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DESIGN PREPARE AND EVALUATION OF EZETIMIBE SELF EMULSIFYING DRUG DELIVERY SYSTEM

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Abstract:

The composition of Ezetimibe loaded loaded SEDDS was optimized using 3^2 facorial design. The impact of the formulation parameters on mean globule size and percentage drug load were studied by applying the analysis of variance and regression models. Several formulation and process variables were evaluated and optimized by response surface methodology. The optimum formulation was prepared by response optimizer through desirability function and the experimental values were found to be in close agreement with the predicted values. Optimized formulation was further subjected to stability studies. Optimal Ezetimibe SEDDS contains sunflower oil as oil phase, labrasol as a surfactant and transcutol HP as cosurfactant (Smix) in the ratio of 67.586% oil and 52.529% % w/w Smix formulates SEDDS with lower droplet size (169.7nm), PDI (0.2), and zeta potential (-31.8 mv) and percentage drug load (87.2%) values.. It was concluded that the smaller particle size and drug load more the release of drug which results in better bioavailability. The in vitro evaluation parameters such as emulsification time, viscosity determination, cloud point measurement, turbidity measurement, refractive index and spectroscopic optical clarity test were performed and the results were foundwithin the limits for all formulations of two drugs. The stability studies revealed that there was no change in particle size and percentage drug load for the two drugs after 6months. The in vitro drug release from optimized Atorvastatin SEDDS formulation were found to be 99.75% after 90 min. It was extremely higher in comparison to the marketed formulation and API suspension. In-vitro drug release studies closely indicate that optimized formulations obey first order kinetics and the mechanism of drug release was by fickian diffusion. The results further concluded that SEDDS can be explored as a potential drug carrier for dissolution enhancement of Atorvastatin other poorly soluble drugs.

Key words: *Design Prepare, Evaluation, Ezetimibe, Self-Emulsifying Drug Delivery System*

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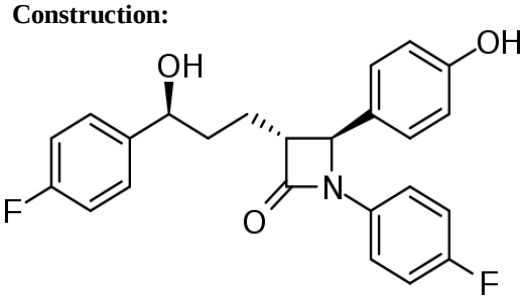
INTRODUCTION:

SEDDS formulations can be simple binary systems: lipophilic phase and drug, or lipophilic phase, surfactant and drug. The formation of a SEDDS requires the use of a co-surfactant to generate a micro emulsion. SEDDS formulations are characterized by in vitro lipid droplet sizes of 200 nm–5 mm and the dispersion has a turbid appearance. Self-emulsifying drug delivery systems (SEDDS) are mixtures of oils and surfactants, ideally isotropic, and sometimes containing cosolvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation.[1-5] Recently, -SEDDS have been formulated using medium chain tri-glyceride oils and non-ionic surfactants, the latter being less toxic. Upon per oral administration, these systems form fine emulsions (or microemulsions) in gastro-intestinal tract (GIT) with mild agitation provided by gastric mobility .[6-12] Ezetimibe is a lipid-lowering compound that hinders intestinal cholesterol as well as related phytosterol absorption The exploration as well as study of this drug began in the early 1990's, where intravenous administration of radio-labelled compound in rats causing succeeding localization of the drug within enterocytes at the digestive villus, bring about research studies of checking out the result of ezetimibe on digestive tract cholesterol absorption. Ezetimibe moderates its blood cholesterol-lowering result by selecting the absorption of cholesterol as well as phytosterol via the small intestine by transforming fat-soluble vitamins as well as nutrients without absorption The main target of ezetimibe is cholesterol transportation healthy protein Niemann-Pick C1-Like 1 (NPC1L1) protein is revealed in the enterocyte/ digestive tract lumen (aplitic) .and also hepatobiliary (canalicular) user interface and plays a role in promoting complimentary cholesterol's internalization into the entosite with the adapter healthy protein 2 (AP2) complex and also clatrin.

Construction:



The aim of the proposed research work was to develop a novel o/w self- emulsifying drug delivery system (SEDDS) for poorly soluble BCS system class II drugs of Ezetimibe with novel manufactured oils, an assortment of edible natural oils and surfactants/co-surfactants with the utilization of Design of Experiments and factorial designing. The proposed investigated work was selected because of simplicity in the basic procedure of creation and to scale up with the least framework.

The purpose of the present research work was to systematically investigate theinteraction, the quadratic effects of formulation variables (independent variables) of SEDDS on desired responses; to develop a model that would yield an optimized SEDDS of Ezetimibe. A 13-run factorial design with 2 factors and 3 levels, including 4 replicates at the centre point was used for fitting a second order response surface. The estimation of the coefficients for the second order polynomial model was performed by regression analysis. The model adequacy was checked by an F-test and the determination of correlation coefficient (R^2). Evaluation of prepared formulations for cloud point measurement, emulsification time, particle size, polydispersity index (PDI), zeta potential, viscosity, spectroscopic optical clarity, refractive index, turbidity measurementand percentage drug loading.

METHODOLOGY:

STANDARD CALIBRATION CURVE OF EZETIMIBE IN METHANOL

UV Spectroscopy (λ max)

The absorption maximum of the standard solution of Ezetimibe wasscanned between 200- 400 nm regions on UV- visible spectrophotometer.

Preparation of standard stock solution

An accurately weighed quantity of about 50 mg of Ezetimibe was taken in 50 ml volumetric flask and dissolved in sufficient quantity of methanol followed by sonication[127] in a bath sonicator (Sonica 2200MH) provided with a power supply of 305 Watts during heating at a temperature of 60 C for 10 minutes and finally diluted to 50 ml with methanol to obtain the concentration of 1000 µg/ml. From this solution, 5 ml was pipetted out in a 50 ml volumetric flask and volume was made up with methanol to obtain the concentration of 100 µg /ml.

Preparation of calibration curve

From the stock solution, 2, 4-, 6-, 8,10- and 12-ml appropriate aliquots were pipetted out from standard stock solution into the series of 100 ml volumetric flask and the volume was made up to the mark with methanol to get the concentration of 2- 12 µg/ml of the drug. The absorbance at various concentrations was measured against methanol as blank at 247 nm using a UV-visible spectrophotometer.

PREPARATION OF BUFFER SOLUTIONS

Preparation of 0.2M Potassium dihydrogen phosphate

Accurately weighed 27.218g of potassium dihydrogen orthophosphate was dissolved in 1000ml of distilled water.

Preparation of 0.2M sodium hydroxide

Accurately weighed 8.0g of sodium hydroxide was dissolved in 1000 ml of distilled water.

Preparation of Phosphate Buffer pH 6.8

Phosphate buffer pH 6.8 was prepared according to I.P. 2007. A measured quantity of 50 ml of 0.2M potassium dihydrogen phosphate and 22.4 ml of 0.2M sodium hydroxide were taken in 200ml volumetric standard flask and diluted with freshly prepared distilled water to produce the required volume.

Preparation of phosphate buffer pH 7.4

Phosphate buffer pH 7.4 was prepared according to I.P. 2007. A measured quantity of 50 ml of 0.2M potassium dihydrogen phosphate and 39.1 ml of 0.2M sodium hydroxide were added in 200ml volumetric standard flask and diluted with freshly prepared distilled water to produce the required volume.

DEVELOPMENT OF CALIBRATION CURVE OF EZETIMIBE IN PHOSPHATE BUFFER pH 6.8

Preparation of standard stock solution

An accurately weighed quantity of about 10 mg of Ezetimibe wastaken in 100 ml volumetric flask and dissolved in sufficient quantity of phosphate buffer of pH 6.8 and finally diluted with the same buffer to obtain the concentration of100 µg/ ml.

Preparation of calibration curve

From the stock solution 2, 4, 6, 8,10and 12-ml appropriate aliquots were pipetted out from standard stock solution into the series of 100 ml volumetric flask and the volume was made up to the mark with phosphate buffer pH 6.8 to get concentration of 2-12 µg/ml of the drug. The absorbance at various concentrations was measured against blank (phosphate buffer pH 6.8)

PREPARATION OF SEDDS

Optimum ratios of oil and Smix were selected from the phase diagrams. SEDDS formulations were prepared by dissolving the drug in Smix mixtures along with gentle vortexing and sonicating and then by adding oil[134]. The effects of the formulation variables for different batches were studied by preparing with each batch of SEDDS formulation containing single dose of Atorvastatin with varying amounts of oil and Smix using 3^2 factorial designs as illustrated in Table 14a and Table 14b. Then the final formulation was equilibrated in water bath at 37°C for 48 h before carrying out the droplet size, polydispersity index and dissolution. The optimized formulations are prepared by the same method.

EXPERIMENTAL DESIGN: 3^2 FULL FACTORIAL DESIGN

A 3^2 full factorial design factor was used to explore and optimize the main effects, interaction effects and quadratic effects of the formulation ingredients on the *in-vitro* performance of liquid SEDDS. A total of 13 experimental runs, including 4 replicates at the centre were generated and evaluated by using Design-Expert software (version 10.0.2.0, Stat-Ease Inc., Minneapolis, U.S.A.) which are summarized inTable 14a and Table 14b. The purpose of the replication was to estimate experimental error and increase the precision by computing a model independent estimate of the process standard deviation. The significant response factors studied for assessing the quality of the SEDDS formulation were particle/globule size (Y_1) and drug loading (Y_2).The data obtained after the each response was fitted to quadratic polynomial model explained by the following non-linear equation $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_1X_1^2 + \beta_2X_2^2 + E$. where Y is the response of the dependent variables; β_0 to β_2 are the regression coefficients; and X_1, X_2 are independent variables. All the two responses were optimized by using the desirability function approach by fixing the constraints in range and minimizing the particle size (Y_1) and maximizing the drug load (Y_2).

RESULTS AND ANALYSIS

PREFORMULATION STUDY

Melting point determination

The melting point of Ezetimibe determined as per standard IP procedure was found to be 160□C. The results obtained were within the melting point range as mentioned in The Merck's Index.

FT-IR studies for Ezetimibe

From Figure it was illustrated that the IR spectrum of Ezetimibe showed the characteristic peaks of aromatic N-H stretching at 3364.93 cm^{-1} and the asymmetric stretching of C=O of amide group at 1651.12cm^{1}. However, similar peaksof symmetric C=O stretching were observed at 1579.75 cm^{-1} and O-H stretching at 3566.50 cm^{-1}. The characteristic peaks were observed at the wave numbers 1510.31 cm^{-1} and 1424.48 cm^{-1} due to the C=C ring stretching. The peak found at 1317.43 cm^{-1} was due to CH_3/CH_3 deformation bending vibration at the plane. The two characteristic bands were observed at 3735.28 cm^{-1} and 3055.35 cm^{-1}due to the O-H stretching associated with the hydrogen bond. From the above study, it was inferred that the drug sample was identified as Ezetimibe.

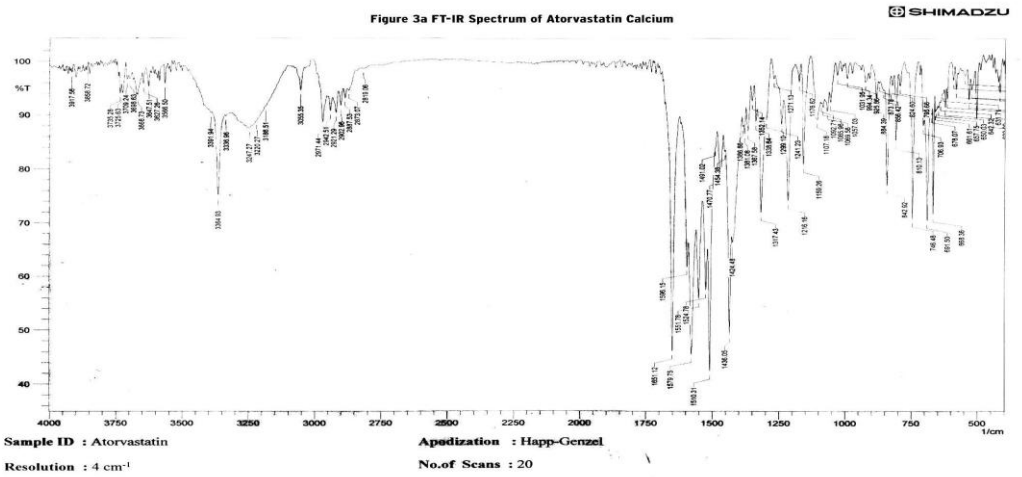


Fig. 1. FT-IR spectrum of Ezetimibe

UV spectroscopic method analysis of Ezetimibe

Linearity and range for calibration curve of Ezetimibe in methanol

The straight-line calibration graph was obtained in the concentration of 2-12µg/ml of the Ezetimibe in methanol. The linear regression equation was found to be y=0.045x+0.003 with the correlation co efficient (r^2) of 0.999. The calibration curve was illustrated in Fig. and from the linear regression data (r^2 value), it can be concluded that the analyzed concentration of the drug solution followed linearity.

Table 1: Calibration data for Ezetimibe in methanol

S.No.	Concentration (µg/ml)	Absorbance
1.	2	0.0913
2.	4	0.1908
3.	6	0.2836
4.	8	0.3774
5.	10	0.4625
6.	12	0.5465

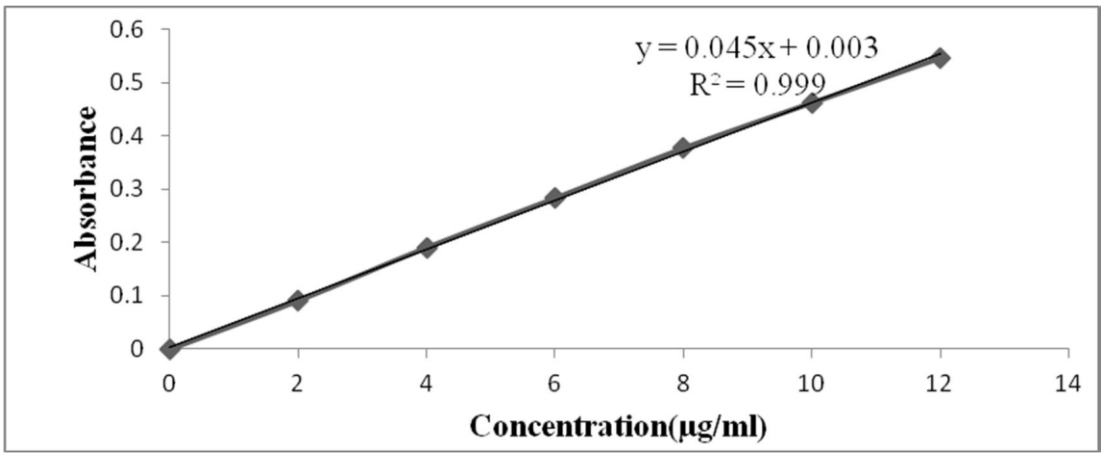


Fig. 2. Calibration curve of Ezetimibe in methanol

Linearity and range for calibration curve of Ezetimibe in phosphate buffer pH 6.8

The straight line calibration graph was obtained in the concentration 2-12 µg/ml of the Ezetimibe phosphate buffer pH 6.8. The linear regression equation for Ezetimibe in phosphate buffer pH 6.8 is y=0.012x+0.001 with the correlation co efficient of 0.999. The calibration curve was illustrated in Fig. and from the linear regression data (r^2 value), it can be concluded that the analyzed concentration of the drug solution followed linearity.

Table 2: Calibration data for Ezetimibe in phosphate buffer pH 6.8

S.No.	Concentration (µg/ml)	Absorbance
1.	2	0.0265
2.	4	0.0529
3.	6	0.0795
4.	8	0.1046
5.	10	0.1279
6.	12	0.1535

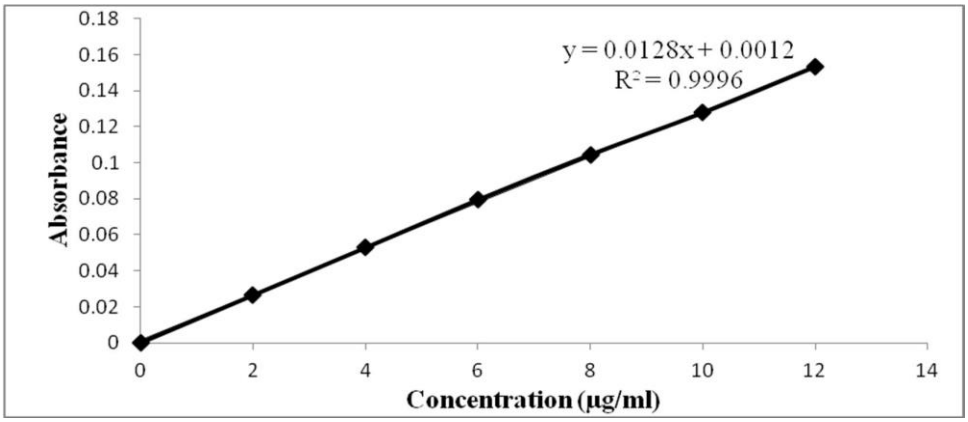


Fig. 3. Calibration curve of Ezetimibe in phosphate buffer pH 6.8

Variables selected for development of SEDDS

Based on the feasibility of micro emulsion formation at extreme values, the range for each component was selected as follows: oil (40-80%), Smix (30-70%) for Ezetimibe and oil (15-25%). The slack variable was taken as water content as it is present in the larger amount in a gastrointestinal tract. The dependent variables which are significantresponse factors studied for assessing the quality of SEDDS are particle size (Y_1) and % drug loading (Y_2). The optimization of the SEDDS was done using by 3 level 2 factorial design. From the preliminary solubility and ternary phase diagram studies theamount of sunflower oil (X_1) as lipophile and the amount of surfactant mixture (X_2) of labrasol and transcutol HP were selected as the two independent variables for the development of Ezetimibe SEDDS. The three levels of each factor were used to construct experimental design. The levels for sunflower oil (40, 60 80), labrasol and transcutol HP (30, 50, 70) for a formulation of Ezetimibe SEDDS were selected from the preliminary study.

Table 3: Variables for Ezetimibe in 3^2 full factorial Design

Independent Variables[(a)]	**Levels**		
	Low (-1)	**Middle (0)**	**High (-1)**
X_1: Amount of oil added (mg)	40	60	80
X_2:Amount of Smix in ratio of 3:1 added (mg)	30(22.5:7.5)	50(37.5:12.5)	70(52.5:17.5)
Dependent Variables	**Constraints**		
	Range		**Goal**
Y_1: Particle size (Globule Size in nm)	In the range		Minimize
Y_2: % drug loading	In the range		Maximize

(a) Oil: Sunflower oil; Surfactant: Labrasol; Cosurfactant: Transcutol HP

(a) Oil: Peceol; Surfactant: Labrasol; Cosurfactant: Transcutol HP

STATISTICAL ANALYSIS OF THE DESIGNED EXPERIMENT

The range of oil (X_1), Smix (X_2) were delimited as independent variables; 3^2 full factorial design was performed to optimize SEDDS with constraints on globule size and drug load as the Response Surface methodology (RSM) requires 13 the observed responses. All the data were fitted to the second order quadratic model and validation of the model was carried out by analysis of variance (ANOVA) test, lack of fit test and correlation coefficient (R^2). The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($p<0.05$) effect of factors on a response. It was observed that for responses Y_1, and Y_2, quadratic fitting was significant (p-value <0.05). For the Y_1 response of Ezetimibe, the "Lack of Fit F-value" of 32.97 implies the Lack of Fit is significant. There is only a 0.28% chance that a "Lack of Fit F-value" this large could occur due to noise. For the Y_2 response of Ezetimibe response, the lack of fit was The "Lack of Fit F-value" of 1.93 implies the Lack of Fit is not significant relative to the pure error, the "Lack of Fit F-value" of 182.64 implies the Lack of Fit is significant. There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise. For the Y_2 response of The "Lack of Fit F-value" of 2.02 implies the Lack of Fit is not significantrelative to the pure error. There is a 25.33% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good while calculating the correlation coefficient (R^2) for the responses Y_1, and Y_2 the confidence that the regression equations would predict the observed value better than mean were more than 83.22%, 93%, respectively for Ezetimibe. The corresponding coefficients which showed the quantitative effects of independent variables (X_1 and X_2) and their interactions on the responses are shown in the Tables .The coefficients (Factor intercepts) ($X_1 \cdot X_2$,) and those with the higher order terms (X_1^2, X_2^2) indicate the interactions and quadratic effects, respectively.

Table 4: Execution of 3^2 Experimental Design and coding of actual values of independent variables for factorial design with the observed responses for Ezetimibe

Std	Run	Formulation Code (FC)	Oil (mg)	Smix (mg)	Y_1(Particle size) (nm)	Y_2(%Drug Loading)
7	1	AF1	-1(40)	+1 (70)	106.8±4.08	81.8±6.63
4	2	AF2	-1(40)	0 (50)	172±7.5	83.1±4.54
6	3	AF3	+1(80)	0 (50)	290±4.9	91.5±2.78
10*	4	AF4*	0 (60)	0 (50)	112.4±8.5	85.1±2.71
13*	5	AF5*	0 (60)	0 (50)	128.5±5.68	84.3±3.05
9	6	AF6	+1(80)	+1 (70)	285±8.6	87.6±1.65
5	7	AF7	0 (60)	0 (50)	137.9±5.5	88.7±1.1
2	8	AF8	0(60)	-1 (30)	197.6±5.65	75.1±2.75
8	9	AF9	0 (60)	+1 (70)	233.1±3.44	86.1±4.37
3	10	AF10	+1 (80)	-1 (30)	229.7±4.98	89.1±4.53
11*	11	AF11*	0 (60)	0 (50)	140.2±3.0	85.7±4.70
1	12	AF12	-1 (40)	-1 (30)	415±8.7	70.1±2.25
12*	13	AF13*	0 (60)	0 (50)	114.9±7.1	86.9±1.21

Y_1: Particle size; Y_2: Drug Load; *Centre point Formulations

The coded and actual values for the factors used in the 3^2 factorial design forEzetimibe at three levels are stated as below

Factors		Factor Level used		
		Low level	**Mid Value**	**High Value**
Coded value	X_1 & X_2	-1	0	+1
Actual value	X_1	40	60	80
Actual value	X_2	30	50	70

X_1 is the % amount of sunflower oil in mg
X_2 is the % amount of Smix (Labrasol and Transcutol) in mg.

Analysis of Variation and Regression

Table 5: Analysis of Variance in the regression models for Ezetimibe

Source		DF	Sum of Squares	Mean Square	F Value	p-Value	
Y_1(Globule Size in nm)	Model	5	83517.68	16703.54	6.94	0.0122*	Significant
	A-Oil	1	2049.80	2049.80	0.85	0.3867	
	B-Smix	1	7877.13	7877.13	3.27	0.1133	
	AB	1	33033.06	33033.06	13.73	0.0076**	Significant
	A^2	1	15552.15	15552.15	6.46	0.0385*	Significant
	B^2	1	9741.60	9741.60	4.05	0.0841	
	Residual	7	16841.75	2405.96			
	Lack of Fit	3	16187.12	5395.71	32.97	0.0028**	Significant
	Pure Error	4	654.63	163.66			
	Cor Total	12	1.004E+005				
Y_2 (Drug Loading in %)	Model	5	382.82	76.56	18.59	0.0006**	Significant
	A-Oil	1	183.71	183.71	44.60	0.0003**	Significant
	B-Smix	1	74.91	74.91	18.19	0.0037**	Significant
	AB	1	43.56	43.560	10.58	0.0140*	Significant
	A^2	1	5.02	5.02	1.22	0.3031	
	B^2	1	79.10	79.10	19.20	0.0032**	Significant
	Residual	7	28.83	4.12			
	Lack of Fit	3	17.04	5.68	1.93	0.2669	Insignificant
	Pure Error	4	11.79	2.95			
	Cor Total	12	411.65				

Table 6: Correlation Coefficients for Two Responses for Ezetimibe

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	%CV
Y1	0.8322	0.7123	-0.5672	7.629	49.05	24.88
Y2	0.9300	0.8799	0.5375	16.864	2.03	2.41

Table 7: Factor coefficients and their corresponding p-values for Ezetimibe

Factors	Y_1		Y_2	
	Regression Coefficient	Probability value (p-value)	Regression Coefficient	Probability value(p-value)
Intercept	135.117		86.0862	
X_1	18.4833	0.3867	5.53333	0.0003**
X_2	-36.2333	0.1133	3.53333	0.0037**
$X_1.X_2$	90.875	0.0076**	-3.3	0.0140*
X_1^2	75.0397	0.0385*	1.34828	0.3061
X_2^2	59.3897	0.0841	-5.35172	0.0032**

Significant model terms at: ** $p<0.01$, * $p<0.05$.

ANALYSIS OF VARIANCE FOR PARTICLE SIZE (Y_1) AND % DRUGLOAD (Y_2)

Ezetimibe SEDDS

The observed values of particle size for 13 formulations as shown in Table varied from 106.8 nm to 415 nm and % drug load varied from 70.1% to 91.5% for Ezetimibe. Two-way analysis of variance (ANOVA) can be applied to determine statistical significance of each model coefficient and least significant difference as post hoc test was performed.

Effect of formulation variables on particle size (Y_1)

The polynomial equation derived for particle size for Ezetimibe is given by

$Y_1 = 135.12+18.48*X_1-36.23*X_2 +90.88*X_1X_2 + 75.04*X_1^2 +59.39 *X_2^2$-Equation 1.

with R^2= 0.8322, adjusted R^2= 0.7123 and % CV= 24.88.

For the particle size, the model F value of 6.94 with a low probability value of (p value 0.05) implies a high significance for the full regression model which is shown in Table. R^2 values of full models are 0.8322 indicating the excellent correlation between the independent variables in the models. The adjusted R^2 value was 0.7123 for the full model indicating a better model as illustrated in Table. An increase in % CV shows moderate precision and reliability of the conducted experiments. The large SS_R and small SS_r values tend to occur for models that accurately describe the experimental data as shown in Table. A significant (p=0.0076) synergistic interaction between oil and Smix was observed which as illustrated in Table and equation 1. The quadratic regression coefficient of A^2 was statistically significant. The quadratic effect of oil showed significant synergistic effect (p=0.0385) influence on particle size of Ezetimibe SEDDS. The % CV was found to be 24.88 which were considered to be a high value for the response Y_1 variable of particle size. It was concluded that the interaction between Smix and oil increases the particle size and hence both the factors are highly significant.

Effect of formulation variables on % drug load (Y_2)

The second order polynomial equation derived for % drug load of Ezetimibe is given by

$Y_2= 86.09+5.53*X_1+3.53*X_2-3.30X_1X_2+1.35* X^2-5.35* X_2^2$- Equation 2

with R^2= 0.9300, adjusted R^2= 0.8799 and % CV= 2.41.

Linear regression and residual plot analysis

The residual analysis is one method to check model adequacy. After model fitting was performed residual analysis was conducted to validate the assumptions in ANOVA. The residual analysis includes case statistics to identify examine diagnostic plots such as normal probability of studentized residuals, a distribution plotof studentized residuals against the predicted values, an outlier T plot and a Box cox plot. For the normal probability plots of the studentized residuals, the number of standard deviations of the actual values from their respective predictive values, a straight line is created indicating no abnormalities or significant deviation from the linearity. The normal probability plot of the residuals depicted for Ezetimibe revealed that the systematic deviations from the expectations. In residuals plot where the residuals are plotted against the normal values of the model depicted that the points are nearby to a diagonal line which implied that the errors are normally dispersed and are individually independently depicting a homogenous error variances indicating a well fitted model. Residuals from the fitted model are normally distributed therefore all the major assumptions of the model have been validated. The plots are shown in Fig for Ezetimibe depicted an agreeable correlation between the predicted and actual values of responses. In this study, the normality is satisfactory as all residual plots are distributed along a straight line. It is inferred that the confidences for the fitness of the regression equations to theobserved values are more than 95% for all responses.

Contour plots and response surface analysis

A polynomial model describing relationship between response and factors of aresponse surface is known as response surface analysis. A model is graphically visualized by drawing 2D contour plots or 3D response plots. The 2D contour plots show the isoresponse lines as a function of two factors. The 3D response represents the response in 3D dimension. Contour plots and surface response plots are diagrammatic representation of the values of the response. These plots are useful to project the magnitude of effects for each variable and interactions. It can also explain the relationship between independent variables and dependent responses. Response surface methodology provides a mathematical trend that can find optimum level of experimental factors required for a given response. The two dimensional contour plot and the three-dimensional response surface plots are graphical representations of the regression equation and express two independent variables at once against the for Y_1 and Y_2 responses which are useful to study the effect of the factors on the responses. With the increasing surfactant (coefficient isnegative) in the formulation, droplet size is decreased. In Table For Ezetimibe, it can be seen that all independent variables showed significant main effects interaction effects and the quadratic effect of X_1 ($p < 0.05$) for % drug load; the most prominent effect being the amount of oil (X_1) added ($p = 0.0003$). For particle size, theinteraction effect was found to be X_1X_2 being the amount of oil and Smix added ($p = 0.0076$) and the quadratic effect of X_1 was found to be significant ($p=0.0385$). In the independent variable X_1 was found to be significant ($p=0.0080$). From Fig , Fig it was clearly observed when the level of Smix concentration was increased from low to high the response Y_1 (particle size) was decreased. From Fig, Fig. it was illustrated that when the level of oil concentration was increased from low to high the response Y_1 (% drug load) was increased. The contour plot of Ezetimibe showed that the denser central optimum area with good average particle size between150-200nm as shown.The contour plot Y_2 of % drug loading showed denser region between 85% and 90% as illustrated in Fig. Both the responses Y_1 and Y_2 are thus analyzed by the diagrammatic contour plots.

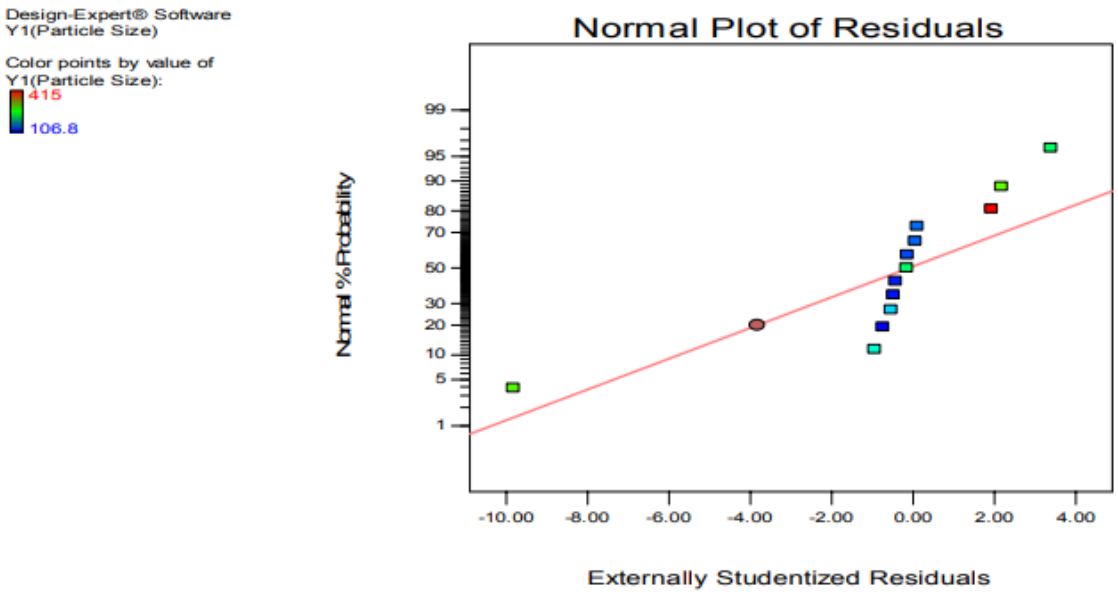


Fig. 4. Normal Residual plot Y_1 of Ezetimibe

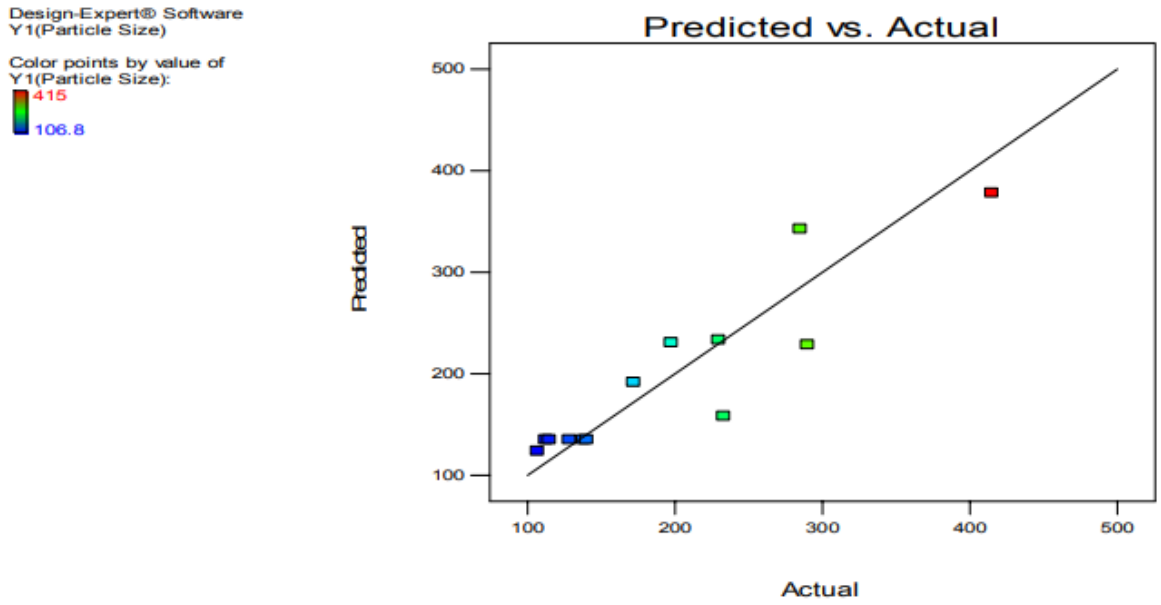


Fig. 5 Linear correlation plot of Y_1 of Ezetimibe

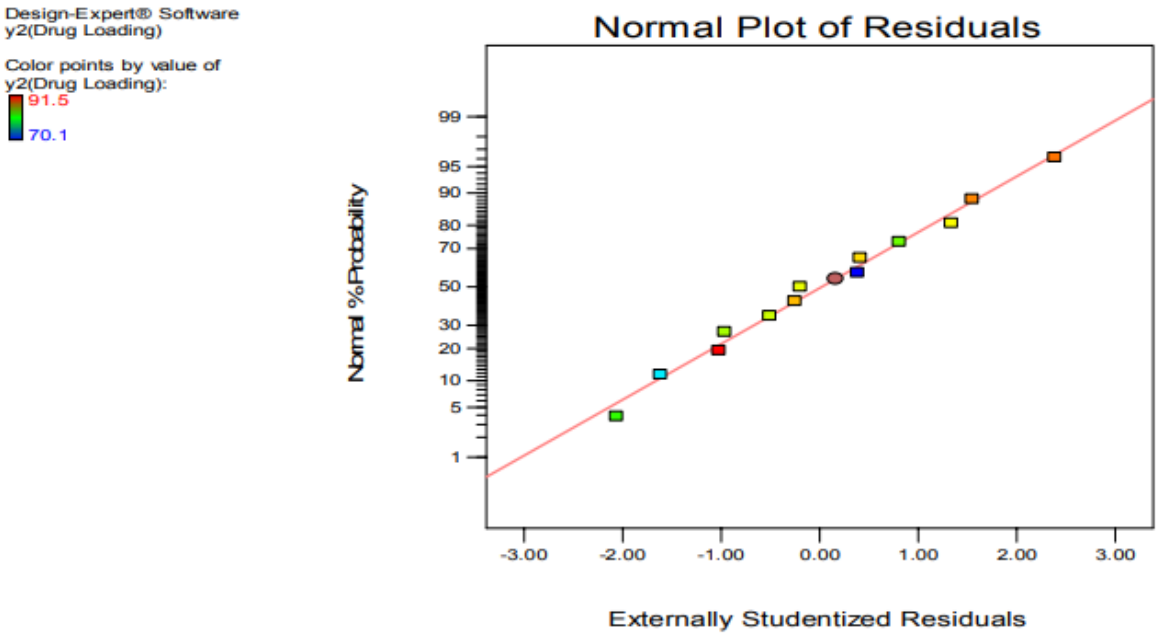


Fig. 6 Normal Residual plot Y_2 of Ezetimibe

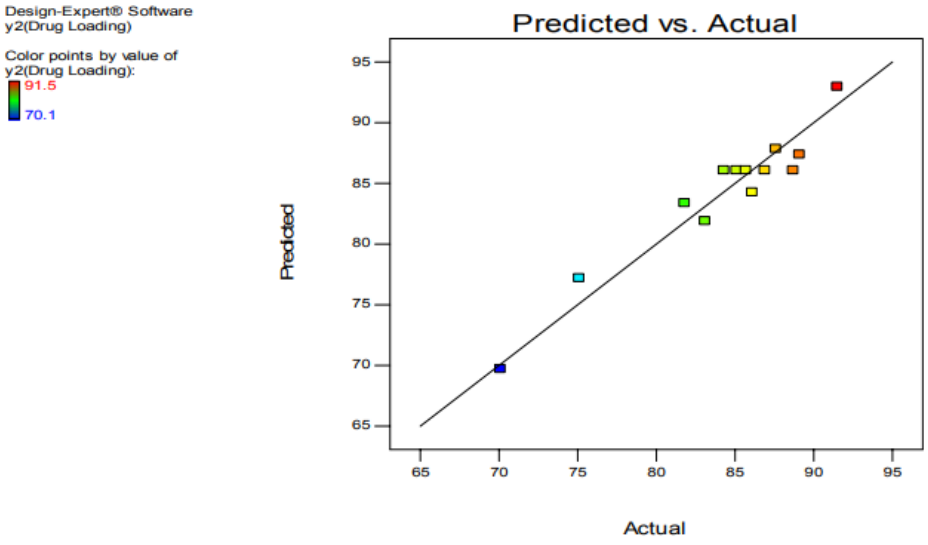


Fig. 7 Linear correlation plot Y_2 of Ezetimibe

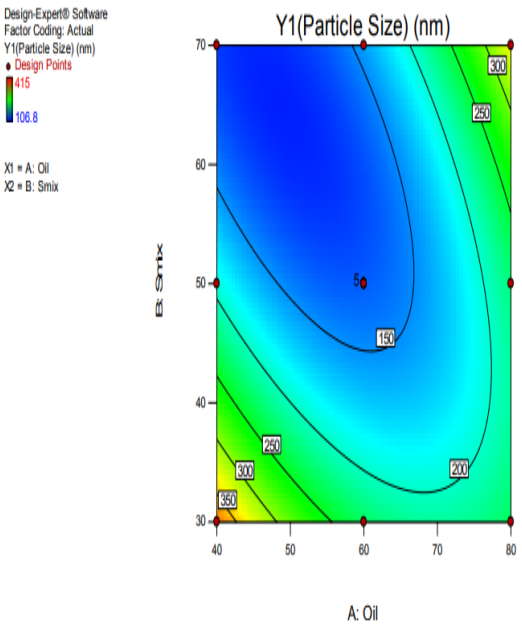


Fig 8 Contour plot Y_1 of Ezetimibe

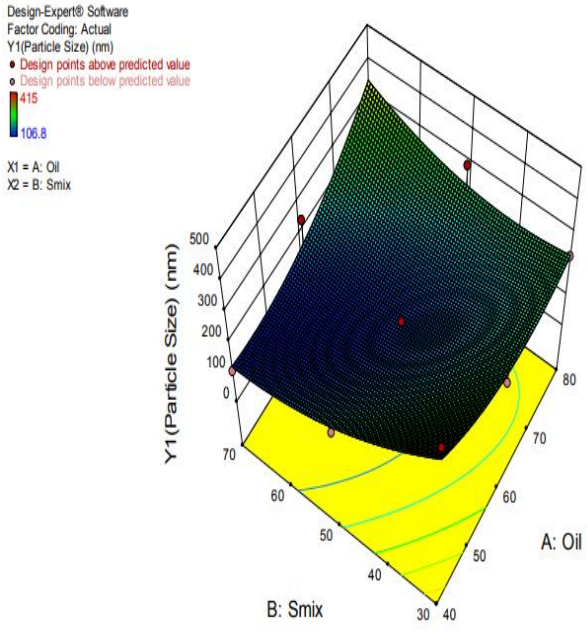


Fig 9 Response surface plot Y_1 of Ezetimibe

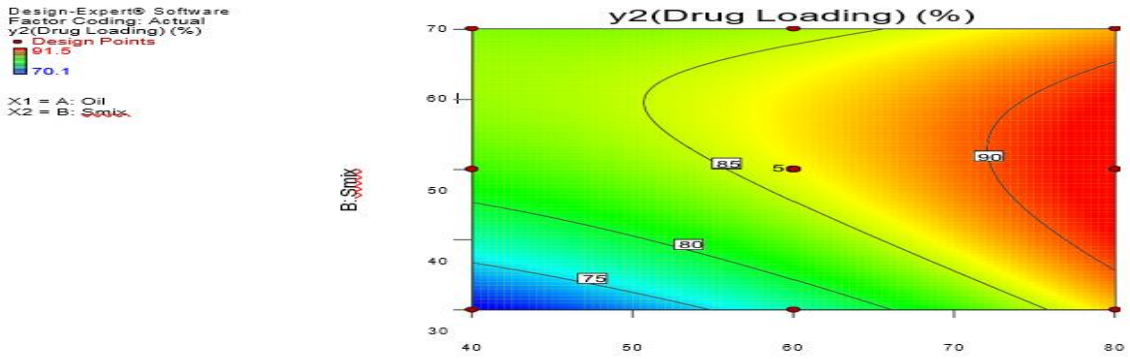


Fig 10 Contour plot Y_2 of Ezetimibe

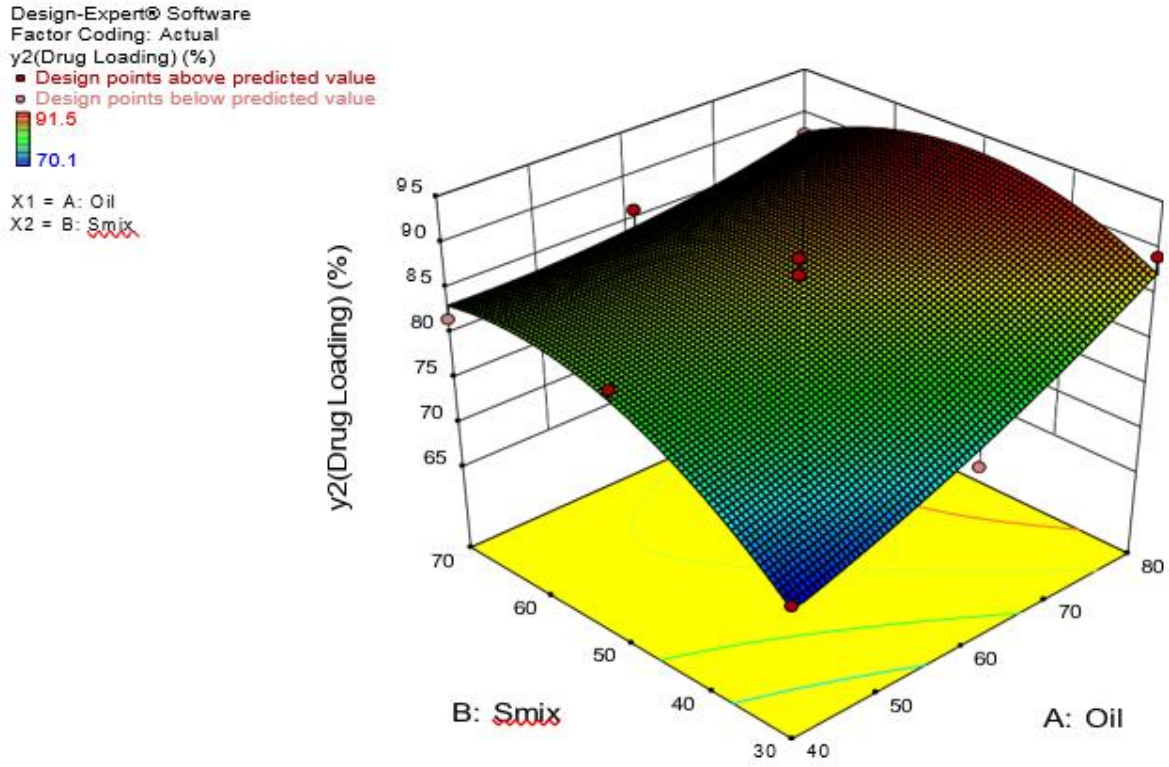


Fig 11 Response surface Y_2 of Ezetimibe

Optimization by using Desirability Function

The optimization criteria included minimum particle size and maximum drug loading in the range. The global desirability value was calculated by combining all the individual desirability functions as the geometric mean by using extensive grid and feasibility search over the domain. The suggested optimized formulation for Ezetimibe consisted of 67.586% oil, 52.529% Smix with the correspondingdesirability (D) value of 0.856 and the predicted response as Y_1=153.651nm, Y_2= 88.582.

Four batches of the optimized formulations were prepared to validate the model adequacy for the prediction, and all the responses were evaluated for each formulation as indicated. It can be concluded that the experimental values were in close agreement with predicted values, indicating the success of the design to evaluate and optimize the SEDDS formulation.

Table 8: Predicted and measured values of responses and corresponding biasness

Ezetimibe responses			
FC	**Particle size (nm)**		
	Predicted value	**Measured value**	**Biasnes %**
AF4	153.650	169.2±3.23	10.12
AF5	153.646	169.4±1.97	10.25
AF11	153.649	168.9±4.23	9.93
AF13	153.636	169.8±1.36	10.52
OPFA	153.651	169.7±3.2	10.45
AF4	88.572	87.2±1.23	1.55
AF5	88.571	87±2.18	1.77
AF11	88.584	86.9±3.24	1.90
AF13	88.586	87.1±2.27	1.68
OPFA	88.582	87.2±2.25	1.57

Biasness %= (predicted value-measured value) ×100/predicted value.

The canonical analysis in the Design Expert software is a mathematical tool for simplifying a second-order polynomial model and simultaneously observing the extreme values of several response surface models. Overlaid contour plots of SEDDS were constructed by two independent variables. The overlaid plots for two response values are illustrated in Fig for Ezetimibe. According to the criteria in present study higher drug loading and lower particle size of the optimized formulation of Atorvastatin SEDDS containing oiland Smix were selected at 67.5761% and 52.5328 %. The particle size and % drug loading of the optimized formulation for Ezetimibe were predicted to be 153.597nm and 88.5782 % as illustrated.

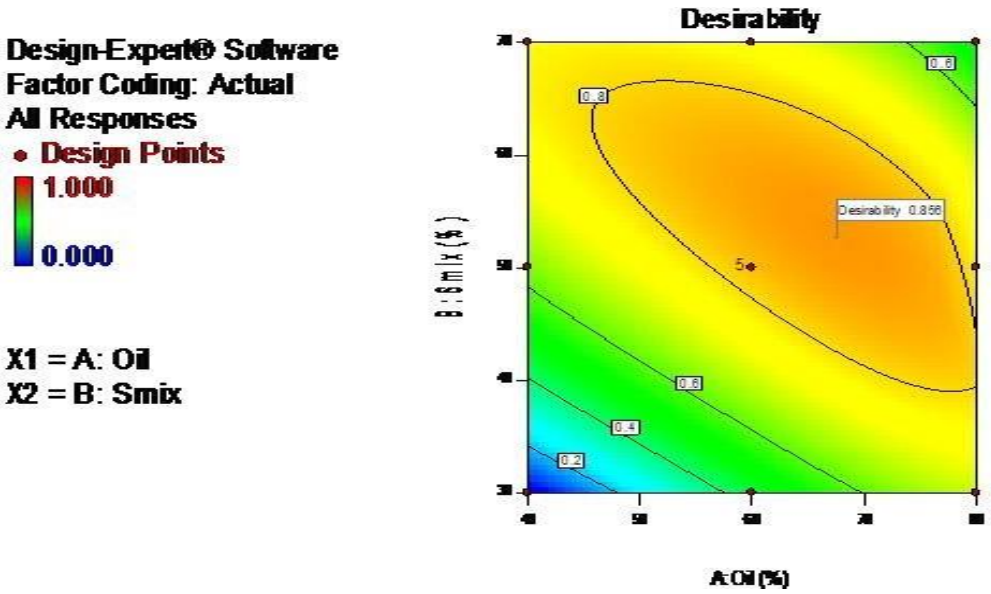


Fig.12: Desirability plot of Ezetimibe SEDDS optimized formulationOPFA

Self-emulsification, drug precipitation and phase separation studies

For all the SEDDS formulations the visual observation of self-emulsification study was recorded and evaluated on visibility grades as explained in section

. The results of graded formulations.

In this study, formulations AF4, AF5, AF11, AF13, OPFA (optimized formulations) for Ezetimibe.

Table 9: Self -emulsification and drug precipitation of EzetimibeSEDDS

Formulation Code	Visibility grade	Phase separation	Precipitation
AF1	IV	+	++
AF2	III	+	++
AF3	IV	+	++
AF4*	I	X	XX
AF5*	II	X	XX
AF6	III	+	++
AF7	IV	X	++
AF8	V	+	++
AF9	III	+	++
AF10	IV	+	++
AF11*	I	X	XX
AF12	III	+	++
AF13*	II	X	XX
OPFA	I	X	XX

X -- No phase separation, XX -- No precipitation, + -- phase separation and ++ -- precipitation.

ASSESSMENT OF EMULSIFICATION TIME STUDIES

The ease of emulsification was suggested to be related to the ease of water penetration into the colloidal or gel phases formed on the surface of the droplet.

Table 10: Refractive index, Turbidity, Optical clarity, Polydispersity index, Viscosity, Cloud point measurement and Emulsification time of SEDDS formulations of Ezetimibe

FC	Refractive Index ± SD (n=3)	Turbidity (NTU)	Absorbance	Polydispersity index ±SD (n=3)	Viscosity (cps) ±SD(n=3)	Cloud point measurement (□C) ± SD(n=3)	Emulsification time (sec)
AF1	1.3343±0.0006	132	0.402	0.171±0.01	253±2.65	78±3.46	132
AF2	1.3352±0.0003	146	0.487	0.244±0.005	262±2.66	73±3.61	119
AF3	1.3366±0.0005	210	0.529	1.097±0.2	264±1.73	75±5.57	121
AF4*	1.3331±0.0002	90	0.455	0.381±0.03	280±2.31	77±3.46	138
AF5*	1.3334±0.0002	94	0.432	0.377±0.06	291±3.51	74±3.46	126
AF6	1.3345±0.0003	168	0.517	0.148±0.012	272±4.58	78±5.20	112
AF7	1.3363±0.0006	320	0.456	0.379±0.06	269±2.89	75±3.61	95
AF8	1.3358±0.0004	357	0.493	0.292±0.03	254±2.66	75±4.36	82
AF9	1.3349±0.0004	92	0.501	0.128±0.04	249±2.08	79±4.58	75
AF10	1.3347±0.0006	96	0.497	0.386±0.04	263±0.56	77±5.20	62
AF11*	1.3330±0.0003	91	0.466	0.343±0.065	259±1.53	75±3.61	64
AF12	1.3352±0.0002	93	0.629	0.224±0.005	266±4.04	76±2.65	67
AF13*	1.3333±0.0002	95	0.452	0.333±0.005	260±3.56	75±1.73	69
OPFA	1.3330±0.0002	92	0.425	0.2±0.013	258±2.23	72±1.28	61

SPECTROSCOPIC CHARACTERIZATION OF OPTICAL CLARITY

The absorbance of the studied aqueous dispersion of Ezetimibe SEDDS ranged between 0.402 to 0.529 which indicates that optically clear and oil droplets formed are to be in a state of finer dispersion.

TURBIDITY MEASUREMENT

The turbidity of SEDDS was performed determined as per procedure and turbidity for all optimized formulations were found to below 100NTU which shows the stability of SEDDS.

VISCOSITY DETERMINATION

From viscosity determination, it was observed that as the concentration of oil increased, viscosity of formulations decreased. Overall, the viscosity of the undiluted liquid SNEDDS was found less than 10,000 cpswhich imply that the developed SEDDS can be filled in soft gelatin capsules.

CLOUD POINT MEASUREMENT

For all the formulations the cloud point was found to be below 80□C. From the above result, it can be concluded that a stable micro emulsion of SEDDS can be formed at physiological temperature *in- vivo.*

DETERMINATION OF REFRACTIVE INDEX (RI)

The RI of the prepared formulations was determined using Abbe refractometer. It is indicated from the results that the isotropic nature of the formulations was found to be in range of 1.3330±0.0002 to 1.3366 ± 0.0005 for Ezetimibe. The closure of the formulations RI value to water indicated the transparency property of the formulations. The results indicated that RI values increased with increase in concentration of oil and corresponding decrease in aqueous content. AF3 exhibited the highest RI value of 1.3366 ± 0.0005 for Ezetimibe in which the oil concentration was 80% as indicated.

DROPLET SIZE, ZETA POTENTIAL AND POLYDISPERSITYINDEX (PDI) ANALYSIS

The PDI for all the formulations were less than 0.5 (AF3-1.097) and the formulations with Smix showed lower PDI values thus indicating the uniform size distribution. The results of PDI were shown**.** After drug addition there was no significant difference in PDI values indicating no interference of the drug with the performance of the spontaneous emulsification.

Among the formulations the optimized Ezetimibe SEDDS (OPFA) was found to have a mean globule size of 169.7nm with a PDI 0.2, and zeta potential -31.8mV.

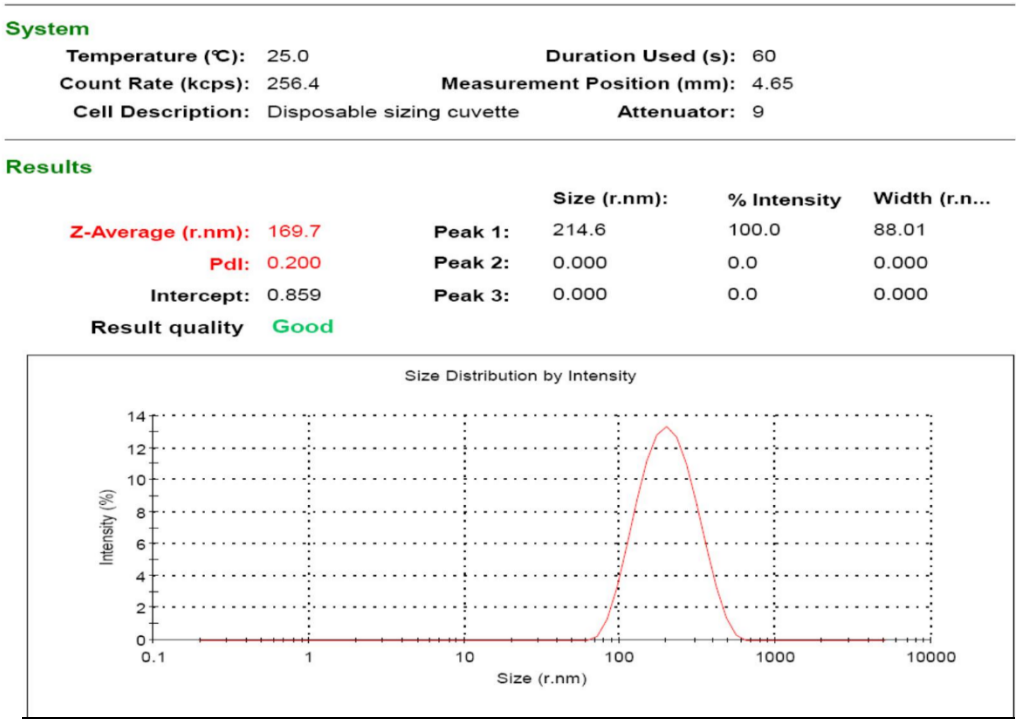


Fig. 13 Particle size of optimized formulation OPFA for EzetimibeSEDDS

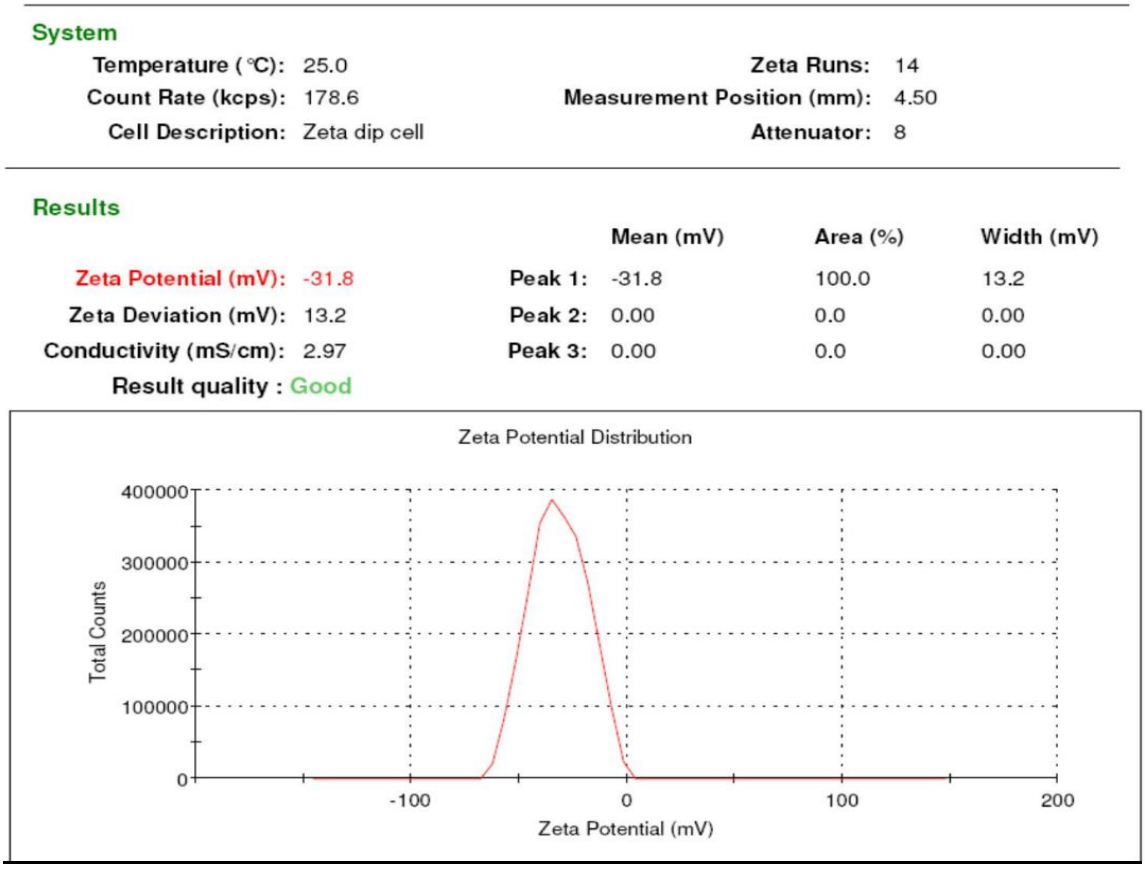


Fig. 14 Zeta potential of optimized formulation OPFA for EzetimibeSEDDS

DRUG LOADING

The Atorvastatin SEDDS formulations were subjected to drug loading studies. The drug content was carried out by UV-visible spectrophotometer (Shimadzu UV-1700) and the drug loading was performed as per procedure described in section 5.12.11. A linear calibration curve was obtained at 247nm for Ezetimibe ith a correlation coefficient (r^2) of 0.999.The drug content of Ezetimibe was calculated from the Beers Lambert's law equation Y = 0.045.concentration + 0.003 (r^2 = 0.999; p < 0.001)]. It was clearly inferred increase in Smix concentration enhances maximum drug load in SEDDS.

***IN VITRO* DISSOLUTION STUDIES**

The *in vitro* drug release studies were performed as per procedure described under 5.12.12 for Ezetimibe SEDDS. The *in vitro* dissolution profile of Ezetimibe optimizedformulations OPFA, AF4, AF5, AF11 and AF13 carried out by USP II dissolution apparatus in phosphate buffer pH 6.8. It could be suggested that spontaneous micro-emulsification resulted in the faster rate of drug release into the aqueous phase in the form of small and mono dispersed droplets[139]. The drug content was calculated from the Beers Lambert's law equation of Y =0.012.concentration+0.001 (r^2 = 0.999; P < 0.001) for Ezetimibe

Table 11: Cumulative percent release of Ezetimibe from various formulations

Time in min	AF1*	AF5*	AF11*	AF13*	OPFA SEDDS	API	Marketed Tablet
0	0	0	0	0	0	0	0
5	29.56±0.69	28.89±0.88	27.45±0.59	25.56±1.25	26.21±0.74	38.69±1.24	33.21±2.03
10	34.58±2.08	38.56±0.63	33.46±1.28	32.45±0.19	39.3±0.23	47.56±0.75	45.23±1.12
20	52.56±1	55.33±2.02	56.59±0.56	57.53±0.73	58.36±0.45	65.22±1.12	60.33±2.21
30	74.23±1.59	73.52±1.94	75.56±1.50	74.87±0.22	72.66±0.32	80.45±1.23	79.54±1.64
40	76.89±1.38	76.26±0.55	77.62±1.20	78.66±0.16	79.5±0.18	86.23±1.56	85.62±0.54
50	84.98±1.27	82.56±1.16	83.32±1.30	84.98±0.02	86.72±0.16	89.21±2.73	86.74±2.21
60	91.26±2.74	90.21±1.48	90.36±0.17	91.63±0.44	91.3±0.55	92.34±1.23	90.69±1.72
75	92.27±1.78	92.24±2.55	92.48±0.56	93.56±1.22	94.5±0.49	93.86±0.62	92.66±1.54
90	95.85±1.30	96.16±0.72	97.28±1.13	98.56±0.44	99.75±0.31	95.64±1.26	93.31±1.18

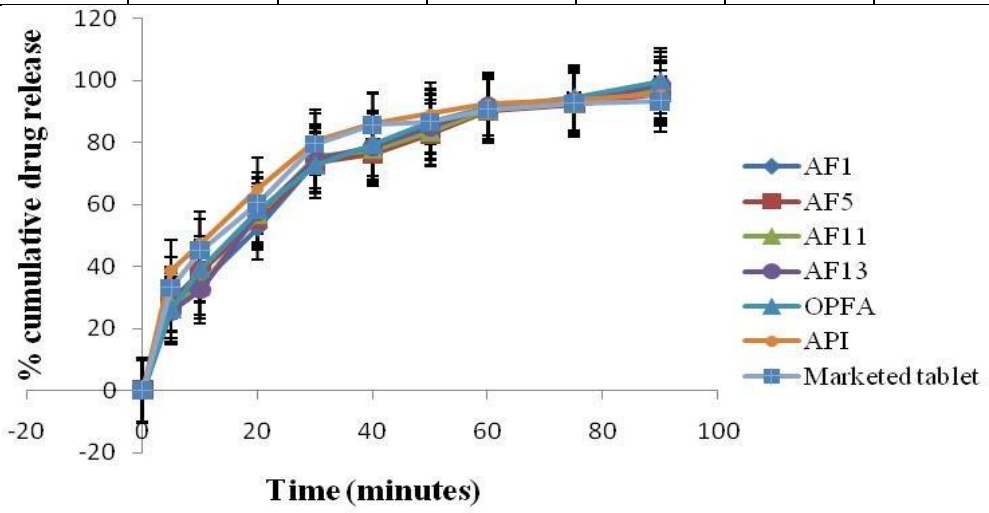


Fig 15 Dissolution comparison graph of API, marketed and optimizedformulation of Ezetimibe SEDDS

Kinetic modeling and Mechanism of drug release of optimized formulations

The dissolution data of optimized formulations OPFA and OPFG showed first order release kinetics with higher correlation coefficient R^2-0.9848 for Ezetimibe. *In vitro* release kinetics data were computed using DD solver and the resultant data were fitted to the Korsmeyer-Peppas exponential equation to establish the mechanism of drug release. The exponent, n has been proposed as indicative of the release mechanism. The 'n' values for OPFA and OPFG was found to be 0.406 and 0.024 which suggested that drug release follows Fickian diffusion controlled mechanism for Ezetimibe.

Table 12: Release kinetic study of optimized formulations for Ezetimibe

FC	Zero order kinetic R^2	First order kinetic R^2	Higuchi Kinetic R^2	Korsmeyer-Peppas	
				R^2	n value
OPFA	0.9569	0.9848	0.9366	0.9701	0.406
OPFG	0.9569	0.9978	0.9519	0.8821	0.024

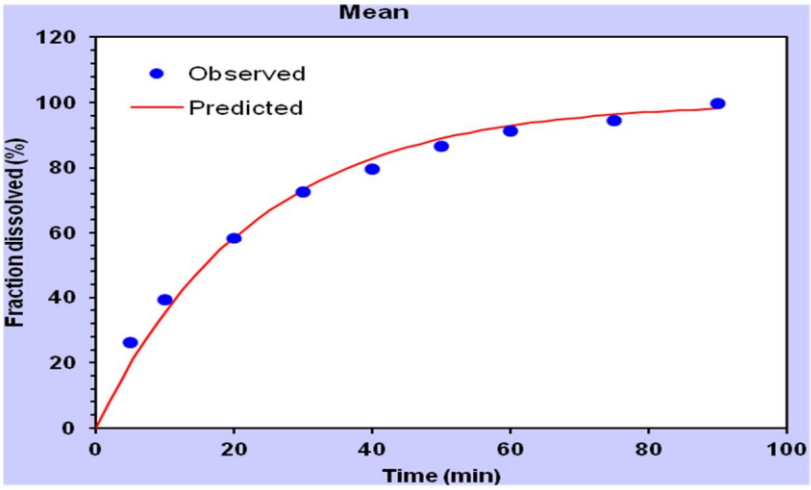


Fig.16. Dissolution first order release kinetics of optimized formulation OPFA

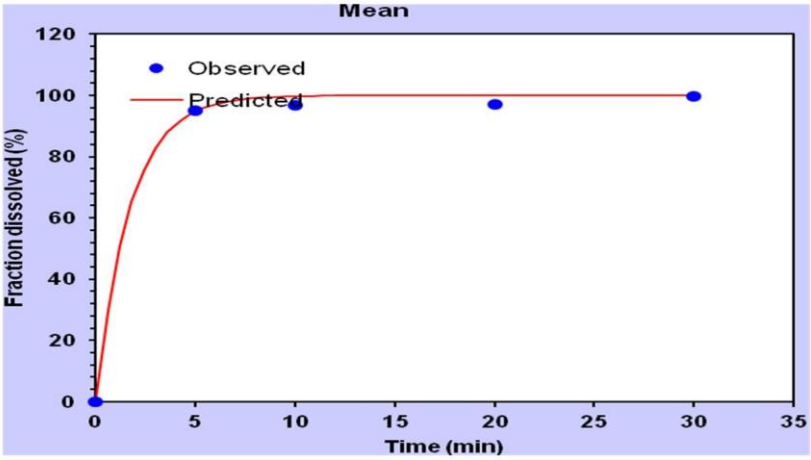


Fig.17. Dissolution first order release kinetics of optimized formulation OPFG

***IN VITRO* DIFFUSION RELEASE STUDY**

Diffusion study was carried out to study the release behavior of formulation from liquid crystalline phase around the droplet using dialysis technique. *In vitro* diffusion profile of Ezetimibe from optimized SEDDS in phosphate buffer (pH 6.8) is given in Table. It was observed that at the end of 12 hour, formulation OPFA SEDDS showed about 99.24% diffusion due to its nano range globule size and presence of surfactant/co-surfactant. It was observed that at the end of 24h, formulation OPFG SEDDS showed about 99.8% and 96.23% for the marketed tablet at the end of 2 hours (Table data only for 2 hours).

Table 13: Percent cumulative drug absorbed through dialysis membrane ofoptimized Ezetimibe SEDDS formulations

Time in hours	AF4*	AF5*	AF11*	AF13*	OPFA SEDDS	Marketed Tablet
0	0	0	0	0	0	0
0.5	82.19±1.23	84.93±1.54	83.45±0.76	82.31±0.78	89.32±2.17	81.25±2.25
1	92.19±0.78	93.42±2.78	92.64±1.23	91.89±0.98	92.22±0.91	90 ±1.14
2	93.75±1.84	94.23±1.66	93.62±2.46	93.16±1.19	93.43±1.56	92±1.98
4	94.94±2.21	94.45±2.56	94.89±0.78	94.23±2.56	95.36±2.45	94 ±2.54
6	96.28±0.73	96.82±0.84	96.4±0.92	96.45±0.74	96.39±1.47	95 ±2.69
8	97.67±0.94	97.14±2.41	97.54±1.47	97.67±1.64	98.56±0.95	96.9±1.85
12	98.45±1.86	98.25±1.78	98.23±2.82	98.21±2.47	99.24±2.26	98.18±0.99

STABILITY STUDIES

The optimized SEDDS of Ezetimibe and Gibenclamide were loadedin soft gelatin capsules (Size 3).They were stored under cold condition (4-8□C) at refrigerator and room temperature (25□C) were subjected to stability studies to evaluate their stability and the integrity of the dosage form. The samples were also charged at elevated temperature of 50□C in stability chambers (Labtech India) with ambient humidity condition.

The formulations were found to be stable at cold, room temperature and at elevated temperatures when the samples were analyzed for its particle size and % drug loading after first and 6 months. It was also seen that the formulation was compatible with the soft gelatin capsule shells, as there was no sign of capsule shell deformation. Furthermorethe formulation was found to show no phase separation and drug precipitation. Thus, the studies confirmed the stability of the developed formulation and its compatibility with soft gelatin capsules.

Table 14: Stability studies of optimized Ezetimibe SEDDSformulations

Temperature (□C)	Particle Size (nm)		% drug load	
	After 1 Month	After 6 month	After 1 month	After 6 month
Cold Temperature (2 -8□C)	173±2.23	176± 1.23	87.2±1.33	83.7±1.89
Room Temperature (25±2□C)	169.7±1.85	171.7±0.86	88.9±2.24	86.2±2.65
Elevated Temperature (50±2□C)	170±2.35	175.6±1.56	85.9±1.42	81.9±2.78

Data expressed as mean ± SD, n=3

SUMMARY AND CONCLUSION:

The purpose of this study was to develop an oral administrable SEDDS of poorly water-soluble drugs of Ezetimibe under Biopharmaceutical classification system of class II classification. Solubility evaluation and ternary phase diagram were carried out to select excipients of SEDDS. The composition of Ezetimibe loaded loaded SEDDS was optimized using 3^2 facorial design. The impact of the formulation parameters on mean globule size and percentage drug load were studied by applying the analysis of variance and regression models. Several formulation and process variables were evaluated and optimized by response surface methodology. The optimum formulation was prepared by response optimizer through desirability function and the experimental values were found to be in close agreement with the predicted values. Optimized formulation was further subjected to stability studies. Optimal Ezetimibe SEDDS contains sunflower oil as oil phase, labrasol as a surfactant and transcutol HP as cosurfactant (Smix) in the ratio of 67.586% oil and 52.529% % w/w Smix formulates SEDDS with lower droplet size (169.7nm), PDI (0.2), and zeta potential (-31.8 mv) and percentage drug load (87.2%) values.. It was concluded that the smaller particle size and drug load more the release of drug which results in better bioavailability. The *in vitro* evaluation parameters such as emulsification time, viscosity determination, cloud point measurement, turbidity measurement, refractive index and spectroscopic optical clarity test were performed and the results were foundwithin the limits for all formulations of two drugs. The stability studies revealed that there was no change in particle size and percentage drug load for the two drugs after 6months. The *in vitro* drug release from optimized Atorvastatin SEDDS formulation were found to be 99.75% after 90 min. It was extremely higher in comparison to the marketed formulation and API suspension. *In-vitro* drug release studies closely indicate that optimized formulations obey first order kinetics and the mechanism of drug release was by fickian diffusion. The results further concluded that SEDDS can be explored as a potential drug carrier for dissolution enhancement of Atorvastatin other poorly soluble drugs.

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