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FORMULATION OPTIMIZATION AND EVALUATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEM OF ATORVASTATIN CALCIUM BY USING DESIGN OF EXPERIMENTS

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ABSTRACT

The aim of the present research was to prepare, evaluate and optimize self emulsifying drug delivery system (SEDDS) of atorvastatin calcium of poor water solubility using design of experiment. A 13-run 3^2 full factorial design with 2 factors and 3 levels, including 4 replicates at the centre point, was used for fitting a 2^{nd} order response surface. After preliminary screening, Sunflower oil, Labrasol as surfactant and Transcutol HP as Co surfactant were taken as independent variables. The dependent factors (responses) were particle size and percentage drug load. The responses were optimized simultaneously by using desirability function, and the results demonstrated marked main and interaction effects of independent factors on responses. The optimized formulation consisted of 67.586% (w/w) oil, 52.529% w/w Smix showed measured responses of particle size 169.7 nm, Percentage drug Load of 87.2%, polydispersity index of 0.2 and Zeta potential of -31.8 mV values. For the optimized formulation, predicted value and experimental value were in close agreement. The *in vitro*

evaluation parameters such as emulsification time, viscosity determination, cloud point measurement, turbidity measurement, refractive index and spectroscopic optical clarity were measured. The *in vitro* drug release from optimized atorvastatin SEDDS formulation was found to be 99.75% after 90 minutes which was highly significant in comparison to the marketed tablet and pure drug. Drug release kinetic of optimized batch showed first order with fickian diffusion type drug release. The optimized formulation was stable at varied temperatures of stability study.

KEYWORDS: Formulation, optimization, evaluation, design of experiments, atorvastatin calcium, self emulsifying drug delivery system.

INTRODUCTION

Dosage form designing for any drug is based on the route of administration. Among the different routes, oral administration is the most preferred throughout the world for patients of all age groups. Though there are numerous advantages for per oral route; there are certain limitations. As per published reports almost 40% of the new chemical entities (NCE) are underutilized due to unfavorable physiochemical properties. The solubility of the NCE to be used as drug plays a crucial role in the final bioavailability. Based on different parameters, drugs are classified under Biopharmaceutical Classification system (BCS). Formulation pharmacist aspires to increase bioavailability through different approaches to increase solubility and bioavailability which includes complexation with cyclodextrins, solid dispersion (suspension), co precipitation, micronisation, salt formation, emulsion, use of micelles, and co grinding. The latest development among the various approaches is to utilize solutions in lipid vehicles containing surfactants that constitute a self-emulsifying drug delivery system (SEDDS). A widely utilized approach for overcoming poor fasted state bioavailability of lipophilic drugs is to effect spontaneous emulsification upon contact of the oil with fluids in the G.I. tract.[1] SEDDSs are isotropic mixtures of oils and surfactants; sometimes it contains co-solvents, and it can be used for the design of formulations in order to improve the oral absorption of highly lipophilic compounds. SEDDSs emulsify spontaneously to produce fine oil in-water emulsions when introduced into an aqueous phase under gentle agitation. SEDDS can be administered orally in soft gelatin capsules and form fine, relatively stable oil-in-water emulsions upon aqueous dilution. Self-emulsifying formulations spread readily in the gastrointestinal tract (GIT), and the GI motility of the stomach and the intestine provide the necessary agitation for self emulsification. These

systems have the advantage that the drug in dissolved form and the small droplet size provides a large interfacial area for the drug absorption. SEDDSs typically produce emulsions with a droplet size between few nanometers (100–300 nm) to several microns (< 5µm) while self micro emulsifying drug delivery systems (SMEDDS) form transparent micro-emulsions with a droplet size of less than 50 nm. SEDDS are physically stable formulations that are easy to manufacture, but when compared with emulsions, which are metastable dispersed forms. The statistical Design of Experiments (DoE) matrix based multi factor method is a systematic alternative to single variable experimentation which constructs useful predictive model to optimize the levels of each critical variable and comes up with the best possible (optimum) combination of excipients and the processes within the total multidimensional experimental region. This is in contrast to the traditional one factor at a time (OFAT) method of optimization which doesn't take interaction and quadratic effects of formulation or process variables into consideration and is generally based on trial-and-error method. DoE is also used in testing robustness of the manufacturing process.[2] Hence, the value of DoE for screening, investigating and optimizing experimental parameters, minimizing operational cycle times, including time to obtain regulatory approval, and direct cost saving cannot be disputed. In the present study, self emulsifying drug delivery system (SEDDS) of atorvastatin calcium, a Biopharmaceutical Classification System (BCS) class II, antihypertensive drug was chosen as a formulation system and 3^2 factorial designs, a statistical DoE which uses response surface methodology (RSM) was used to understand and optimize the formulation system. However, it is reported that the absolute bioavailability (*F*) of atorvastatin is 12% after a 40 mg oral dose.[3] The aim of the present research work was to systematically investigate the main, interaction and the quadratic effects of formulation variables (independent variables) of SEDDS on desired responses; and to develop a model that would yield an optimized SEDDS of atorvastatin calcium. A 13 run 3^2 factorial design with 2 factors and 3 levels, including 4 replicates at the centre point, was used for fitting a 2nd-order response surface. The estimation of the coefficients for the second order polynomial model was performed by regression analysis and the model adequacy was checked by an *F*-test and the determination coefficient (R^2). All the responses were optimized simultaneously by using desirability function. An *in vitro* drug release study was performed and it was compared with marketed tablet and pure drug.

MATERIALS AND METHODS

Atorvastatin calcium was supplied by Goodman Pharmaceuticals (Pondicherry, India). Capryol PGMC, transcutol HP, peceol, labrasol, labrafil M 1944 CS, labrafil M 2125 CS were obtained as free gift samples from Gattefosse (Saint-Priest Cedex, France).Virgin sesame oil, virgin coconut oil, olive oil, mustard oil, sunflower oil, rice bran oil and corn oil were purchased from local market. The dialysis membrane of molecular mass cut off 12,000-14,000 daltons; pore diameter of 2.4 nm and dialysis membrane clips were purchased from (Himedia Labs Pvt Ltd, Mumbai). All the other chemicals were of analytical reagent grade and used as received without purification.

UV spectroscopy method: The standard plot of atorvastatin calcium in methanol with the concentrations of 2, 4, 6, 8, 10, 12 µg/ml was measured at 247 nm by UV double beam spectrophotometer (Shimadzu). The calibration equation for the standard plot was found to be Y = 0.045x + 0.003 and the regression coefficient (R2 = 0.999) was used for all calculations. The results of the standard curve preparation are shown in Table 1 and Figure 1.

Table 1 Data of atorvastatin calcium calibration curve by UV spectroscopy method

Concentration (µg/ml)	2	4	6	8	10	12
Absorbance	0.0913	0.1908	0.2836	0.3774	0.4625	0.5465

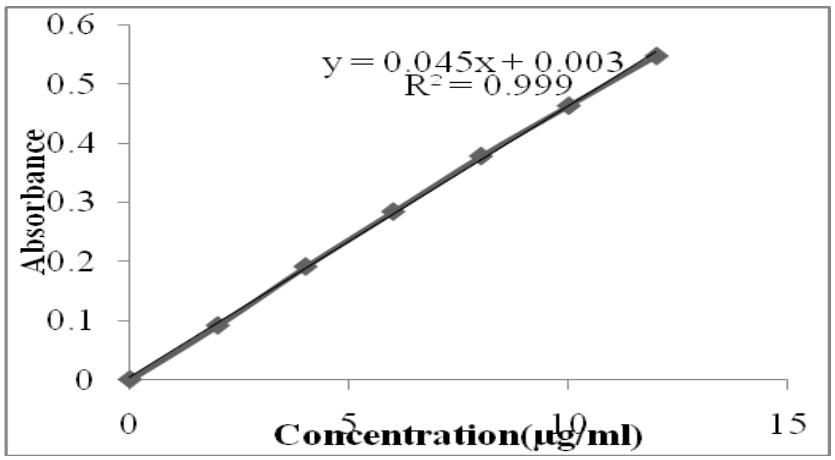

Fig. 1 calibration curve of Atorvastatin Calcium

Screening of excipients

Solubility studies: The solubility of atorvastatin calcium in various oils, surfactants, co-surfactants was measured using shake flask method.[4] An excess amount of Atorvastatin calcium (approximately 200 mg) was added to 2 ml of each of vehicle in screw capped glass vials followed by vortex mixing for 30 sec using vortex mixer. Mixtures were shaken for 48 h

at 30^0C in a thermostatically controlled shaking water bath, followed by equilibrium for 24 hr. Mixtures were then centrifuged at 3000 rpm for 10 min and the supernatant was filtered through a millipore membrane filter (0.45µ). Samples were suitably diluted with methanol and the final drug concentration was obtained by UV spectroscopic method at 247 nm. The experiment was repeated in triplicates. The results are represented as mean value (mg/ml) ± SD.

Construction of ternary phase diagram: Based on the results of saturation solubility studies in Table 2, sunflower oil, labrasol and transcutol HP for atorvastatin calcium were selected as oil, surfactant and co-surfactant respectively. The percentage limit of surfactant, co-surfactant and oil used herein was selected by considering their acceptable safe dose and decided on the basis of the requirements stated according to the lipid formulation classification system introduced by Pouton.[5] A ternary phase diagram was constructed for the system containing oil-surfactant-co-surfactant by Chemix School software version 3.51. The grading method reported by Craig *et al.*[6] was modified and adopted in this study. A series of self emulsifying systems were prepared with varying weight percentage of oil, surfactant and co-surfactant. Since the drug incorporated in the SEDDS may have some effect on self emulsion boundary, every system in the series also consisted of 10% w/w for atorvastatin calcium. The extreme and middle level of the independent variables consisting of oil, surfactant and co-surfactant were selected for further study.

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 (vortex mixer-Spinix, Japan) and sonicating (bath sonicator-B.N.Scientific Enterprise, India) and then by adding oil.[7] 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 3. Then the final formulation was equilibrated in water bath at 37°C for 48 h before carrying out the droplet size, polydispersity index (PDI) 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 in Table 3a and Table 3b. 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), 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–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).

Evaluation of prepared SEDDS

Self Emulsification, Drug precipitation, Phase Separation and Assessment of emulsification time: The different compositions of self emulsifying drug delivery system (SEDDS) were categorized on speed of emulsification, clarity, and apparent stability of the resultant emulsion. Visual assessment was performed by drop wise addition of the preconcentrate (SEDDS) into 100ml of distilled water. This was done in a glass beaker at room temperature, and the contents were gently stirred with glass rod. Precipitation was evaluated by visual inspection of the resultant emulsion after 24 hours. The formulations were then categorized as clear transparent or transparent with bluish tinge (Grade I), bluish white appearance (Grade II), bright white emulsion (Grade III), non clear dull grey white emulsion with a slight oil appearance (Grade-IV), turbid appearance (Grade V), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours).

The emulsification time of SEDDS formulations was determined in a USP dissolution tester Type II (Electrolab, India). The SEDDS formulation equivalent to single dose of the drug was added drop-wise to 500 mL of distilled water maintained at 37 ± 0.5°C. Gentle agitation was provided by a paddle rotating at 50 rpm. The emulsification time was recorded manually.[8] The phase separation study was performed by subjecting the optimized formulation at elevated temperature (50±2°C) and centrifugation (Centrifuge-Eppendorff 5415D at 5000rpm for 5 min.

Spectroscopic characterization of optical clarity: Each SEDDS formulation equivalent to single dose of atorvastatin calcium were diluted with 500 mL of distilled water. The absorbance values of each emulsion at 0, 10, 20, and 30 min post-dilutions were measured by a UV spectrophotometer (Shimadzu) at 400 nm.[9]

Turbidity measurement: The turbidity measurements in nephlometric turbidity unit (NTU) were performed on the resultant emulsion stored in a screw capped sample vials using turbidimeter (Elico D-10-model 331, Japan). 0.5 ml of the SEDDS formulation was introduced into 250 ml of distilled water in 500 ml conical flask under action of magnetic stirrer (Remi instruments) rotating at constant speed. The emulsification was done at room temperature.[10]

Viscosity determination: The viscosity of the prepared SEDDS formulations as such without being diluted was measured by Brookfield viscometer (Brookfield DV-III Ultra Rheometer) using spindle C 16-1 at 25±0.5 ˚C.[11]

Cloud point measurement

The formulated SEDDS was diluted with 50ml water in a beaker which is placed on a water bath with gradually increasing temperature until the diluted formulation turned cloudy. It mainly insists about the stability of microemulsion at body temperature.[12]

Determination of refractive index

The clarity of microemulsion could be estimated by measuring the refractive index of the formulations.[13] The SEDDS formulations were diluted 100 times with water. The refractive index of the system was measured by an Abbe refractometer (Remi instruments) by placing 1 drop of solution on the slide and it compare with water.

Droplet size, zeta potential and polydispersity index (PDI) analysis

The mean droplet size, zeta potential and polydispersity index of formulations was determined by using Malvern Nano Zeta sizer-90 (Malvern Instruments Ltd., Malvern, UK). Light scattering was monitored at 25^{o}C at a 90^{o} angle.[14] The dispersed formulations were measured after dilutions (1:100). Each determination was done in triplicate. Droplet size distribution of all batches and the zeta potential of the final microemulsions were determined immediately using particle size and zeta potential analyzer.

Drug loading efficiency

The prepared SEDDS containing equivalent to one dose of the drug was added in 50ml volumetric flask containing methanol and mixed well. The extracted solution was suitably diluted and analyzed for its drug content using UV visible spectrophotometer.[15]

$$\text{Drug loading efficiency} = \frac{\text{Amount of drug in known amount of formulation}}{\text{Initial drug load}} \times 100$$

***In vitro* dissolution studies for atorvastatin calcium**

The optimized SEDDS formulations were filled into soft gelatin capsules and were stored at room temperature for 24 h to allow complete solidification of the systems before use. The *in vitro* drug release[16] profiles of atorvastatin of SEDDS, plain atorvastatin, and marketed atorvastatin calcium tablet (Storvas 10mg Ranbaxy Laboratories Ltd) were studied using USP dissolution apparatus II **(**Electrolab**).** The dissolution medium consisted of 900 mL of freshly prepared pH 6.8 phosphate buffer maintained at 37 ± 0.5°C and the speed of the paddle was set at 100 rpm. Capsules were held to the bottom of the vessel using copper sinkers. At regular time intervals, 5 ml samples were withdrawn and replaced with equal volumes of fresh medium to maintain the volume and sink conditions. Samples were then filtered using a membrane filter (0.45 µm, Whatman) and drug concentration was obtained by UV spectroscopic method at 247nm. All measurements were done in triplicate.

Stability Studies

The optimized SEDDS was stored under cold condition (4-8°C) at refrigerator (Samsung), room temperature and at elevated temperature (50±2°C) at stability chamber (Humidity chamber-Labtech). After every 1 month samples were analyzed for phase separation, transparency, globule size% drug load.[17]

RESULTS AND DISCUSSIONS

Solubility Study

The solubility of the drug in the various vehicles is presented in Table 2 and figure 2. Sunflower oil (30.13±0.02 mg/mL), labrasol (89.23±0.015 mg/ mL) and transcutol HP (38.620±0.28 mg/mL) for atorvastatin calcium were chosen as oil (X_1), surfactant and cosurfactant (Smix-X_2) respectively.

Table 2 Solubility of Atorvastatin calcium in various excipients

S. No.	Excipients	Solubility (mg/ml)
1.	Virgin sesame oil	15.36±0.006
2.	Virgin coconut oil	25.37±0.015
3.	Sunflower oil	30.13±0.02
4.	Corn oil	4.86±0.030
5.	Mustard oil	10.35±0.01
6.	Rice bran oil	12.29±0.040
7.	Olive oil	17.62±0.010
8.	Peceol	12.84±0.021
9.	Labrasol	89.23±0.015
10.	Labrafil 1944CS	1.78±0.011
11.	Labrafil 2125	1.62±0.012
12	Capryol PGMC	2.22±0.006
13.	Transcutol HP	38.62±0.28

* Values are mean±S.D (n=3)

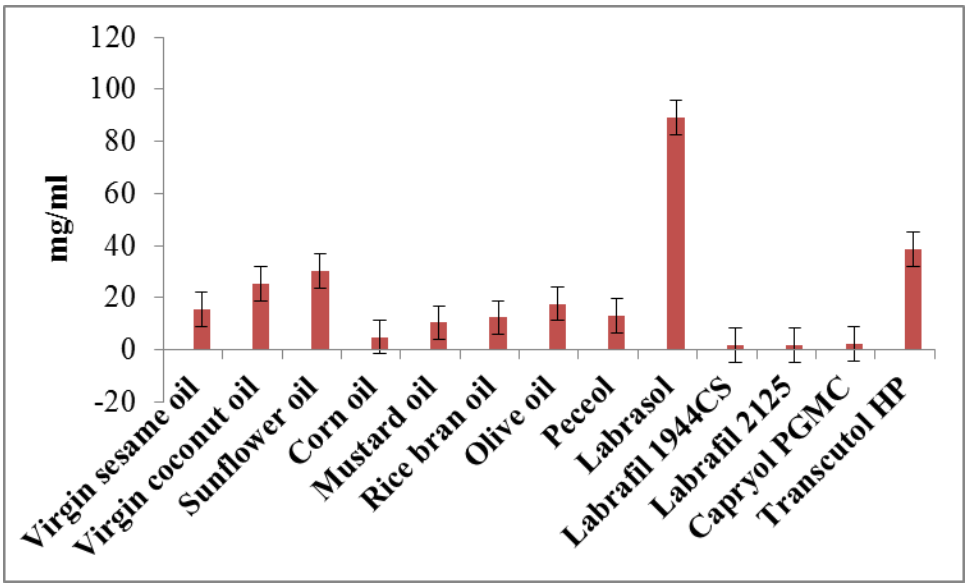

Fig. 2 Solubility profile of atorvastatin calcium

The components used in the SEDDS formulations solubilize the maximum amount of the drug which showed large self-emulsification domain in ternary phase diagram. Selection of the vehicles was also done considering the safety and compatibility of the excipients with soft gelatin capsule.

Construction of ternary phase diagram: The blue-shaded region in the ternary phase diagram (Figure 3) represents the efficient self-emulsifying region where desired visual observation characteristics were observed for clarity of the solution, no phase separation,

rapidity and spontaneity of the emulsion formation. The range and level for each component (independent variables) was selected as: oil (40–80%), surfactant (22.5–52.5%), co-surfactant (7.5–17.5%) for atorvastatin as shown in Table 3. The red lines in the Figure 3 indicate the boundary of the level used in the 3^2 factorial design study and the polygonal area bounded by all the red lines indicate the region from which optimum formulation is to be selected. As the water is always in considerable abundance and oil volume fraction is low, it was safely supposed that only oil/water emulsion was formed, and no other dispersed and bicontinuous pseudophases were formed.

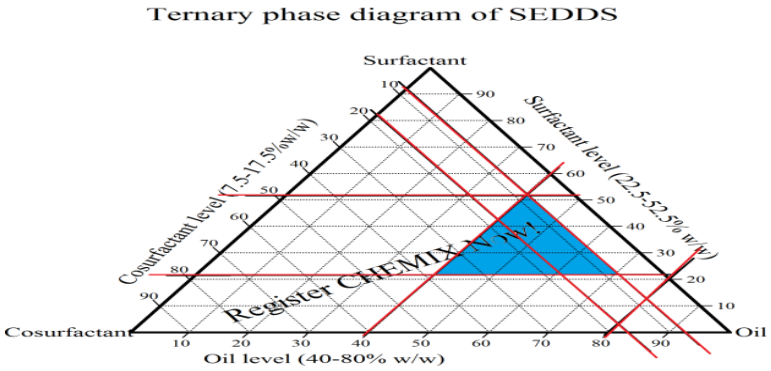

Fig. 3 Ternary phase diagram of Atorvastatin calcium

Blue-shaded region represents the self emulsifying domain and the red line indicates the levels taken in the 3^2 factorial design.

Table 3 Variables for atorvastatin calcium 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 Surfactant: Cosurfactant (3:1) of ratio 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

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 experiments and the observed

responses are summarized in Table 4. 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 response. Various statistical evaluations of models for each response are depicted in the Tables 5 and Table 6 for atorvastatin calcium. As shown in Table 5 at 5% significance level, it was observed that for responses Y_1, and Y_2, quadratic fitting was significant (p-value <0.05). For Y_1 response of atorvastatin calcium 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 Y_2 response of atorvastatin calcium response the lack of fit was The "Lack of Fit F-value" of 1.93 implies the Lack of Fit is not significant and there is a 26.69% chance that a "Lack of Fit F-value" this large could occur due to noise. 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 (Table 6) for atorvastatin calcium. 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 7. 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. For all the models the predicted R^2 value is reasonable agreement with the Adjusted R^2 value. Adequate precision values higher than 4 for all responses confirmed that the predicted models can be used to navigate the design space defined by full factorial design. A positive value represents an effect that favours the optimization and negative value indicates an inverse relationship between the factor and response.

Table 4 Execution of 3^2 experimental design and coding of actual values of independent variables for factorial design with the observed responses for atorvastatin calcium

Std	Run	Formulation Code (FC)	Oil (%)	Smix (%)	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

Table 5 Analysis of variance in the regression models for atorvastatin calcium

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 atorvastatin calcium

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 atorvastatin calcium

Factors	Y_1		Y_2	
	Coefficient	P Value	Coefficient	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 % drug load (Y_2) of Atorvastatin calcium SEDDS: The observed values of particle size for 13 formulations (Table 4) varied from 106.8 nm to 415 nm and % drug load varied from 70.1% to 91.5% for atorvastatin calcium. The polynomial quadratic equations for two dependent variables (particle size, % drug load) in coded factors are obtained according the data of experimental design and parameters (Table 4) for factorial 13 formulations.

The equations derived for particle size and Percentage drug load for atorvastatin calcium

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

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

The values of the coefficient X_1, X_2 were substituted in the equation to obtain the theoretical values of Y. The above equations 1 indicate as the factor X_2 increases the response Y_1 decreases and is indicated by negative coefficient value of dependant variable in which the Smix concentration is increased the particle size is decreased. The possible explanation is that when the lower concentration of oil and higher concentration of Smix added facilitate the increase in water penetration and the mixture become hydrophilic causing decrease in particle size. The positive coefficient for independent value X_1 indicates the positive effect on dependent variable Y_1 that increase in concentration of oil increases the particle size from above equations 1. In equations 2 the positive coefficient for independent value X_1 indicates the positive effect on dependent variable Y_2 that increase in concentration of oil increases the % drug load.

3.3.1. Linear regression and residual plot analysis

The quality of fit of the model, residual plots and linear correlation plots of the observed values verses the predicted values were depicted in Figure's 4a, 4b, 4c, 4d of atorvastatin calcium for responses Y_1 and Y_2. The plots showed the points fairly close to straight lines indicating good model.

Contour plots and response surface analysis

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 (Figure's 5a, 5b, 6a, 6b for atorvastatin calcium) which are useful to study the effect of the factors on the responses. The contour plots led to the determination of the regions where acceptable values of the response are obtained. In

Table 7 for atorvastatin calcium 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), the Smix (X_2) (p =0.0037), the interaction effect (p = 0.0140), and the quadratic effect (X_1^2) where p=0.0032 were found to be significant. For particle size the interaction 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). From Figure 5b it was clearly observed when the level of Smix concentration was increased from low to high the response Y_1 (particle size) was decreased. With the increasing surfactant (coefficient is negative) in the formulation, droplet size decreased. Zhao *et al.*, also reported similar effect of surfactant on the droplet size.[18] From Figure 6b it was illustrated that when the level of oil concentration was increased from low to high the response Y_1 (% drug load) was increased.

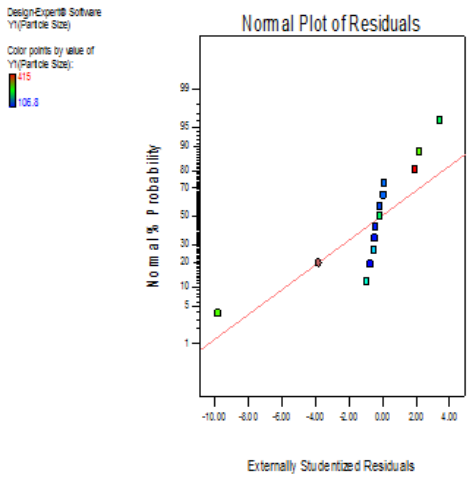

Fig. 4(a) Normal Residual plot of Y_1

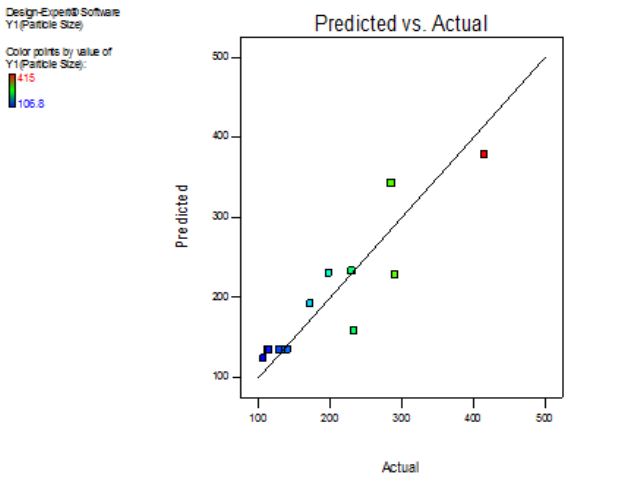

Fig. 4(b) Linear correlation plot of Y_1

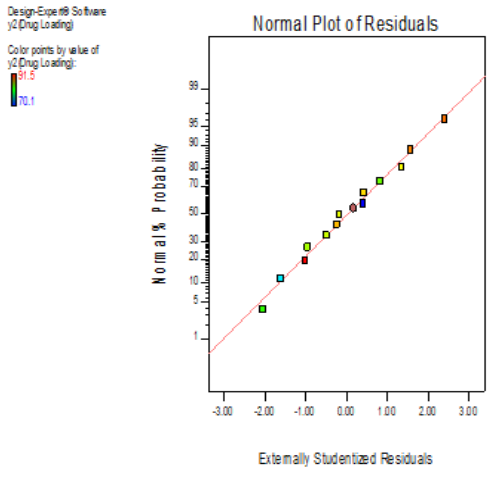

Fig. 4(c) Normal Residual plot of Y_2

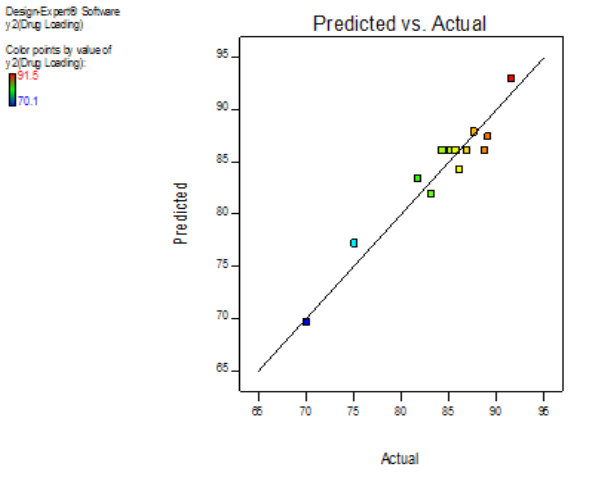

Fig. 4(d) Linear correlation plot of Y_2

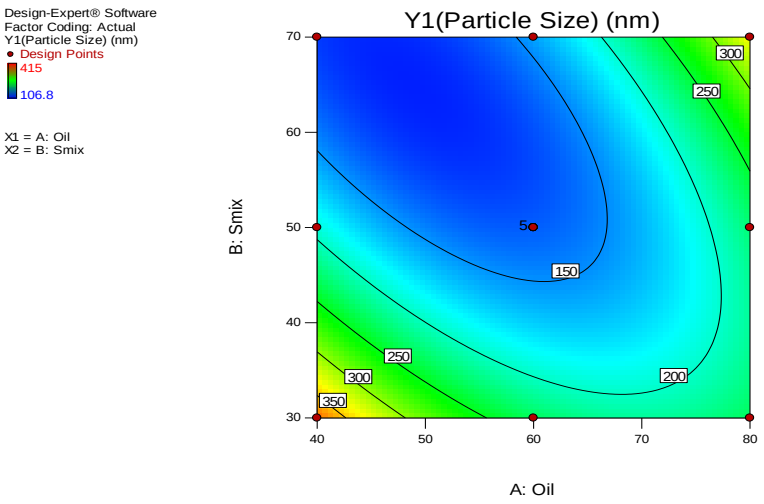

Fig. 5a Contour plot of Y_1

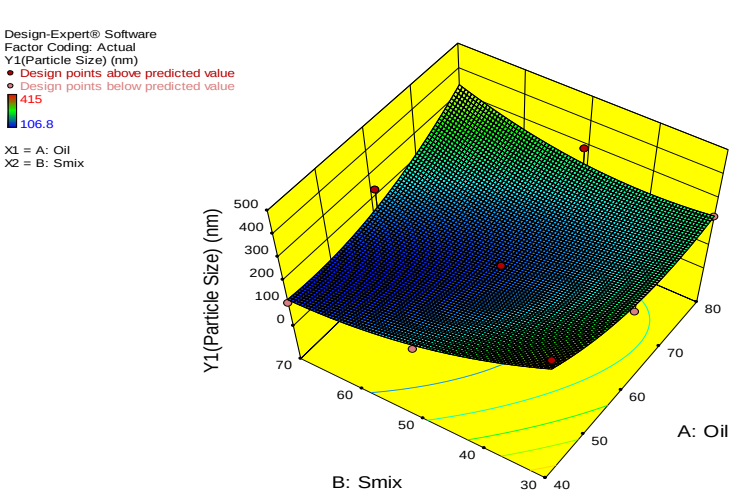

Fig. 5b Response surface plot of Y_1

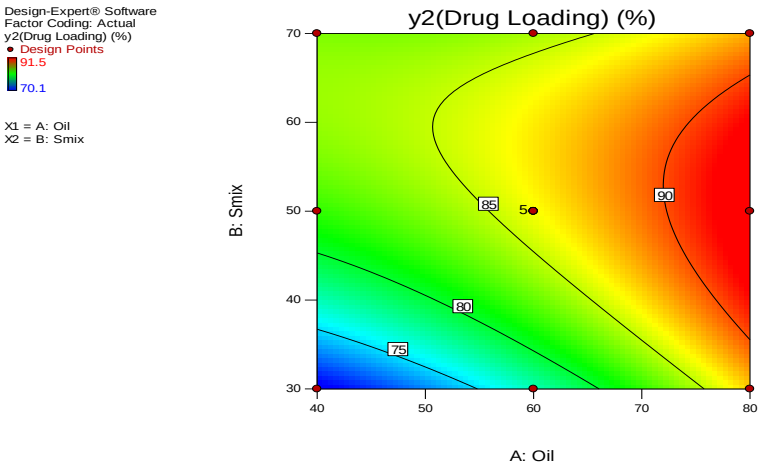

Fig. 6a Contour plot of Y_2

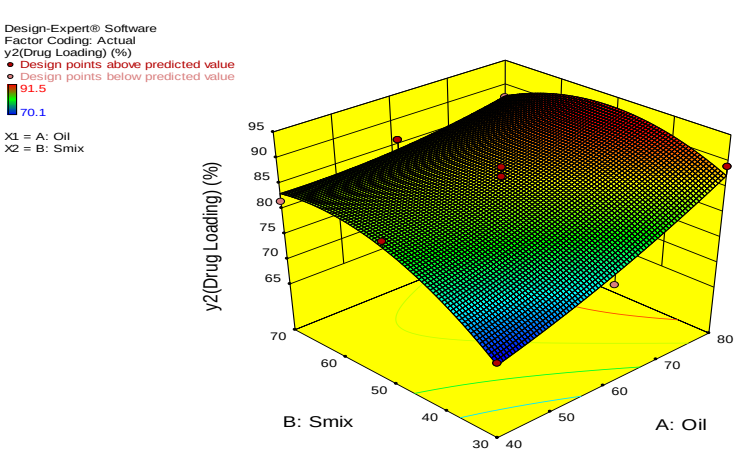

Fig. 6b Response surface of Y_2

Optimization by using desirability function

The model polynomial equations was generated to relate the dependent and independent variables, the process was optimized for all two responses simultaneously by using desirability function..The optimum formulation was selected based on the criteria of attaining the constraints of variables responses. 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 atorvastatin calcium consisted of 67.586% oil, 52.529% Smix with the corresponding desirability (*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 (Table 8). 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. The overlay plot for two response values is illustrated in Figure 7.

Table 8 Predicted and measured values of Responses and corresponding biasness

Atorvastatin calcium responses				
FC	**Particle size (nm)**			
	Predicted value	**Measured value**	**Biasness %**	
AF4	153.650	169.2±3.23	10.12	GF1
AF5	153.646	169.4±1.97	10.25	GF2
AF11	153.649	168.9±4.23	9.93	GF7
AF13	153.636	169.8±1.36	10.52	GF12
OPFA	153.651	169.7±3.2	10.45	OPFG
Drug loading %				
AF4	88.572	87.2±1.23	1.55	GF4

AF5	88.571	87±2.18	1.77	GF4
AF11	88.584	86.9±3.24	1.90	GF5
AF13	88.586	87.1±2.27	1.68	GF11
OPFA	88.582	87.2±2.25	1.57	OPFG

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

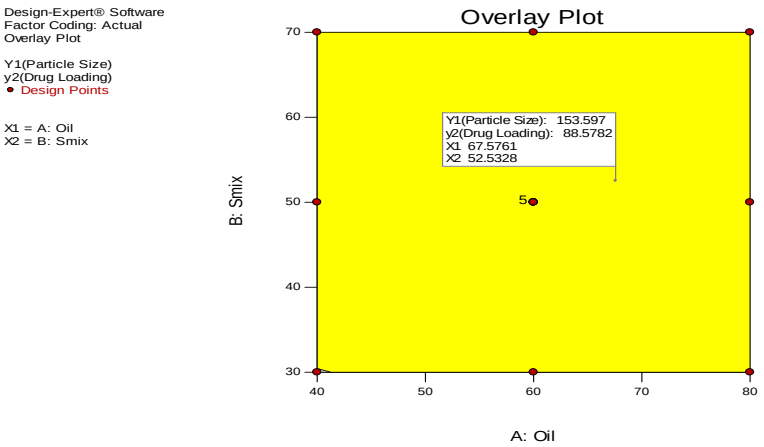

Fig. 7 Overlay plot for atorvastatin calcium SEDDS

Self emulsification, drug precipitation assessment of emulsification time studies

In the study formulations AF4, AF5, AF11, AF13 and OPFA (optimized formulation) there was good stability without any signs of drug or excipient precipitation or phase separation and the results are shown in Table 9.The emulsification time studies showed (Table 10) indicated the spontaneous emulsification for all formulations.

Table 9 Self emulsification and drug precipitation of atorvastatin calcium SEDDS

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

Table 10 Refractive index, turbidity, optical clarity, polydispersity index, viscosity, cloud point measurement and emulsification time of SEDDS formulations of atorvastatin Calcium

FC	Refractive Index ±SD (n=3)	Turbidity (NTU)	Absorbance	PDI ±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

As shown in Table 10 the absorbance of the studied aqueous dispersion of atorvastatin calcium SEDDS varied between 0.402 to 0.629 which indicated that optically clear and oil droplets are thought to be in a state of finer dispersion.

Turbidity measurement

The turbidity of SEDDS was performed determined as per procedure and turbidity for all formulations were found to below 100 NTU which shows the stability of SEDDS and the results were shown in Table10.

Viscosity determination

From viscosity determination, it was observed that as the concentration of oil increased viscosity of formulations decreased (Table10). Overall, the viscosity of the undiluted liquid SNEDDS was found less than 10,000 cps; it implied that the developed SEDDS can be filled in soft gelatin capsules.

Cloud point measurement

All the formulations were found to be below 80°C and the cloudiness formed above the body temperature of above 37°C which inferred that without any precipitation of drug the SEDDS formulations were stable as shown in Table10.

Determination of refractive index

It can be seen from the Table 10 the refractive index of the majority of the prepared formulations have similar values as that of distilled water (1.3330±0.0002 n.d.) at 28 ± 0.5°C were found to be clear as water (Table10).

Droplet size, zeta potential and polydispersity index (PDI) analysis

The PDI for all SEDDS formulations was found to be below 0.5 which found to be uniform size distribution. Among the formulations the optimized atorvastatin calcium SEDDS was found to have a mean globule size of 169.7nm (Figure 8) with a PDI 0.2, and zeta potential -31.8mV. The higher Zeta potential of optimized SEDDS indicates that microemulsion was stable.

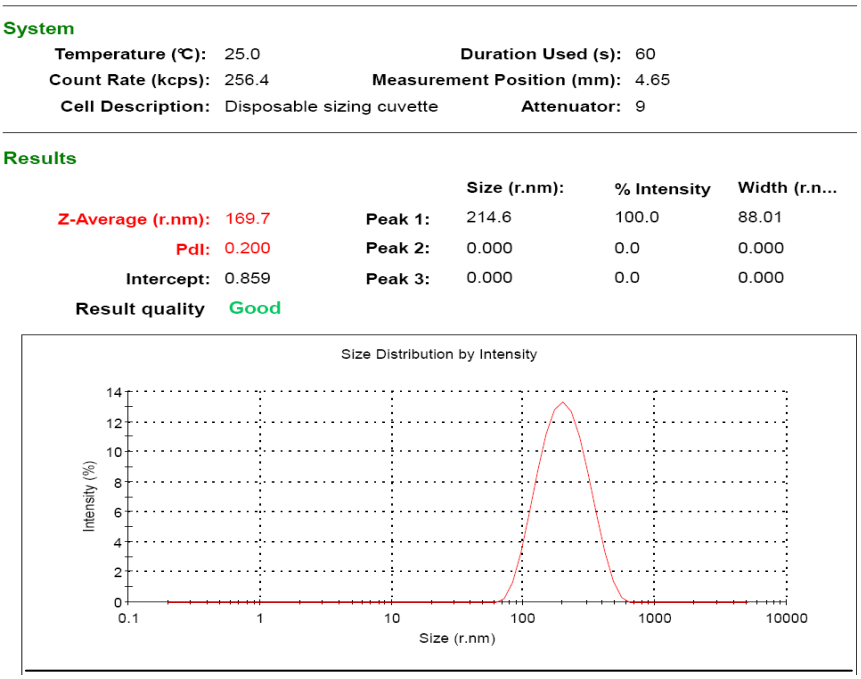

Fig. 8 Particle size of atorvastatin calcium SEDDS

Drug loading

The drug loading for optimized formulation of atorvastatin calcium was found to be 87.2% respectively. It was clearly inferred increase in Smix concentration enhances maximum drug load in SEDDS.

***In vitro* dissolution studies**

The *in vitro* dissolution profile of atorvastatin calcium optimized formulations OPFA, AF4, AF5, AF11 and AF13 in phosphate buffer pH 6.8 was significantly higher than with drug

powder and marketed tablet as shown in the Table 11 and Figure 9. 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.[19])

Table 11 Cumulative percent release of atorvastatin calcium from various formulations

Time in min	AF1*	AF5*	AF11*	AF13*	OPFA SEDDS	Pure Drug	Marketed product
0	0	0	0	0	0	0	0
5	21.56±0.69	22.89±0.88	23.45±0.59	24.56±1.25	26.21±0.74	5.69±1.24	18.21±2.03
10	34.58±2.08	31.56±0.63	33.46±1.28	32.45±0.19	39.3±0.23	7.56±0.75	24.23±1.12
20	48.56±1	49.33±2.02	48.59±0.56	49.53±0.73	58.36±0.45	11.22±1.12	36.33±2.21
30	68.23±1.59	66.52±1.94	65.56±1.50	67.87±0.22	72.66±0.32	13.45±1.23	42.54±1.64
40	77.89±1.38	76.26±0.55	75.62±1.20	74.66±0.16	79.5±0.18	16.23±1.56	45.62±0.54
50	79.98±1.27	79.56±1.16	78.32±1.30	79.98±0.02	86.72±0.16	19.21±2.73	48.74±2.21
60	83.26±2.74	84.21±1.48	83.36±0.17	82.63±0.44	91.3±0.55	22.34±1.23	53.69±1.72
75	85.27±1.78	87.24±2.55	86.48±0.56	85.56±1.22	94.5±0.49	24.86±0.62	58.66±1.54
90	93.45±1.30	92.16±0.72	93.28±1.13	94.56±0.44	99.75±0.31	25.64±1.26	62.31±1.18

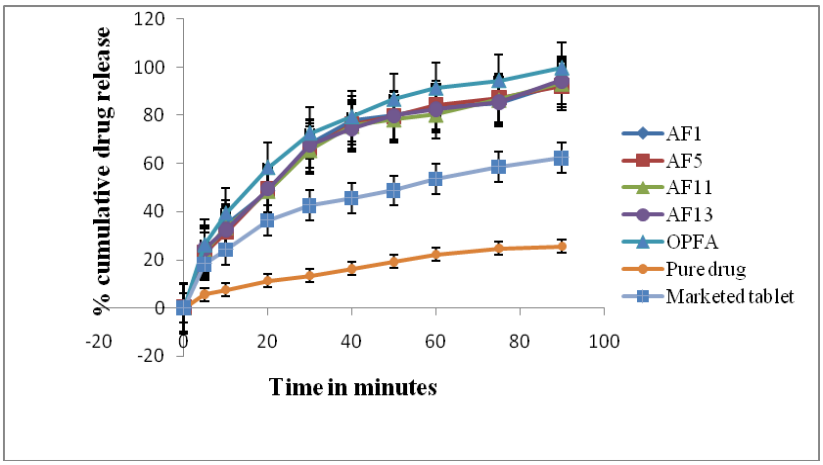

Fig. 9 Percentage cumulative drug release of various formulations.

Kinetic modeling and mechanism of drug release of optimized formulations

The dissolution data of optimized formulations OPFA showed first order release kinetics with higher correlation coefficient R^2-0.9848 for atorvastatin calcium as shown in Table 12. *In vitro* release kinetics data were computed using DD solver, which is an excel plug-in module 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 was found to be 0.406 which suggested that drug release follows fickian diffusion controlled mechanism.

Table 12: Release kinetic study of optimized formulations for atorvastatin calcium

FC	Zero order kinetic R^2	First order kinetic R^2	Higuchi Kinetic R^2	Korsemeyer-Peppas	
				R^2	n value
OPFA	0.9569	0.9848	0.9366	0.9701	0.406

Stability studies: The optimized formulations (OPFA) for atorvastatin calcium were found to be stable for 6 months and there was no significant change in the drug loading and particle size as given in Table 13.

Table 13 Stability studies of optimized atorvastatin calcium SEDDS formulations

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

CONCLUSION

In this present work the influence of formulation factors (Oil and Smix) on two responses of particle size and loading were studied using 3^2 factorial design. The two factors showed significant effect on two responses. The optimized formulation of atorvastatin calcium SEDDS was developed which contain 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 with lower droplet size (169.7nm), PDI (0.2), and Zeta potential (-31.8 mV) and % drug load (87.2%) values. The *in vitro* drug release from optimized Atorvastatin SEDDS formulation was found to be 99.75% after 90 min which was extremely higher in comparison to the marketed formulation and pure drug. It was concluded that the smaller particle size with maximum drug load more the release of drug which results in better bioavailability. The results further concluded that SEDDS can be explored as a potential drug carrier for dissolution enhancement of atorvastatin calcium and other poorly soluble drugs.

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