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-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.

Various oils, surfactants and cosurfactants were 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 °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:C:o) ratios were prepared and diluted with water by water titration method. From the different trials, 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:CoS) mixed with oil at room temperature (25 °C). The ratio of S:CoS 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:CoS:oil...
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 nanomulsion domain corresponding to the use of oils and surfactant: cosurfactant mixing ratio. To determine the effect of rosuvastatin calcium on nanomulsion 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 spectrophotometrically 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. Turbidity 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 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 using Brookfield viscometer (Middleboro, USA) at 25 ±C. 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. Nanomelusions 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) | Actual value in % |
| | | 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 |
| B (Cosurfactant-Cremophor EL) | 1 | 65 |
| C (Oil (1:1)-Pecol:Ethylolate) | 0 | 20 |
| | 1 | 35 |
| | 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. Furthermore, Labrasol, Cremophor EL and Propylene glycol also showed very high solubility of rosuvastatin calcium. In contrast, Lauroglycol FCC, Labrafac M2125 CS, Labrafac 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 Pecoctol: 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 Pecocel: Ethyl oleate was tried. Finally, based upon clarity of emulsion, Pecocel: Ethyl oleate and Labrasol: Cremophor EL was selected for further investigation.

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 °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].
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:

\[
R = \beta_aA + \beta_bB + \beta_cC + \beta_{ab}AB + \beta_{ac}AC + \beta_{bc}BC + \beta_{abc}ABC
\]

Where \( R \) is the dependent variable and \( \beta \) 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**

\[
R_1 = 43.90A + 22.95B + 41.00C - 4.50AB - 16.20AC - 3.50BC - 188.76 ABC
\]

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 revealed that mean 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, \( \beta_{ab}, \beta_{bc}, \) and \( \beta_{abc} \) had a negative value which showed a synergistic effect on mean globule size it had a positive value.

**% Transparency**

\[
R_2 = 95.50A + 99.50B + 3.28AC + 1.40BC + 28.56ABC
\]

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, \( \beta_{ac} \) had a negative value which showed an antagonistic effect of % transparency. \( \beta_{ab}, \beta_{bc}, \) and \( \beta_{abc} \) had a synergistic effect on % transparency because they had a positive value.

**Amount of drug diffused in 10 min**

\[
R_3 = 67.39A + 93.72B + 68.81C + 29.70AB - 17.80AC + 0.58BC - 173.22ABC
\]

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, \( \beta_{ac} \) and \( \beta_{abc} \) had a negative value which showed an antagonistic 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                  | 81.41±1.78               |
| F6               | 0          | 0.5           | 0.5    | 31.10±1.50       | 98.00±2.10                  | 71.61±2.10               |
| F7               | 0.33       | 0.33          | 0.33   | 29.87±1.40       | 98.40±1.30                  | 76.12±2.10               |

n=6

Table 3: Summary of regression analysis of significant factors

| Responses                     | Coefficients of parameters | \( \beta_a \) | \( \beta_b \) | \( \beta_c \) | \( \beta_{ab} \) | \( \beta_{ac} \) | \( \beta_{bc} \) | \( \beta_{abc} \) | \( R^2 \) |
|-------------------------------|-----------------------------|-------------|-------------|-------------|---------------|---------------|---------------|---------------|--------|
| 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      |
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 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**

### Table 5: Checkpoint prediction

| Batch code | Coded value | Actual value in % |
|------------|-------------|-------------------|
|            | A           | B                  | C                  |
| CP         | 0.342       | 0.391              | 0.267              |
|            | A           | B                  | C                  |
|            | 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           | 29.0±1.03 nm       |
| % Transparency                    | 98.65 %            | 98.25±0.67 %       |
| % Amount of drug diffused in 10 min | 74.03 %         | 74.9±1.44 %        |

$n=6$
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±0.02 mV. 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 emulsion viscosity near to the water.

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 its 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±0.02 mV. 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 emulsion viscosity near to the water.

| Batch | Drug content (%) | Self-emulsification time (s) | Refractive index | % Transmittance |
|-------|-----------------|-----------------------------|-----------------|----------------|
|       | 0.1 N HCL       | Distilled water             | 0.1 N HCL       | Distilled water |
| F1    | 0.98±0.01       | 2.4±0.10                    | 1.4±0.01        | 3.9±0.01       |
| F2    | 0.97±0.02       | 1.2±0.01                    | 1.3±0.07        | 9.4±0.07       |
| F3    | 0.95±0.03       | 1.5±0.03                    | 1.2±0.03        | 9.5±0.04       |
| F4    | 0.93±0.04       | 1.9±0.04                    | 1.3±0.04        | 9.5±0.05       |
| F5    | 0.92±0.05       | 1.7±0.05                    | 1.3±0.06        | 9.5±0.06       |
| F6    | 0.91±0.06       | 2.0±0.06                    | 1.3±0.07        | 9.5±0.07       |
| F7    | 0.90±0.07       | 2.3±0.08                    | 1.3±0.07        | 9.5±0.08       |

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

![Fig. 7: Droplet size analysis of SNEDDS formulation (F2)](image)

![Fig. 8: Diffusion profile of various SNEDDS formulations](image)

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

| Batch | Viscosity (cps) | Before dilution | After dilution (distilled water) | Particle size (nm) | Zeta potential | PDI |
|-------|----------------|-----------------|---------------------------------|-------------------|---------------|-----|
| F1    | 216.4±2.51    | 61.8±0.07       | 43.9±0.01                       | -0.49±0.03 mV     | 0.063±0.01   |
| F2    | 315.5±3.56    | 1.27±0.11       | 22.9±0.44                       | -0.49±0.02 mV     | 0.062±0.03   |
| F3    | 210.4±0.46    | 1.0±0.11        | 41.4±0.64                       | -0.49±0.03 mV     | 0.127±0.01   |
| F4    | 265.5±3.55    | 1.2±0.17        | 32.3±0.36                       | -0.49±0.01 mV     | 0.100±0.02   |
| F5    | 262.5±3.33    | 1.19±0.15       | 46.5±0.75                       | -0.49±0.02 mV     | 0.118±0.02   |
| F6    | 213.6±3.61    | 1.23±0.15       | 31.1±0.25                       | -0.49±0.03 mV     | 0.067±0.02   |
| F7    | 247.7±0.67    | 1.25±0.11       | 29.8±0.66                       | -0.49±0.01 mV     | 0.190±0.01   |

n=6

Table 8: Evaluation of viscosity, particle size, zeta potential and % PDI of SNEDDS
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) Before dilution | % Transmittance | Dispersion time (sec) | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|-----------|--------------------|---------------------|-------------------------------|----------------|----------------------|----|----|----|----|----|----|----|
| 0         | 25±3 °C            | 99.6±0.54           | 315.5±1.64                   | 99.50±0.21     | 13±1                 | No*| No*| No*| No*| No*| No*| Slight* |
| 1         | 40±2 °C/75±5%      | 99.5±0.24           | 312.4±1.87                   | 99.50±0.11     | 12±2                 | No*| No*| No*| No*| No*| No*| Slight* |
| 2         | 25±3 °C            | 99.4±0.76           | 316.6±2.97                   | 99.35±1.34     | 13±1                 | No*| No*| No*| No*| No*| No*| Slight* |
| 3         | 40±2 °C/75±5%      | 99.4±0.24           | 312.7±0.34                   | 99.14±3.71     | 14±2                 | No*| No*| No*| No*| No*| No*| Slight* |
|           | 25±3 °C            | 99.4±0.60           | 316.2±1.25                   | 99.22±1.57     | 13±1                 | No*| No*| No*| No*| No*| No*| Slight* |
|           | 40±2 °C/75±5%      | 99.3±0.12           | 315.3±0.65                   | 99.16±0.45     | 13±1                 | No*| No*| No*| No*| No*| No*| Slight* |
|           | 25±3 °C            | 99.4±0.76           | 315.7±1.86                   | 99.15±1.75     | 12±2                 | No*| No*| No*| No*| No*| No*| Slight* |
|           | 40±2 °C/75±5%      | 99.3±0.62           | 314.6±0.45                   | 99.18±1.24     | 13±1                 | No*| No*| No*| No*| No*| No*| Slight* |

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* | No* | Slight* |
| Freezethaw cycle       | Drug precipitation | No* | No* | No* | No* | No* | No* | No* | No* |
|                       | Phase separation | No* | No* | No* | No* | No* | No* | No* | No* |
|                       | Drug precipitation | No* | 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 shown 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. SNEDDS 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 dissolution 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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