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Original Article

USE OF SIMPLEX LATTICE DESIGN IN DEVELOPMENT OF ORAL SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM CONTAINING ROSUVASTATIN CALCIUM

NISHANT OZA*, SWATI SAGAR, AKRUTI KHODAKIYA

C. U. Shah College of Pharmacy and Research, Wadhwan City, Gujarat, India
Email: ozanishant@gmail.com

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ABSTRACT

Objective: The aim of the present work was to enhance the solubility of rosuvastatin calcium by self-nano emulsifying drug delivery system (SNEDDS) using mixtures of oil, cosolvent, surfactant and cosurfactant.

Methods: Based on solubility study and emulsification efficiency, Preliminary investigations of various oils, surfactants and cosurfactants were carried out for the selection of the proper SNEDDS ingredients. Pseudo-ternary phase diagrams were constructed to identify the efficient self-emulsification region. A series of SNEDDS formulations were prepared using labrasol: cremophor EL with a combination of peceol: ethyl oleate by using the simplex lattice design. Prepared formulation evaluated for refractive index, turbidimetric, droplet size, zeta potential and polydispersity index, self-emulsification, stability tests, viscosity and *in vitro* diffusion studies.

Results: The best formula for SNEDDS in the current study were: 15% oil (peceol: ethyloleatein 1:1 ratio), 50% Labrasol and 35% Cremophor EL. All the SNEDDS batches globule size was found to be varied from 22.90±1.50 nm to 43.90±1.40 nm. and no significant variations in globule size were observed after 3 mo stability studies. All the batches % transparency was found to be varied from 95.40±1.40% to 99.50±1.10% and drug diffused in 10 min varied from 63.65±1.51% to 93.72±1.46 %.

Conclusion: The data suggest the use of rosuvastatin calcium SNEDDS to offer the potential for delivery and it increases the aqueous solubility and bioavailability of the drug.

Keywords: SNEDDS, Rosuvastatin Calcium, Simplex lattice design, Peceol, Ethyl Oleate, Labrasol, Cremophore EL.

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INTRODUCTION

Orally available drugs must be a sufficient soluble and permeable through the gastrointestinal tract. Almost two-thirds of the new drug candidates are poorly water-soluble, which is commonly associated with low bioavailability, high intra-and inter-subject variability, and lack of dose suitability. Lipid-based formulations offer the opportunity to enhance the absorption of lipophilic drugs. Being a nanosized, self-nano emulsifying drug delivery system (SNEDDS) offers a strong alternative to the more conventional oral formulations of lipophilic compounds. SNEDDS are isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, one or more hydrophilic solvents and cosolvents: surfactants that have forming fine oil-in-water emulsions upon mild agitation followed by dilution in aqueous media [1, 2].

The model drug for the current study had been selected from the biopharmaceutical classification system (BCS) class II. Rosuvastatin calcium is a lipid-lowering drug was an attractive candidate for the current study because it is a lipophilic compound with partition coefficient; log P = 4.81 and low aqueous solubility (0.00936 mg/ml). The current rosuvastatin calcium commercially marketed dosage forms is tablets and these show low (about 20%) and erratic oral bioavailability [3, 4]. The aim of the present study is bioavailability enhancement of rosuvastatin calcium and find the optimum formula of rosuvastatin calcium SNEDDS followed by characterization.

MATERIALS AND METHODS

Materials and reagents

Rosuvastatin calcium was gifted by Mepro Pharmaceuticals Pvt. Ltd., Surendranagar, Gujarat, India. Peceol, Labrasol, Transcutol P, Labrafil M, Labrafil M, Lauroglycol FCC and Capryol 90 were gifted by Gattefosse India Pvt. Ltd, Mumbai, India. Cremophore EL was gifted from BASF India Ltd., Mumbai, India. Sefsol was gifted from Nikko Chemicals, Japan. Polyethylene glycol 400, Propylene glycol, Tween 80, Tween 20, Span 20, Span 80, Oleic acid, Castor oil, Olive oil, Cotton-seed oil, Sesame oil and Almond oil were purchased from Seva fine chemical ltd, Ahmedabad, Gujarat, India. Methanol AR grade was purchased from SD fine chem Ltd, Mumbai, India. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Solubility study and screening of surfactants, cosurfactant and oil

Screening of surfactants and oil was done by the equilibrium solubility method. An excess quantity of rosuvastatin calcium was added to 2 ml of excipients and mixed in a vial. The mixtures in vials were shaken at 25±1.0 °C for 48 h using a rotary shaker (Remi, Mumbai, India). Then, mixtures were centrifuged at 5000 rpm for 15 min. The supernatant was separated and the drug was extracted in methanol. The drug content was analyzed by using shimadzu 1700 UV-visible spectrophotometer at 244 nm. Several trials were taken with different ratios of surfactants, cosurfactants and oils to select the proper combination of surfactant: cosurfactant: oil. Preliminary selection of 0.5 ml surfactant: cosurfactant: oil (S_{mix}: $_{oil}$) ratios were prepared and diluted with water by water titration method. From the different trails, ratios which gave clear emulsion on dilution were selected for further study [5, 6].

Drug excipient interaction study

Drug excipient interaction study was carried out by differential scanning calorimetric (DSC). DSC thermograms of the rosuvastatin calcium and formulation were derived from a DSC with a thermal analysis performed by an automatic thermal analyzer system (DSC 60, Shimadzu, Japan). The analysis was performed at a rate of 10 °C/min from 50 °C to 250 °C under a nitrogen flow of 20 ml/min [7, 8].

Development of pseudo-ternary phase diagram

Pseudo ternary phase diagrams of oil, surfactant: cosurfactant (S: CoS) and water were developed using the water titration method. Aliquot of surfactant: cosurfactant mixture (S_{mix}) mixed with oil at room temperature (25 °C). The ratio of S_{mix} to oil was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 (%v/v). Deionized water was added in small increments (≤5% v/v) to the mixture of $S_{mix/oil}$ and

stirred in a vortex shaker for 2 min (Remi, Mumbai, India). Concentration of water at which turbidity to transparency and transparency to turbidity transitions occurred was derived from weight measurements. These values were used to determine the boundaries of the nanoemulsion domain corresponding to choose the value of oils and surfactant: cosurfactant mixing ratio. To determine the effect of rosuvastatin calcium on nanoemulsion boundary, phase diagrams were constructed with the drug. Pseudo ternary phase diagrams were plotted using Tri plot version 4.1.2 [9-11].

Evaluation of rosuvastatin calcium SNEDDS

Drug Content: Drug was extracted from SNEDDS by dissolving in 25 ml methanol. Then the methanolic extract was separated out and drug content in methanolic extract was analyzed spectro-photometrically UV Visible spectrophotometer (Shimadzu 1700) at 244 nm, against the standard methanolic solution of Rosuvastatin calcium.

Self-Emulsification Time*:* The emulsification time of SNEDDS was determined by USP-II, dissolution apparatus. Each formulation was added dropwise into 500 ml with purified water at 37˚C and 50 rpm. Emulsification time was assessed visually.

Refractive Index: SNEDDS was added to 250 ml 0.1 N hydrochloric acid and 250 ml purified water at 50 rpm on a magnetic plate at ambient temperature. Then Refractive index of the system was measured by using an Abbe's Refractometer [12, 13].

Turbidimetric: SNEDDS was added to 250 ml 0.1 N hydrochloric acid and 250 ml purified water at 50 rpm on a magnetic plate at ambient temperature. Turbidity of the system was measured by measuring % transmittance at 694 nm in the UV-Visible spectrophotometer.

Droplet Size, Zeta Potential and Polydispersity Index (PDI): Droplet size and zeta potential were determined using Particle size analyzer (Zetatrac, Microtrac). It is controlled by Microtrac FLEX Operating Software Particle size analyzer uses a high-frequency AC electric field to oscillate the charged particles. The Brownian motion power spectrum is analyzed with the Modulated Power Spectrum (MPS) technique, a component of the power spectrum resulting from oscillating particles. Samples were diluted to 250 ml with purified water and placed into cuvette to measure particle size, PDI and zeta potential [14, 15].

Dilution and Aqueous Phase Composition: Robustness of SNEDDS to the dilution and effect of aqueous phase composition were studied. Optimized formulation was dispersed in 250 ml of distilled water and 0.1 N HCL with gentle stirring. Resulting emulsion was kept at 25±2 °C. Emulsion was evaluated for drug precipitation, phase separation and size over the period of 24 h.

Viscosity: Viscosity was measured by using Brookfield viscometer (Middleboro, USA) at 25 ℃. Spindle S61 was selected for the measurement of various formulations. Viscosity of SNEDDS was measured at 30 rpm before dilution and after dilution with aqueous phase (250 ml).

In vitro Diffusion Studies: *In vitro* diffusion studies were carried out by dialysis technique. In this method, one end of dialysis membrane tubing (12 cm in length) was with thread and diluted SNEDDS was filled in it. Then, another end of the tubing was also secured with thread and it was allowed rotating freely in the dissolution vessel of USP-II, dissolution test apparatus (Electrolab TDT-08L, USP). Dissolution apparatus contained 250 ml pH 6.8 phosphate buffer maintained at 37±0.5 °C and stirred at 50 rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through Whatman filter paper (No. 41), were analyzed spectrophotometrically at 244 nm for drug content [16, 17].

Stability Study: Chemical and physical stability of rosuvastatin calcium SNEDDS were assessed at 40±2 °C/75±5% RH and 25±3 °C as per ICH guidelines. It was stored in a glass vial and subject to a stability chamber over a period of 3 mo. Samples were withdrawn after 3 mo and assessed for physical appearance, dispersion time, % transmittance, viscosity and drug content.

Accelerated Stability Tests by Centrifugation and Freeze-Thaw Cycle: Rosuvastatin calcium SNEDDS were diluted with 250 ml aqueous phases (distilled water and 0.1 N HCL) and centrifuged (Remi, Mumbai, India) at 5000 rpm for 30 min. In addition, it was subjected to a freeze-thaw cycle by storing it at-20 °C for 24 h and then for another 24 h at 40 °C. Nanoemulsions were observed visually for phase separation and drug precipitation, whereas their physical stability was assessed by measuring globule size before and after centrifugation and freeze-thaw cycle [18, 19].

Optimization of rosuvastatin calcium SNEDDS by using simplex design

A simplex lattice design was used to optimize for SNEDDS. In this design, three factors were evaluated by changing their concentrations simultaneously and keeping their total concentration constant. The simplex lattice design is a three-component system and it's represented by an equilateral triangle as shown in fig 1**.** Seven batches of SNEDDS were prepared, including three vertexes (*A*, *B*, *C*), three half-way points between vertices (*AB*, *AC*, *BC*), and one center point (*ABC*). Code representations of formulation with actual and transformed values are shown in table 1**.** The concentrations of surfactant, cosurfactant and oil were selected as independent variables. Mean globule size, percent transparency and amount of drug diffuse through dialysis membrane in 10 min were taken as responses. The responses of seven formulations were used to fit an equation for the simplex lattice model which can predict properties of all possible formulations using of Design Expert 8.0.5 [20-22].

Table 1: Simplex lattice design of rosuvastatin calcium SNEDDS

Formulation	Code	Concentration (Transformed value)		
		Surfactant	Cosurfactant	Oil
F1	A	1	0	0
F2	B	0	1	0
F3	C	0	0	1
F4	AB	0.5	0.5	0
F5	AC	0.5	0	0.5
F6	BC	0	0.5	0.5
F7	ABC	0.33	0.33	0.33
Code		**Transformed value**	**Actual value in %**	
A (Surfactant Labrasol)		0	50	
		1	65	
B (Cosurfactant-Cremophor EL)		0	20	
		1	35	
C {Oil (1:1)-Peceol: Ethyloleate}		0	15	
		1	30	

RESULTS AND DISCUSSION

Solubility study and screening of surfactants, cosurfactant and oil

SNEDDS consists of a mixture of oil, surfactant, cosurfactant and drug. When SNEDDS introduced to an aqueous phase, the mixture should form a clear and monophasic at room temperature. It should have good solvent properties that allow the drug to be present in solubilised form. The results of the solubility of rosuvastatin calcium in various vehicles were shown in fig. 2 and 3. Rosuvastatin calcium had the highest solubility in oleic acid with comparison to other lipid vehicles. Among oils, olive oil, cottonseed oil and almond oil were having miscibility problems with the selected surfactants, as well as they shown lesser solubility of rosuvastatin calcium, so they were rejected. Rosuvastatin calcium had the highest solubility in Transcutol P as compare to other surfactant and cosurfactant. Further Morelabrasol, Cremophor EL and Propylene glycol also showed very high solubility of rosuvastatin calcium. In contrast, Lauroglycol FCC, Labrafil M2125 CS, Labrafil M 1944 Cs, Tween 80, Tween 20 and Span 80 were rejected due to a comparatively lesser solubility. Lutrol F 68 was having a solid-state so if it was used precipitation might have occurred on storage, so it was also rejected.

From this study, it reveals that transparent emulsion was not formed by using Sefsol 218, Oleic acid, Castor oil, Sesame oil with different surfactants and cosurfactants. Transparent emulsions were formed by using Peceol: Labrasol, Propylene glycol: Ethyl oleate with Cremophor EL but these, combinations showed phase separation on higher dilutions. On the other hand Ethyl oleate with Cremophor EL: Propylene glycol, formed gel-like structures when it diluted with water. So the combination of Labrasol: Cremophor EL and Peceol: ethyl oleate was tried. Finally, based upon clarity of emulsion, Peceol: Ethyl oleate and Labrasol: Cremophor EL was selected for further investigation.

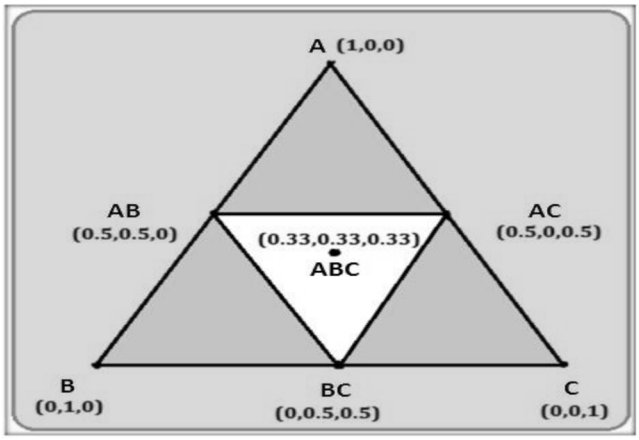

Fig. 1: Equilateral triangle representing simplex lattice design for three components

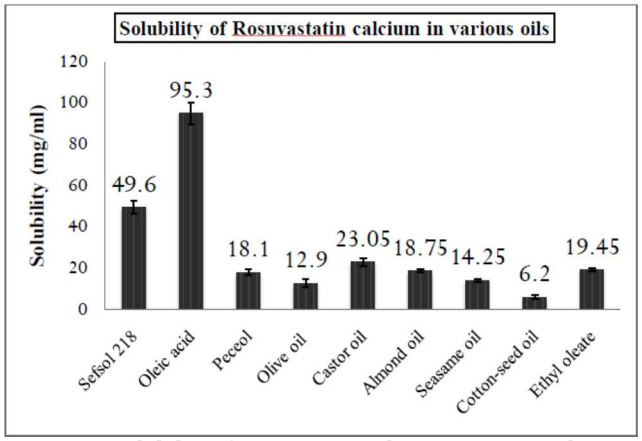

Fig. 2: Solubility of rosuvastatin calcium in various oils

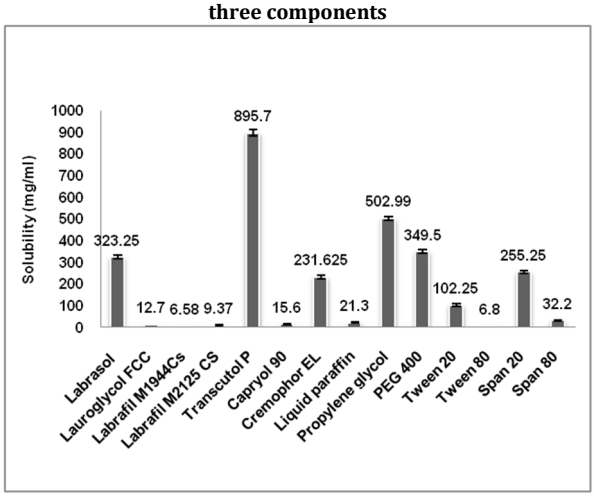

Fig. 3: Solubility of rosuvastatin calcium in various surfactants

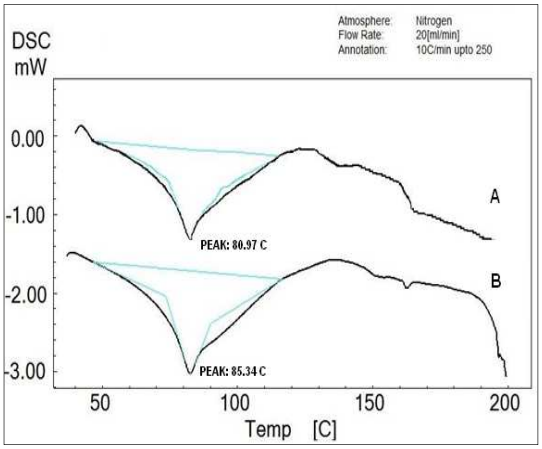

Fig. 4: DSC spectra of formulation mixture of drug, oil, surfactant and cosurfactant (a), pure drug (b)

Drug-excipient interaction study

The DSC results provided both qualitative and quantitative information about the physicochemical state of the drug present in the formulation. The thermogram of rosuvastatin calcium showed a melting endothermic peak at 85.34 °C and a formulation mixture containing rosuvastatin calcium showed a melting endothermic peak at 80.97 ^{0}C as shown in fig. 4. The thermogram of the drug does not change after mixing with oil, surfactant and cosurfactant indicates the compatibility of oil, surfactant and co-surfactant with the drug. The peaks in both the thermogram show that there is no significant interaction between drug and excipients [23, 24].

Pseudo ternary phase diagram

Pseudo ternary phase diagrams were constructed to identify the self-nano emulsifying regions and optimize the concentration of oil as shown in fig 5. The efficiency of emulsification was good when Labrasol: Cremophor EL concentration was more than 50% in a formulation. It was observed that increasing concentration of surfactants also increased the spontaneity of the self-emulsification region. Therefore, a higher concentration of surfactant higher self-emulsifying region in phase diagrams. The ratio of surfactant: cosurfactant was very effective in a stable and efficient SNEDDS formation. The phase diagrams were constructed at ratio of surfactant: cosurfactant 1:1, 2:1, 3:1 and 4:1. However, the stability of self-emulsifying droplets 1:1, 3:1 and 4:1 was decreased and precipitation after a few hour. So, ratio of 2:1 was chosen in the formulation. To determine the effect of drug addiction on the nano-emulsion boundary, phase diagram was constructed in the presence of the drug. No significant changes were observed in phase diagram regions after drug loading [25, 26].

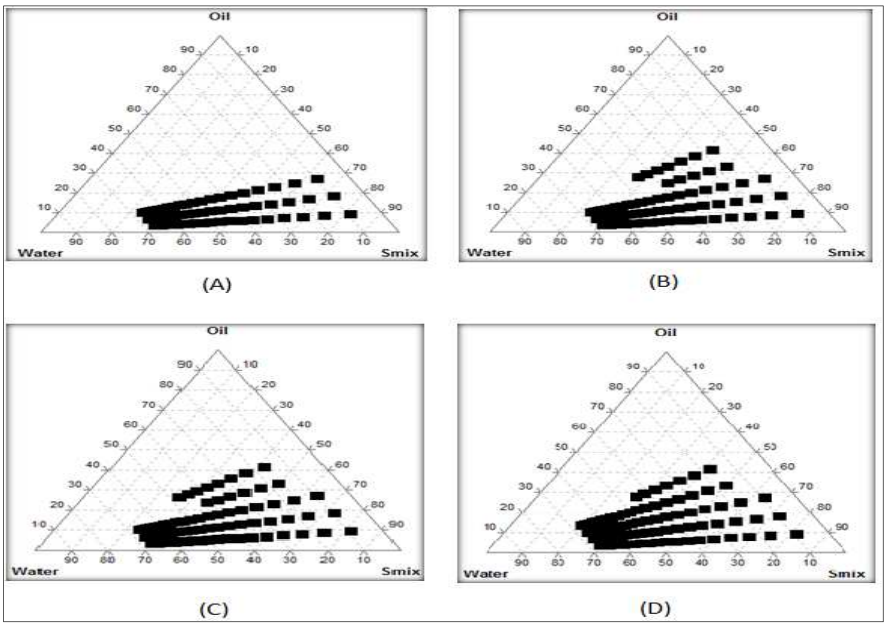

Fig. 5: Pseudo-ternary phase diagrams (A) S: CoS ratio 1:1 (B) S: CoS ratio 2:1 (C) S: CoS ratio 3:1 (D) S: Cos ratio 4:1

Optimization of rosuvastatin calcium SNEDDS by using simplex design

Simplex lattice design was used to optimize the rosuvastatin calcium SNEDDS. The concentrations of surfactant (A), cosurfactant (B) and oil (C) were chosen as the independent variables. The equation for simplex lattice model is described as follows:

$$R = \beta aA+\beta bB+\beta cC+\beta abAB+\beta acAC+\beta bcBC+\beta abcABC$$

Where *R* is the dependent variable and *β*i is the estimated coefficient for the factor (A/B/C). The major effects (*A, B,* and *C*) represent average results of changing one factor at a time from its low to high value, the interactions *AB, BC, AC,* and *ABC*. The results of their mean droplet size (R1), % transparency (R2) and the amount of drug diffused in 10 min (R3) were given in table 2 [26, 27].

Mean globule size

R1 = 43.90A+22.95B+41.00C-4.50AB+16.20AC-3.50BC-188.76 ABC

All the SNEDDS batches globule size was found to be varied from 22.90±1.50 nm to 43.90±1.40 nm. As seen from fig. 6 (I), the Contour plot reveled that means globule size is less when the amount of B is increased. Here, it can be predicted that Cremophor EL has the highest effect on mean globule size. Additionally, βab, βbc, and βabc had a negative value which showed a synergistic effect on mean globule size. βac had an antagonistic effect on mean globule size it had a positive value.

% Transparency

R2 = 95.50A+99.50B+95.80C+3.28AB-1.00AC+1.40BC+28.56ABC

All the SNEDDS batches % transparency was found to be varied from 95.40±1.40% to 99.50±1.10%. As saw from fig 6 (II), the contour plot revealed that B has highest effect on % transparency. Additionally, βac had a negative value which showed an antagonistic effect of % transparency. βab, βbc, and βabc had a synergistic effect on % transparency because they had a positive value.

Amount of drug diffused in 10 min

R3 = 67.39A+93.72B+68.81C+29.70AB-17.80AC+0.58BC-173.22ABC

All the formulation showed drug diffused in 10 min varied from 63.65±1.51% to 93.72±1.46 %. As saw from fig 6 (III), the contour plot revealed that B has the highest effect on the amount of rosuvastatin calcium in 10 min. Additionally, βac and βabc had a negative value which showed an antagonistic effect on rosuvastatin calcium diffused in 10 min. βab and βbc had a synergistic effect on rosuvastatin calcium diffused in 10 min because they had a positive value.

Table 2: Runs and measured responses of rosuvastatin calcium SNEDDS by using simplex design

Formulation code	Formulation components			Mean globule size (nm) (R1)	% Transparency (R2)	% Drug diffused in 10 min (R3)
	Surfactant (A)	Cosurfactant (B)	Oil (C)			
F1	1	0	0	43.90±1.40	95.50±1.60	67.39±1.54
F2	0	1	0	22.90±1.50	99.50±1.10	93.72±1.46
F3	0	0	1	41.50±2.60	95.80±1.30	68.81±2.10
F4	0.5	0.5	0	32.30±1.60	98.32±2.00	87.98±1.40
F5	0.5	0	0.5	46.50±2.80	95.40±1.40	63.65±1.51
F6	0	0.5	0.5	31.10±1.50	98.00±2.10	81.41±1.78
F7	0.33	0.33	0.33	29.87±1.40	98.40±1.30	71.61±2.10

n=6

Table 3: Summary of regression analysis of significant factors

Responses	Coefficients of parameters							
	βa	βb	Bc	βab	βac	βbc	βabc	R^2
Mean globule size	43.90	22.95	41.00	-4.50	16.20	-3.50	-188.76	1
% Transparency	95.50	99.50	3.28	3.28	-1.00	1.40	28.56	1
Amount of drug diffused in 10 min	67.39	93.72	68.81	29.70	-17.80	0.58	-173.22	1

Table 4: Analysis of variance (ANOVA) of the dependent variable

Source of variation	DF	SS	MS	*F*	*P*
Mean globule size					
Regression	1	32.32	32.32	63660000.0	<0.001
% Transparency					
Regression	1	0.74	0.74	63660000.0	<0.001
% Amount of drug diffused in 10 min					
Regression	1	27.22	27.22	63660000.0	<0.001

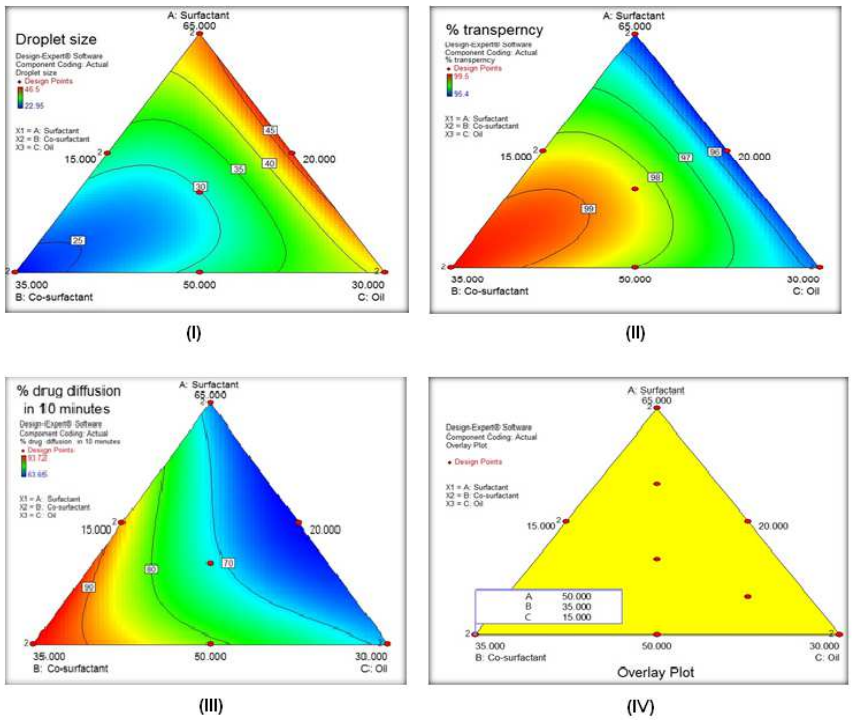

Fig. 6: Contour plots (I) mean globule size (ii) % transparency (iii) amount of drug diffused in 10 min (iv) superimposed ternary contour plot of the three responses

The Summary of regression analysis of significant factors and results of ANOVA shown in table 3 and table 4 respectively. It suggested that *F, as well as P values,* are significant. Counter plots as shown in fig 6, it reveals that an inverse relationship exists between mean globule size and % transparency. As the globule size of SNEDDS increases, % transparency decreases. Direct relationship exists between % transparency and % amount of drug diffusion. As the % transparency of formulation increases, the amount of rosuvastatin calcium diffused in 10 min were also increases. In order to obtain both high % transparency, high amount of rosuvastatin calcium diffused in 10 min and smallest possible mean globule size, the appropriate ratio of components was chosen for the optimized formulation, which consisting of oil (15%), surfactant (35%), cosurfactant (50%).

Validation of design

One extra checkpoint was taken and the checkpoint batch was prepared as shown in table 5. The checkpoint batch was evaluated for all three dependent variables. The practically obtained responses of the checkpoint batch were compared with the calculated responses from the simplex equations shown in table 6. Practically, obtained responses are closer to the predicted response. Closeness of the value justifies the validation of design [28, 29].

Table 5: Checkpoint prediction

Batch code	Variable level					
	Coded value			Actual value in %		
	A	B	C	A	B	C
CP	0.342	0.391	0.267	57.13	25.87	19.0

Table 6: Evaluation of checkpoint batches and comparison with the predicted value

Variable	Predicted response	Practical response
Mean globule size	28.70 nm	29.01±1.03 nm
% Transparency	98.65 %	98.25±0.67%
% Amount of drug diffused in 10 min	74.03 %	74.9±1.44%

n=6

Table 7: Evaluation of drug content, self-emulsification time, refractive index% and %transmittance of SNEDDS

Batch	Drug content (%)	Self-emulsification time (s)	Refractive index		% Transmittance	
			0.1 N HCL	Distilled water	0.1 N HCL	Distilled water
F1	098.91±0.51	24.67±4.18	1.41±0.13	1.40±0.06	43.85±1.21	95.50±1.21
F2	099.63±0.21	12.67±3.73	1.33±0.11	1.33±0.07	93.64±2.14	99.50±0.43
F3	098.07±0.55	15.33±3.06	1.38±0.07	1.37±0.13	02.30±0.12	95.80±1.33
F4	100.35±0.44	19.33±2.57	1.40±0.08	1.38±0.11	87.15±2.01	98.32±1.14
F5	099.37±0.83	17.00±2.96	1.39±0.09	1.34±0.06	03.10±0.10	95.40±1.15
F6	102.07±0.25	20.33±1.90	1.37±0.05	1.35±0.04	61.10±1.33	98.00±2.16
F7	097.77±0.45	23.67±0.58	1.40±0.10	1.38±0.05	42.90±1.21	98.40±2.13

n=6

Selection of optimized batch

Batch F2 was selected as an optimized batch in order to obtain high % transparency and higher % diffusion and the smallest mean globule size. The appropriate ratio of components for optimized formulation F2 was, oil (15%), surfactant (35%), cosurfactant (50%).

Evaluation of rosuvastatin calcium SNEDDS

The results of all batches of SNEDDS showed drug content variation range from 98.91% to 102.07%. Emulsification time is an important parameter for SNEDDS and all the formulation was prepared nanoemulsion within 24 s. Refractive index and % transmittance of various formulations were shown in table 7. Batch F2 had refractive index and % transmittances are similar in water, so it's proving the transparency of the system. Droplet size distribution is a critical factor to evaluate SNEDDS. The smaller droplets have a larger interfacial surface area that will be provided for drug absorption. The optimized formulation (F2) has found droplet size 22.95±1.50 nm and it's shown in fig. 7. Generally, an increase of electrostatic repulsive forces between droplets prevents the coalescence of droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. Rosuvastatin calcium SNEDDS (F2) was diluted with distilled water and resulted in zeta potential was found-8.40±0.02mV. According to the study, positively charged droplets could have better interaction with the mucus of the gastrointestinal tract, because intestinal cell interior carry negative charges with the presence of mucosal fluid. Here, F2 formulation has a positive potential, it was likely to facilitate intestinal absorption of rosuvastatin calcium. [30, 31] Effect of Dilution and aqueous phase composition results indicated that SNEDDS can be diluted up to 1,000 fold without any phase separation or drug precipitation and it's remained stable over a 24 h. Aqueous phase composition also did not affect the physical stability of the resulting emulsion. Viscosity data were shown in table 8. It was observed that before dilution the formulation having higher viscosity and after dilutions with water up to 250 ml the emulation viscosity near to the water.

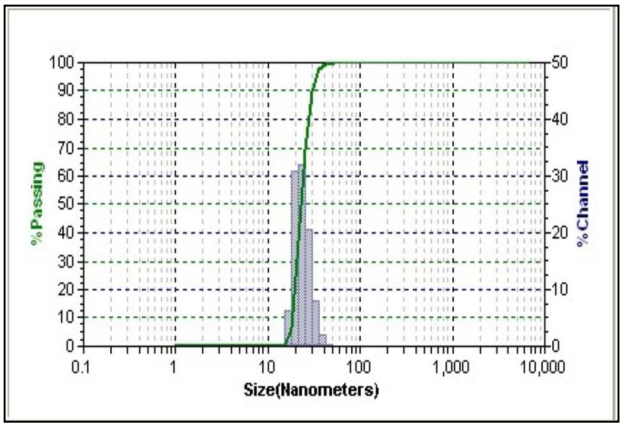

Fig. 7: Droplet size analysis of SNEDDS formulation (F2)

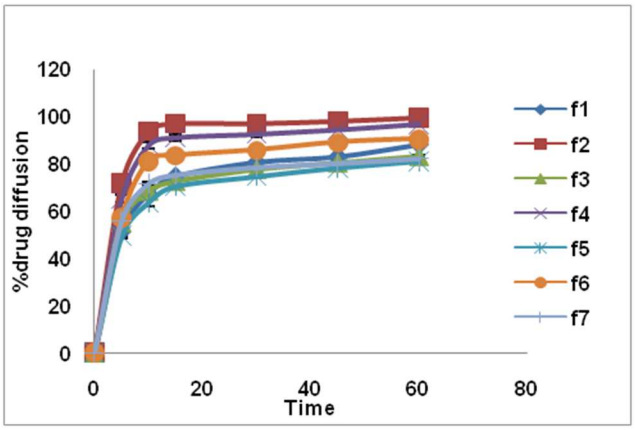

Fig. 8: Diffusion profile of various SNEDDS formulations

Table 8: Evaluation of viscosity, particle size, zeta potential and % PDI of SNEDDS

Batch	Viscosity (cps)		Particle size (nm)	Zeta potential	PDI
	Before dilution	After dilution (distilled water)			
F1	216.44±2.51	1.18±0.07	43.90±0.11	-0.49±0.03mV	0.063±0.01
F2	315.56±3.56	1.27±0.11	22.95±0.44	-8.40±0.02mV	0.692±0.03
F3	210.44±0.46	1.08±0.11	41.44±0.64	-0.49±0.03mV	0.127±0.01
F4	265.55±3.55	1.20±0.17	32.38±0.36	-0.49±0.01mV	0.100±0.02
F5	262.55±1.33	1.19±0.15	46.58±0.75	-0.49±0.02mV	0.118±0.02
F6	213.67±3.61	1.23±0.15	31.18±0.25	-0.49±0.03mV	0.067±0.02
F7	247.78±0.67	1.25±0.11	29.87±0.66	-0.49±0.03mV	0.190±0.01

n=6

***In vitro* diffusion studies**

The drug diffusion profile of different SNEDDS is shown in fig 8. Order of drug diffusion through the dialysis membrane was F2>F4>F6>F7>F3>F1>F5. It shows that increasing the droplet size of nanoemulsion decrease the diffusion rate of the drug. Optimized batch F2 was given more than 95% release in 15 min. It suggests that rosuvastatin calcium dissolved in SNEDDS and

diffused due to the small droplet size. SNEDDS was given a faster rate of drug release in the aqueous phase which affects bioavailability.

Stability studies of rosuvastatin calcium SNEDDS

No change in physical parameters such as homogeneity and clarity of SNEDDS was observed during stability studies. The stability data of rosuvastatin calcium SNEDDS at stated storage conditions is shown in table 9. Interestingly, it was shown that no decline in rosuvastatin calcium content which was observed at the end of three months indicating that rosuvastatin calcium remained chemically stable in SNEDDS. Furthermore, other parameters such as self nanoemulsion efficiency, % transmittance viscosity and dispersion time remained unchanged at all storage conditions during the entire period of study.

Table 9: Stability data of rosuvastatin calcium SNEDDS batch F2

Time (mo)	Storage conditions	Drug content (%w/w)	Viscosity (cps)		% Transmittance	Dispersion time (sec)
			Before dilution	After dilution		
0	25±3 °C	99.63±0.54	315.54±1.64	1.90±0.43	99.50±0.21	13±1
	40±2 °C/75±5%	99.63±0.24	312.44±1.87	1.87±0.64	99.50±1.11	12±2
1	25±3 °C	99.47±1.76	316.64±2.97	1.92±0.65	99.35±1.34	13±1
	40±2 °C/75±5%	99.41±0.24	312.67±0.34	1.89±0.76	99.14±3.71	14±2
2	25±3 °C	99.46±0.76	316.27±1.25	1.92±0.34	99.22±1.57	13±1
	40±2 °C/75±5%	99.37±1.23	315.34±0.65	1.90±0.76	99.16±0.45	13±1
3	25±3 °C	99.41±0.76	315.75±1.86	1.88±0.54	99.15±1.75	12±2
	40±2 °C/75±5%	99.30±0.62	314.67±0.45	1.87±0.23	99.18±1.24	13±1

n=6

Accelerated stability study by centrifugation and freeze-thaw cycle

The effect of centrifugation and freeze-thaw cycling on emulsion is shown in table 10. Accelerated tests were carried under stress conditions. Optimized SNEDDS (F2) did not exhibit any drug precipitation and phase separation after centrifugation. Similarly, it survived freeze-thaw cycling and it was reconstituted without any phase separation or drug precipitation.

Table 10: Accelerated stability of SNEDDS

Accelerated stability	Parameter	Formulation code						
		F1	F2	F3	F4	F5	F6	F7
Centrifugation	Phase separation	Slight*	No*	No*	No*	Slight*	No*	Slight*
	Drug precipitation	No*	No*	No*	No*	No*	No*	No*
Freeze-thaw cycle	Phase separation	No*	No*	No*	No*	No*	No*	No*
	Drug precipitation	No*	No*	No*	No*	No*	No*	No*

* = (same result was noticeable in all 6 formulation) n=6

CONCLUSION

The bioavailability enhancement of most oral lipid-based formulations depends on the ability of the oil vehicle to maintain the drug in solution after dispersion. The SNEDDS was explored successfully for oral delivery of poorly soluble drug rosuvastatin calcium. SNEDDS are isotropic mixtures made up of oil, surfactant, cosurfactant and cosolvent. In an aqueous environment, a homogeneous, isotropic and thermodynamically stable nanoemulsion formed. The formulation of SNEDDS was optimized by a simplex lattice design. Solubility study was showed the highest solubility of rosuvastatin calcium in Transcutol P as compare to other materials. Pseudo ternary phase diagrams were constructed to identify the efficient self-emulsification region. SNEEDS had also shown that after dilution there was no precipitation and phase separation found. No significant variations in globule size were observed after the Stability study. *In vitro* diffusion studies revealed that the release of rosuvastatin calcium from SNEDDS was faster. Nonetheless, there is a clear need for developing methods for tracking the solubilization state of the drug *in vivo*.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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