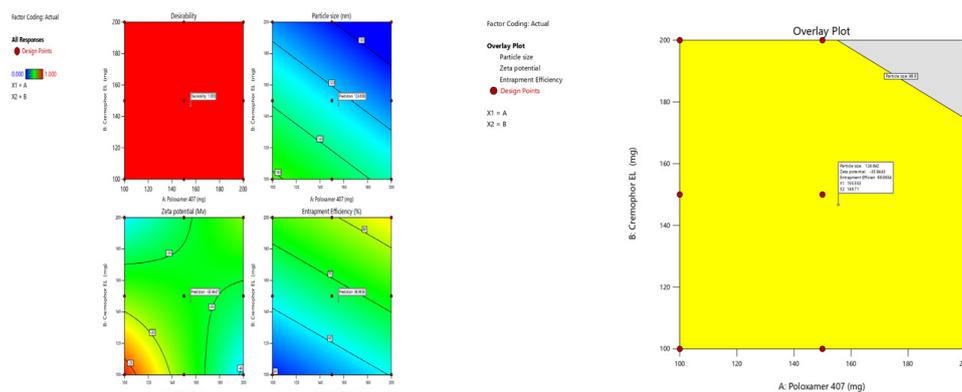


Figure 12: Desirability and overlay plots of Spingomyelin based SLN

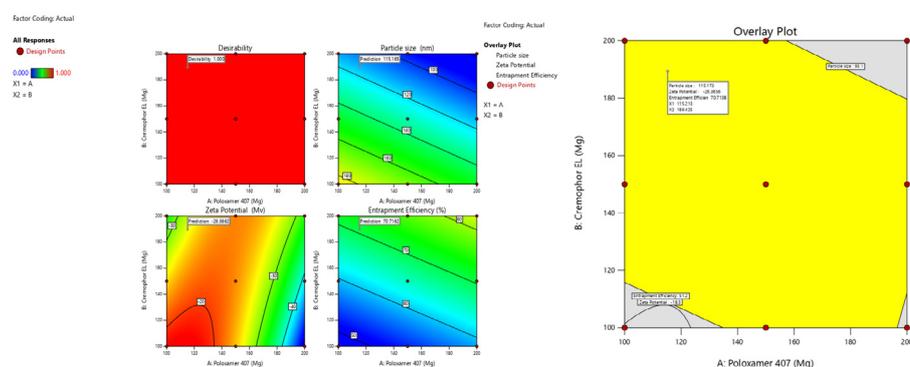


Figure 13: Desirability and overlay plots of Soya Phosphatidyl Ethanolamine based SLN

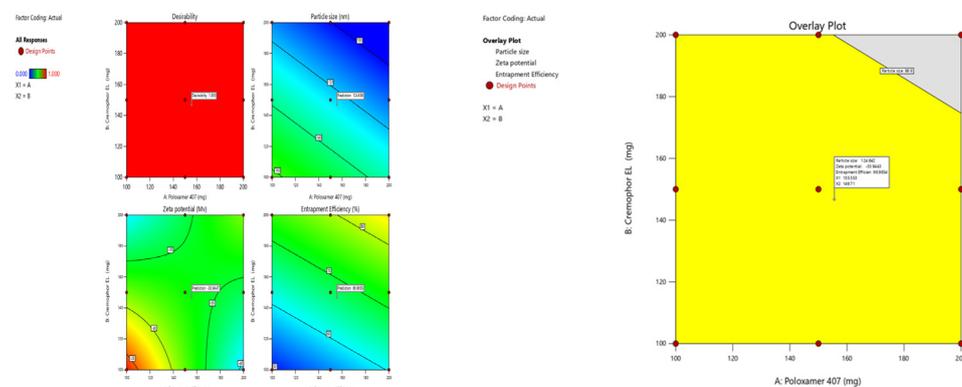


Figure 14: Desirability and overlay plots of Phospholipon 90H based SLN

Table 21: Optimized formula of solid lipid nanoparticles

Solid Lipid Nanoparticles		
Base	Poloxamer 407	Cremophor EL
Sphingomyelin	200 mg	200 mg
Soya Phosphatidyl Ethanolamine	200 mg	200 mg
Phospholipon 90H	200 mg	200 mg

Surface morphology by transmission electron microscopy

Transmission electron microscopy of the tristearin based SHF9 formulation was performed to elucidate the surface morphology as shown in Plate (Figure 15). The SLN obtained were in nanometer-size and spherical in shape with well-defined periphery at 50000X magnification. The size of the solid lipid nanoparticles was found to be in agree-

ment with the Malvern Zetasizer particles size distribution for the selected sample.

DSC

DSC analysis was performed on Doultegravir pure drug, individual phospholipids, physical mixtures prepared with drug and each phospholipid (1:1) and SNF9, SPF9 and SHF9 formulations. DSC thermograms of pure drug, Sphingomyelin, physical mixture of drug and Sphingomyelin and SNF9 formulation prepared with Sphingomyelin are shown. Similarly that of Soya Phosphatidyl Ethanolamine are shown and Phospholipon 90H are shown (Figure 16). The pure drug Doultegravir showed a sharp endothermic peak at 141.9°C. Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H showed sharp endothermic peaks at 60.36°C, 65.19°C and 75.32°C. Physical mixture of pure drug Doultegravir with Sphin-

gomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H showed sharp and broad endothermic peak at 59.45°C and 136.69°C, 64.57°C and 131.34°C, 73.90°C and 137.71°C respectively.

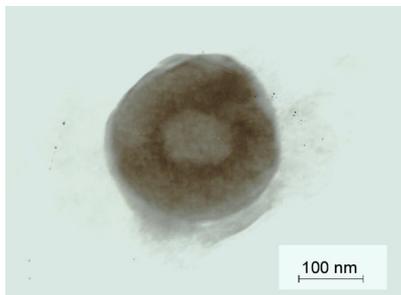


Figure 15: Plate-surface morphology of SHF9 by TEM studies

Doultegravir SLN with Sphingomyelin, Phosphatidyl Ethanolamine and Phospholipon 90H showed a reduced peak at 55.19°C, 61.41°C and 69.66°C shifted towards the lower temperature. The peak corresponding to drug completely disappeared indicating the complete entrapment of the drug in the SLN. This suggested that Doultegravir was not in crystalline state but was in amorphous state in the SLN formulations.

The method of preparation hot homogenization followed by ultrasonication and presence of surfactant could not allow the drug to crystallize (Freitas and Muller, 1999). Melting points of Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon in SLN form were depressed when compared to melting points of corresponding bulk phospholipids. This melting point depression might be due to small particle size (nanometer range), their high specific surface area.

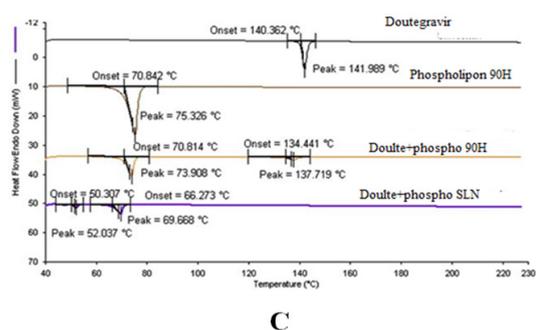
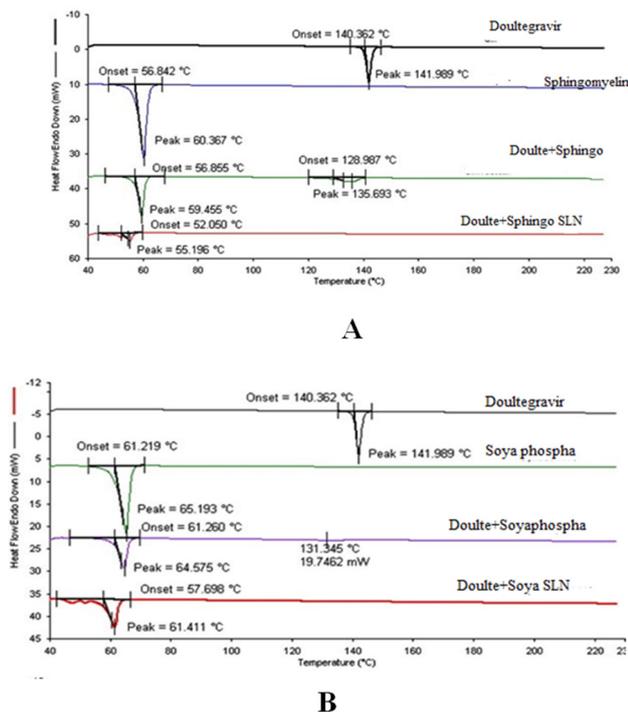


Figure 16: DSC thermograms A) Sphingomyelin; B) Soya Phosphatidyl Ethanolamine and C) Phospholipon 90H based optimized formulations

Powder X-Ray Diffractometry (PXRD)

PXRD patterns of Doultegravir exhibits sharp peaks at diffraction angle around 9.7 indicating crystalline nature of Doultegravir. Results are shown in the Figure 17. All the Phospholipids showed sharp peaks at diffraction angles around 19.5. In the physical mixtures the intensity of the sharp peaks was reduced as compared to the pure drug and phospholipid. The intensities of the peaks were further reduced and broad for simvastatin loaded SLN of Sphingomyelin, Soya Phosphatidyl Ethanolamine and Phospholipon 90H. This suggests that Doultegravir was not in crystalline form in the formulation. However, the characteristic peaks of Phospholipon 90H were most decreased in SLN, Similarly crystallinity of Sphingomyelin, Soya Phosphatidyl Ethanolamine was also decreased in SLN.

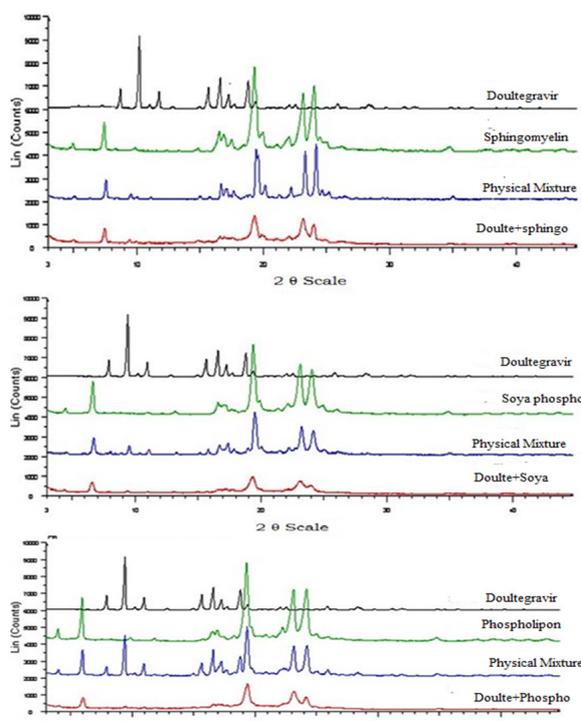


Figure 17: Powder x-ray diffractometry (PXRD) patterns of Doultegravir

In vitro drug release

The drug release testing is a fundamental part of drug product development and manufacturing and is also employed as a quality control tool to monitor batch-to-batch

consistency of the drug release from SLN systems. The SLN drug release mechanism was influenced by various physicochemical factors such as particle size, % entrapment efficiency and poloxamer 407 and Cremophor EL composition. The Franz diffusion process was used for drug release. The *in vitro* release profile of optimized formulations with desired particle size, zeta potential and entrapment efficiency *viz.* SNF9, SPF9 and SHF9 was studied using cellulose membrane as semi permeable membrane. The study revealed that the release of the drug from the formulations depends on the relative amounts of poloxamer 407 and Cremophor EL and phospholipids present.

SLN formulations SNF9, SPF9 and SHF9 released 31.78%, 32.55% and 37.17% of Doultegravir respectively for 36 h. 98% of drug release was observed in 36 h for

pure Doultegravir dispersed in pH 6.8 buffer. The results indicated slower release of Doultegravir from the prepared SLN compared to pure drug which may be due to the lipid nature of the prepared SLN and homogeneous dispersion of the drug in the lipid matrix.

In vitro release kinetics

From Table 22, drug release kinetics of SLN formulations indicated that drug release followed zero order and non-Fickian diffusion anomalous super case-II manner and absence of any burst release, which indicated complete entrapment of drug in the lipid matrix without any adsorption of simvastatin on the surface of SLN. No significant variation was observed in release of Doultegravir from different bases of SLN. The *in vitro* drug release profiles of SLN are shown in Figure 18.

Table 22: Drug release kinetics of SLN formulations

Formulation	Zero order	First order	Higuchi	Peppas	for	for	
SNF9	0.989	0.873	0.982	0.0092	0.928	0.993	0.931
SPF9	0.992	0.904	0.986	0.0092	0.944	0.994	0.944
SHF9	0.998	1.045	0.997	0.0115	0.962	0.995	0.991

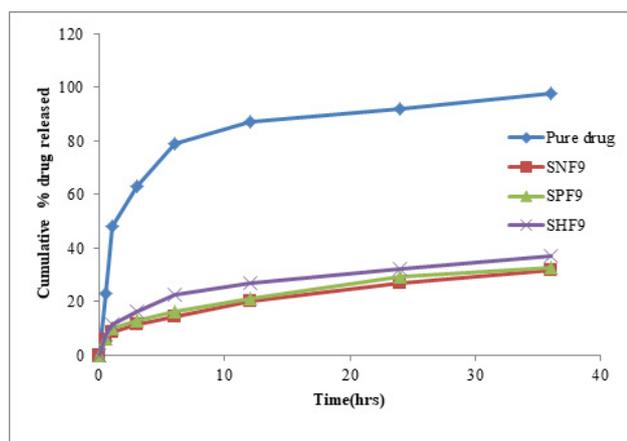


Figure 18: Comparison of cumulative % drug release of pure drug with optimized formulations prepared by different lipids

Conclusion

A 3² full factorial design has been employed to produce Doultegravir loaded SLN by using hot homogenization technique followed by ultra-sonication method. The statistical optimization reduced the number of experiments that were carried out for obtaining formulations with desired properties. The derived polynomial equations, response and contour plots helped in predicting the values of selected independent variables for preparation of optimum SLN with desired properties. Solid lipid nanoparticles showed minimum particles size, optimum zeta potential and higher %EE. This experimental design revealed that Phospholipon 90H based formulation showed better result compared to Sphingomyelin, Soya Phosphatidyl Ethanolamine based formulations which could be due to maximum carbon chain length and high phase transition temperature. The drug excipients characterization parameters reveal that there is no drug-excipients interaction.

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Conflict of Interest

Authors have no conflict of interest to declare.

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