



## Self-emulsifying Drug Delivery System of Rosuvastatin Calcium

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### ABSTRACT

**Running title:** Self-emulsifying drug delivery system of Rosuvastatin Calcium.

**Introduction:** Oral route is the preferred route for higher patient compliance. Rosuvastatin Calcium is BCS class II drug and its oral bioavailability is 20%. To overcome this problem, studies were performed to enhance the solubility of the Rosuvastatin Calcium in order to improve the bioavailability.

**Materials and methods:** Different permutations and combinations of oils such as Castor oil, Cottonseed oil, Olive oil, Soyabean oil, Olyl alcohol, Peceol, Rice bran and Sunflower oil, surfactants Tween 20, Tween 80, Kollisolve PEG 400, Cremophore RH 40, Cremophore EL and co-surfactants PEG 200, PEG 400, Transcutol HP were tried in order to deduce the compatible oils and (co)surfactants to prepare pseudo-ternary phase diagram.

**Results and discussion:** The optimized batch of Rosuvastatin Calcium SEDDS was prepared using Peceol (10%), Tween-20 (30%) and Transcutol (30%) having average particle size 76.88nm, polydispersibility index 0.555 and zeta potential of -24.5 mV. In addition, drug entrapment in the optimized batch was found to be 95.89%. The stability studies data of 3 months shows that the emulsion was stable for at least 3 months with drug entrapment  $91.60 \pm 0.02\%$ . The formulation showed approximately 95% drug release within 15 min as compared to the pure drug, which showed drug release of 36.56% in phosphate buffer pH 6.8.

**Conclusion:** From the above study, it can be concluded that SEDDs can be a promising approach to improve the solubility and in order to improve the oral bioavailability of Rosuvastatin Calcium.

**Keywords:** Rosuvastatin calcium, Formulation, Oral bioavailability

## INTRODUCTION

The oral route is the preferred route of delivery due to its advantage of higher patient compliance and acceptance over other drug delivery routes. However, development of new and existing drug into oral formulations is limited due to their poor water solubility leading to decrease in their oral bioavailability [1]. Various methods have been employed to enhance the solubility of drug including polymeric micelles, liposomes, solid dispersion, Nano-emulsions, cyclodextrin complexes, self-emulsifying drug delivery system (SEDDS) [1-4]. In the oral formulation, drug solubility in the physiological medium is vital for the drug absorption to be able to available at the site of action. SEDDS maintains the drug in a solubilized state in the gastrointestinal tract, also decrease in particle size increases the surface area, which can also increase the solubility of the drug. Hence, SEDDS becomes one of the promising approaches in increasing the drug solubility in order to enhance oral bioavailability and therapeutic effect [5,6].

SEDDS are isotropic mixtures of natural or synthetic oils, surfactants and one or more hydrophilic solvents and co-solvents/surfactants [7,8]. This system then upon mild agitation followed by dilution in aqueous media such as gastric fluid self-emulsifies to form thermodynamically stable micro or nanodroplets of oil in water (o/w) called self-emulsifying micro or nano drug delivery system (SEDDS or SNEDDS). Oral absorption of these poorly absorbed drugs is influenced by the specific components of the SEDDS, which promotes the lymphatic transport of drugs [5].

Rosuvastatin calcium is a calcium salt of [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxyhept-6-enoic acid] belonging to BCS class II having low solubility resulting into decreased oral bioavailability. To overcome this problem, herein the aim of this study was to improve the solubility of the drug which then, in turn, will enhance the oral bioavailability of the drug to give the desired therapeutic effect.

## MATERIALS AND METHODS

Rosuvastatin calcium was obtained as a generous gift sample from Biocon, Bangalore. Peceol (Glyceryl mono-oleate), Transcutol HP was gifted from Gatefosse Ltd., Mumbai India. Cremophore ELP and Cremophore RH 40 were gifted from BASF, India. Tween 20, Tween 80, PEG 400 and PEG 200 were gifted from Mohini Organics, Mumbai, India. All the other chemicals used in this study were analytical or technical grade and were used without further treatment.

### Screening of oils, surfactants and co-surfactants based on the saturation solubility [9-11].

#### *Screening of oils*

Screening of oils was done by determining the relative solubility of the drug in various oils. All the selected oils were GRAS (Generally recognized as safe) listed. Selection of oil was based on the saturated solubility of Rosuvastatin calcium in the oils. The various oils which were used namely- Castor oil, Cottonseed oil, Olive oil, Soyabean oil, Olyl alcohol, Peceol, Rice bran and Sunflower oil; Also, different ratios of oil mixtures were tried such as Castor oil: Soyabean oil (1:1) and Olive oil: Soyabean oil (1:1). The solubility of

Rosuvastatin Calcium in the oil was determined by dissolving the excess amount of the drug (600 mg) in each oil and was placed in the stoppered vial. This mixture was then stirred using vortex mixer for 10 mins, sonicated for 30 mins and kept at 37°C + 0.5°C in orbital shaker for 72 h to obtain equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 15 min and supernatant was filtered through 0.45 µm membrane filter and diluted with methanol. Drug content was quantified by using ultraviolet-visible spectrophotometer (UV-spectrophotometer) at 240.2 nm.

#### *Screening of surfactants and co-surfactants*

The surfactant with high HLB value forms a stable O/W microemulsion. The surfactants Tween 20, Tween 80, Kollisolve PEG 400, Cremophore RH 40, Cremophore EL and co-surfactants PEG 200, PEG 400, Transcutol HP were selected for screening. Saturation solubility of Rosuvastatin calcium in various surfactants and co-surfactants was determined by the procedure mentioned above in then the screening of oils.

**Screening of oils, surfactants and co-surfactants based on the % transmittance**

The oil, surfactant & co-surfactant were heated at 40-50°C for 30 secs in a combination of oil + surfactant and oil + surfactant + co-surfactant. Further, it was diluted with the distilled water in volumetric flask & percent transmittance was determined by ultraviolet-visible spectrophotometer at 638.2 nm.

**Construction of pseudo-ternary phase diagram [12].**

The pseudo-ternary phase diagrams were constructed by water titration method of the homogenous liquid mixture of oils, surfactant & co-surfactant at room temperature. Surfactant and co-surfactant mixture (S-mix) were varied in the ratio of 1:1, 2:1 & 3:1. These S-mix ratios were chosen to reflect increasing concentration of surfactants with respect to co-surfactant for detailed study of the phase diagram in the microemulsion region. A mixture of oil, surfactant & co-surfactant combination was prepared in the ratios from 1:9 to 9:1 in the glass tubes. Nine different combinations of oil and S-mix were made for the study to delineate the boundaries of phases precisely formed in the phase diagram. Each mixture was then titrated with distilled water in a drop wise manner until they showed turbidity which disappeared and formed a clear emulsion after 24 hours. Based on the result, appropriate percentage of oil, surfactant & co-surfactant was selected, correlated in the phase diagram and were used for the preparation of SEDDs. The physical state observed was plotted on a pseudo three-component phase diagram with one axis representing aqueous phase, the second axis representing the oil phase and the third representing a mixture of surfactants, co-surfactant at a fixed volume ratio. The Percent compositions were put in CHEMIX SCHOOL SOFTWARE in order to construct pseudo-ternary phase diagram.

**Preparation of drug loaded microemulsion****Drug loading capacity**

Drug loading capacity was studied by adding the excess amount of Rosuvastatin calcium to the microemulsion formulations and stirring for 72 hours at 250°C. The formulations were then centrifuged and the supernatants were collected. About 0.1 gm of supernatant were mixed together and then centrifuged. The supernatants were collected, appropriately diluted and Rosuvastatin concentration was determined by U.V. Spectrometry method of analysis.

**Loading of 10 mg rosuvastatin calcium into microemulsion formulation**

From pseudo-ternary phase diagrams, the composition of formulation in which the amount of oil phase completely solubilized the drug and which could accommodate the optimum quantity of S-mix and distilled water were selected for the study. A series of microemulsions were formulated by adding Rosuvastatin calcium to the mixture of oils and S-mix ratio 1:1, then an appropriate amount of distilled water was added to the mixture drop by drop and the microemulsion was obtained by stirring the mixture using vortex mixer at room temperature. In all the formulation, the concentration of Rosuvastatin calcium was constant (10mg). All the components used for the formulation of microemulsion were within the limits specified by FDA's Inactive Ingredient Guide (IIG). All the selected formulations were evaluated for the different parameters such as clarity, dilution test, Thermodynamic stability, and percent drug release studies.

**Characterisation of SEDDS [13].****Clarity and percent transmittance**

Microemulsion should be clear and transparent and hence all formulations were diluted with distilled water. Clarity was observed through naked eyes and percent transmittance was measured at 638.2nm using UV-spectrophotometer keeping distilled water as blank.

**Thermodynamic stability studies****Heating cooling cycle**

The selected formulations were kept for three cycles between 4°C and 40°C with storage at each temperature of not less than 24 h. Formulations which were stable at these temperatures were subjected to centrifugation test.

**Centrifugation test:** Passed formulations were centrifuged at 5000 rpm for 30 min. Those formulations that did not show any phase separation were selected for the freeze thaw stress test.

**Freeze thaw cycle** Formulations were subjected to three freeze-thaw cycles between -4C for 24 h followed by 40C for 24 h.

**Self-emulsification time**

The efficiency of self-emulsification was assessed by USP apparatus type II. Dissolved 1 ml of SEDDs in 900 ml of distilled water at  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with rotating speed of 50 rpm to provide gentle agitation. Following grades were given as per the self-emulsification time of SEDDs.

**Grade A:** Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance.

**Grade B:** Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

**Grade C:** Fine milky emulsion that formed within 2 minutes.

**Grade D:** Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (Longer than 2 min).

**Grade E:** Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface [15].

**Robustness to dilution**

Formulations were subjected to 1:100 times dilution with buffer pH 1.2, pH 6.8 and distilled water and phase separation was observed at 12 h and 24 h.

**Particle size, zeta potential and polydispersity index measurement**

The particle size Polydispersity index, and zeta potential and of the selected formulations were determined by laser diffraction analysis using particle size analyzer (Malvern Zeta sizer Nano Series ZS 90). The samples were diluted with a ratio of 1:100 with Millipore water.

**Cloud point measurement**

The optimized SEDDS formulations were diluted with distilled water in the ratio of 1:250. The diluted samples were placed in a water bath and gradually temperature was increased until sudden appearance of cloudiness [14-16].

**Drug content**

Microemulsion contained dose equivalent to 10 mg of drug was dissolved in methanol and quantified at 236.6 nm by U.V. spectrophotometer.

**In Vitro Dissolution study**

The Rosuvastatin calcium SEDD's dose equivalent to 10 mg of

were filled into 0 size hard gelatin capsule. Rosuvastatin calcium release from SEDD's formulations was performed using dissolution test apparatus of USP type I Basket method (Electro lab Etc-11L) with 900 ml phosphate buffer pH 6.8 as a dissolution medium at  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with Basket speed at 50 rpm. 5 ml aliquot was withdrawn for up to 30 min at 5 min of intervals and it was replaced with the same amount of fresh dissolution media to maintain the sink condition. The withdrawn sample was filtered and analyzed by UV-spectrophotometer (SHIMADZU Corporation, Japan).

**Protocol for dissolution study**

- A) Apparatus – USP type I-Basket dissolution apparatus
- B) Medium- Phosphate buffer pH 6.8
- C) Volume-900ml
- D) Speed- 50 RPM
- E) Temperature –  $37 \pm 2^{\circ}\text{C}$
- F) Time intervals- 5, 10, 15, 20, 25, 30 min as per IP.

**Differential scanning calorimetry (DSC) thermogram of pure rosuvastatin and microemulsion formulation**

In Differential Scanning Calorimetry, any drastic changes in the formulation with the thermal the behavior of either the drug or the excipients were visualized. It is a thermodynamic technique where the sample and reference material are subjected to a controlled temperature conditions and the difference in energy inputs between the sample and reference material is measured as a function of temperature. Both the sample and reference are maintained at the same temperature throughout the experiment. The temperature programmed for the DSC analysis is designed such that the sample holder temperature increases linearly as a function of time.

**Procedure**

Differential Scanning Calorimetry (DSC) was used to study the thermal analysis of Drug-Excipients compatibility of Rosuvastatin calcium and Rosuvastatin calcium microemulsion. About 10 mg of the sample was weighed in a standard open aluminum pan and scanned from  $30\text{--}280^{\circ}\text{C}$  at a heating rate of  $10^{\circ}\text{C}/\text{minute}$ . Nitrogen was used as a purge gas through the DSC cell [17].

**Stability studies**

The stability study has done to know the effect of aging and temperature on the percent drug content and in-vitro drug release profile. Stability studies were carried out on the optimized formulation to determine the effect of various excipients on the stability of the drug at  $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$

as per ICH guidelines. The formulation was evaluated based on drug content and in-vitro drug release studies

The study was performed by keeping the prepared optimized batch of self-emulsifying drug delivery system (SEDD's) of Rosuvastatin calcium i.e. ME 2 in air tight high density HDPE bottle pack at  $40^\circ\text{C}$  and relative humidity of 75% ( $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$ ) according to ICH guidelines for the period of 3 months.

**RESULT AND DISCUSSION**

**Screening of oil, surfactant and co-surfactant based on the saturation solubility**

The saturated solubility of Rosuvastatin calcium was carried out in various oils as shown in Figure 1.

The surfactant with high HLB value forms a stable o/w microemulsion. The suitable combination of high and low HLB surfactant and co-surfactant shows better stability of the microemulsion. Solubility study of the drug was tested in different surfactants and the data is given in Figure 2.

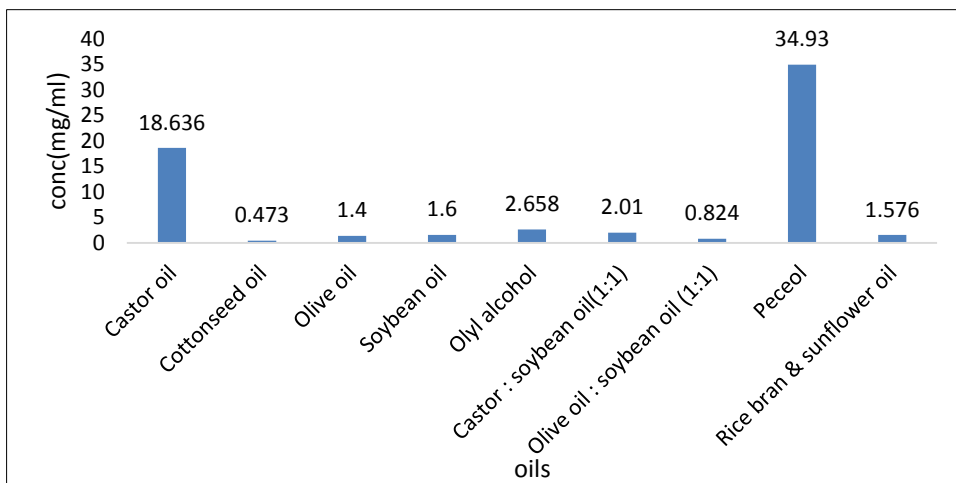


Figure 1: Graphical representation of saturation solubility of rosuvastatin calcium in various oils

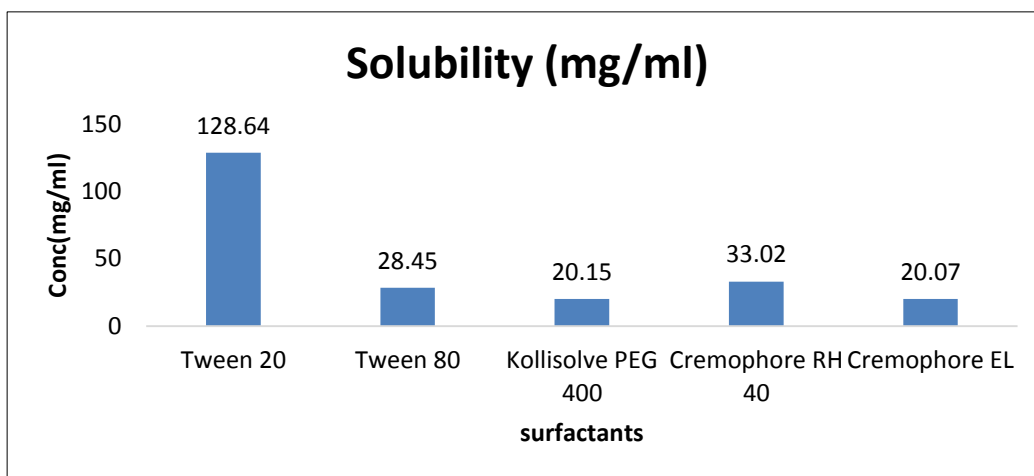


Figure 2: Graphical representation of saturation solubility of rosuvastatin calcium in various surfactants

So, Tween 20 was selected as the surfactant, with HLB value of 16.7 as it showed maximum saturation solubility. For better stability of microemulsion, saturation solubility of

Rosuvastatin calcium was screened for different co-surfactants and is given in Table 1. The drug was found to be more soluble in Transcutol HP i.e. 523 mg/ml with HLB value of 4.2.

Table 1: Graphical representation of saturation solubility of rosuvastatin calcium in various co-surfactants

Sr No.	Co-surfactants	Concentration(mg/ml)
1	PEG 200	429
2	PEG 400	471.2
3	Transcutol HP	523

The drug was found to be more soluble in Transcutol HP i.e. 523 mg/ml with HLB value of 4.2.

**Screening of oil, surfactant and co-surfactant based on the % transmittance**

Percent Transmittance studies of various combinations of oils, surfactant and co-surfactant are illustrated in Table 2.

Table 2: Graphical representation of combination of oil, surfactant, and co-surfactant based on % transmittance

Sr No.	combination of oil-surfactant-co-surfactant	%Transmittance
1	Peceol+Tween 20	73.183
2	Peceol+Tween 80	68.161
3	Peceol+ Transcutol HP	82.642
4	Peceol+Tween 20+Transcutol HP	93.974
5	Peceol+Tween 80+Transcutol HP	89.624

The combination of Peceol, Tween 20 and Transcutol HP showed the highest transmittance hence these are selected for further studies.

**Pseudo-ternary phase diagram**

From all the three phase diagrams, S-mix ratio of 1:1 shows largest microemulsion region as compared to S-mix ratio of 2:1 and S-mix ratio of 3:1.

Figures 3(a), 5(b) and 5(c) shows the ternary plot obtained from different ratios of Peceol (oil), Tween 20 (surfactant),

Madhura Mestry, et al. Int J Pharm Year; 2017(4): 130-139  
 Transcutol HP (co-surfactant) ranging from 1:1, 2:1, 3:1,  
 respectively.

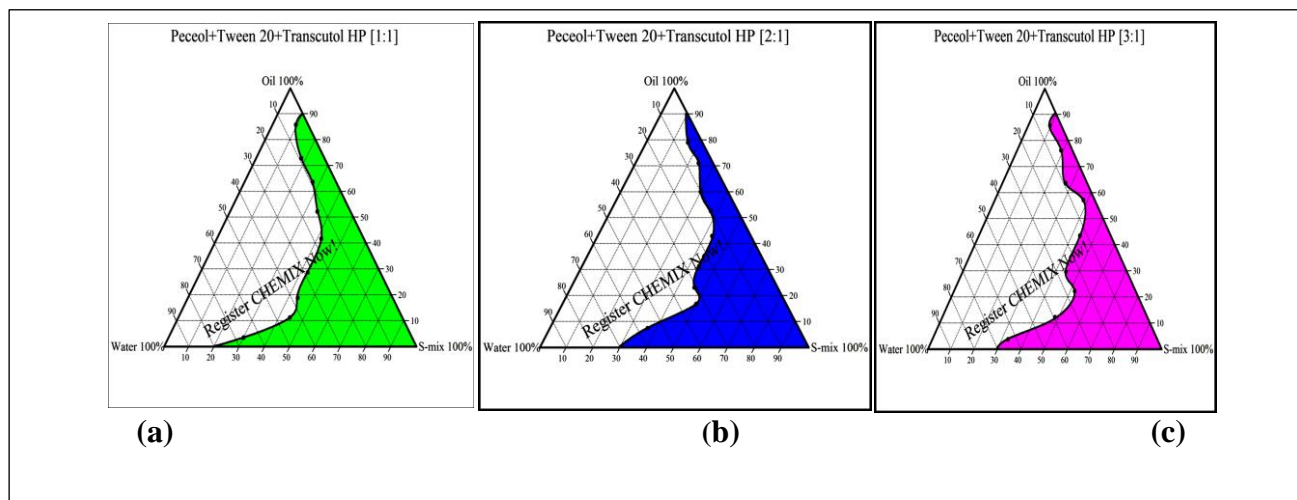


Figure 3: (a) 1:1 Ternary plot (b) 2:1 Ternary plot (c) 3:1 Ternary plot

As shown in Table 3, four micro-emulsion points were selected from the largest micro-emulsion region of the pseudo-ternary

phase diagram of S-mix ratio of 1:1 and these were further optimized and evaluated.

Table 3: Selected batches from ternary plot (1:1)

Micro-emulsion (ME)	Surfactant and Co-surfactant mixture (S-mix) ratio 1:1		
	Peceol (oil)	Tween 20 (surfactant)	Transcutol HP (co-surfactant)
Batches			
ME 1	10%	25%	25%
ME 2	10%	30%	30%
ME 3	10%	20%	20%
ME 4	20%	15%	15%

#### Preparation of drug loaded microemulsion

Loading of Rosuvastatin calcium into microemulsion formulations. Certain points from phase diagrams of Peceol/ Tween 20/ Transcutol HP/ water (S-mix 1:1) were selected from the microemulsion zone. No change was found in the phase behavior of the pseudo-ternary phase diagram when Rosuvastatin calcium 10 mg was included in the formulation. This may be because the formulation and stability of microemulsion consisting of non-ionic surfactants are not affected by the change in the pH or ionic strength.

#### Evaluation of SEDDs

As shown in Table 4, SEDDs were characterized on the basis of % transmittance, clarity and precipitation, dilution test in distilled water and buffer pH 1.2 and buffer pH 6.8, self-emulsification time and thermodynamic stability. Micro emulsion batch ME1 & ME2 was optimized and further evaluated among all micro emulsion batches from ME1 to ME4. Microemulsion batch ME1 & ME2 were further evaluated for Drug content, Particle Size Analysis and zeta potential, Cloud point and In-vitro dissolution study. The results were tabulated in Table 5.

**Table 4: Characterization of SEDDS formulation of rosuvastatin calcium**

Batch code	% Transmittance (%T)	Clarity & precipitation	Dilution test	Self-emulsification		Thermodynamic stability
				Time	Grade	
ME 1	99.516	clear	No Phase-separation	32.6 sec	A	Stable and clear
ME 2	104.224	clear	No Phase-separation	25.6 sec	A	Stable and clear
ME 3	82.382	Turbid and precipitation	Phase separation	1min 9 sec	B	Stable and clear
ME 4	39.2	Turbid and precipitation	Phase separation	1 min 12 sec	B	Stable and clear

**Table 5: Characterization and evaluation of optimized SEDDS formulations of rosuvastatin calcium**

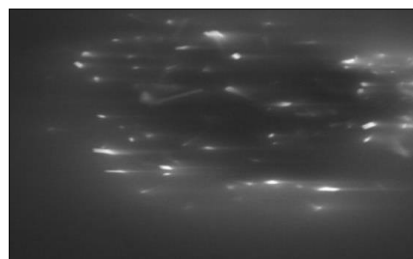
Batch code	%Drug content	% Drug release (within 15 min)	Cloud point(°C)	Particle size (nm)	Zeta potential (mV)	Polydispersibility index
ME 1	92.08	93.30	86°C	158.6	-19.1	0.270
ME 2	95.89	103.41	74°C	76.88	-24.5	0.555

***In Vitro* dissolution study**

From the above evaluation parameters (as mentioned in Table 4 and 5), it was observed that formulation ME 2 exhibited optimum characterization and in-vitro percent drug release (as shown in Figure 4) with pure Rosuvastatin calcium drug and marketed product (Fm). *In-vitro* dissolution study of ME1 and ME2 shows 93.30% and 103.41% drug release within 15 min, whereas pure Rosuvastatin Calcium showed 36.56%. As compared to the drug release profile of pure Rosuvastatin Calcium, Self-Emulsifying Drug Delivery System (SEDDs) of ME 2 showed enhancement in percent drug release within 15 mins.

**SEM (scanning electron microscopy)**

The SEM was used to examine the morphology of prepared microemulsion. Figure 5 depicts spherical shaped globules of optimized microemulsion batch ME 2; in addition, the droplets were dark within bright surroundings.

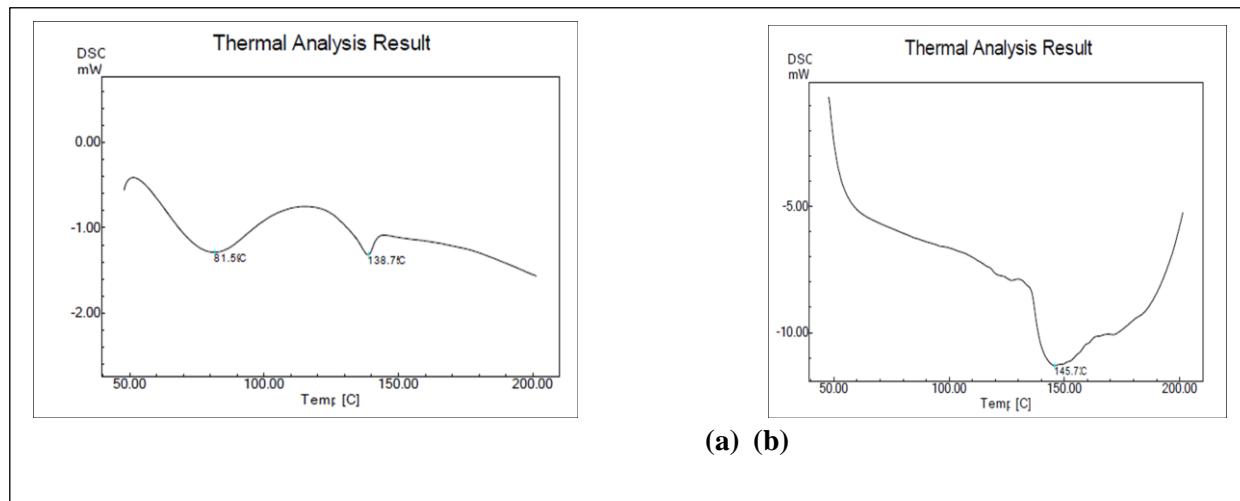
**Figure 5: SEM analysis of optimized formulation**



**DSC thermogram of pure rosuvastatin and microemulsion formulation**

DSC studies were carried out to investigate the compatibility between the drug and excipients used. DSC thermograms of

pure drug Rosuvastatin calcium and microemulsion formulation, ME-2 are shown in Figure 6 (a) and (b) respectively.



**Figure 6: (a) DSC thermogram of pure rosuvastatin calcium (b) DSC thermogram of microemulsion ME 2**

It is indicated from thermograms that well characterized and recognizable endotherms appeared at the temperature of for Rosuvastatin calcium. DSC thermogram of the formulation was far from the endotherm of Rosuvastatin calcium indicating that there may be no interference between drug and excipients used in microemulsion formulation. It is obvious from DSC thermograms that there are no significant shifts in the endothermic peaks, hence it can be concluded that there is no

interaction between drug and excipients.

**Stability studies**

The stability study sample that is an optimized batch of self-emulsifying drug delivery system (SEDD's) i.e. ME 2 were stored over a period of three months. The sample was withdrawn periodically at time intervals of 0, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> month and evaluated for the parameter such as percent drug content, *in-vitro* dissolution study as shown in Table 6.

**Table 6: Stability studies of optimized SEDD's ME 2**

Temperature	Parameter	0 month	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
8 °C ± 2 °C	Drug content (% w/w)	95.89 ± 0.02	95.25 ± 0.03	93.36 ± 0.08	93.10 ± 0.02
	<i>In-Vitro</i> Drug Release(% W/W)	108.12 ± 0.05	106.83 ± 0.05	106.12 ± 0.07	105.43 ± 0.04
25°C ± 2°C / 60% RH ± 5% RH	Drug content (% w/w)	95.89 ± 0.02	95.50 ± 0.02	94.45 ± 0.04	93.85 ± 0.05
	<i>In-Vitro</i> Drug Release(% W/W)	108.12 ± 0.05	108.85 ± 0.01	107.58 ± 0.04	107.46 ± 0.06
40 °C ± 2 °C / 75% RH ± 5 % RH	Drug content (% w/w)	95.89 ± 0.02	94.55 ± 0.03	93.56 ± 0.04	91.60 ± 0.02
	<i>In-Vitro</i> Drug Release(% W/W)	108.12 ± 0.05	108.23 ± 0.04	107.25 ± 0.07	106.25 ± 0.04

Hence, it can be concluded from the results that the developed

tablets were stable and retain their pharmaceutical properties

over a period of 3 months.

### CONCLUSION

The optimized batch of Rosuvastatin Calcium SEDDS (ME2) was prepared using Peceol (10%) as an oil phase, Tween-20 (30%) and Transcutol (30%) as a surfactant and co-surfactant respectively. The average particle size was found to be around 76.88 nm with poly dispersibility index of 0.555 and zeta potential of -24.5 mV. In addition, high drug entrapment efficiency was obtained which were around  $91.60 \pm 0.02\%$  at

the end of 3 months accelerated stability studies. Moreover, the formulation showed approximately 95% drug release within 15 min as compared to the pure drug. Thus, the microemulsion prepared using Peceol, Tween-20 and Transcutol HP increased the oral bioavailability by increasing the effective surface area of drug exposure in the physiological medium.

### REFERENCES

1. L. Wu, Y. Qiao, L. Wang, J. Guo, G. Wang, W. He, L. Yin, J. Zhao. *A. A. P. S. Pharm. Sci. Tech.* **2015**, 16(5), 1051-8.
2. R. Lian, Y. Lu, J. Qi, Y. Tan, M. Niu, P. Guan, F. Hu, W. Wu. *A. A. P. S. Pharm. Sci. Tech.* **2011**, 12(4), 1234-40.
3. T. Vasconcelos, B. Sarmento, *P. Costa. Dru. Disco. Tod.* **2007**, 12(23), 1068-75.
4. X. Hu, C. Lin, D. Chen, J. Zhang, Z. Liu, W. Wu, H. Song. *Inter. J. Pharma.* **2012**, 438(1), 123-33.
5. W. Wu, Y. Wang, L. Que. *Euro. J. Pharma. Biopharma.* **2006**, 63(3), 288-94.
6. S. Onoue, A. Uchida, K. Kuriyama, T. Nakamura, Y. Seto, M. Kato, J. Hatanaka, T. Tanaka, H. Miyoshi, S. Yamada. *Euro. J. Pharma. Sci.* **2012**, 46(5), 492-9.
7. R. N. Gursoy, S. Benita. *Biomed. & Pharmacother.* **2004**, 58(3), 173-82.
8. P. P. Constantinides. *Pharma. Res.* **1995**, 12(11), 1561-72.
9. C. Amrutkar, K. Salunkhe, S. Chaudhary. *W. J. Pharma. Res.* **2014**, 3(4), 2137-51.
10. C. Amrutkar, K. Salunkhe, S. Chaudhary. *W. J. Pharma. Res.* **2014**, 3(4), 2137-51.
11. A. Nasr, A. Gardouh, H. Ghonaim, E. Abdelghany, M. Ghorab. *Inter. J. App. Pharma.* **2016**, 8(1), 13-24.
12. H. K. Syed, K. K. Peh. *Act. Polon. Pharma. Dr. Res.* **2014**, 71(2), 301-9.
13. S. T. Prajapati, H. A. Joshi, C. N. Patel. *J. Pharma.* **2012**.
14. C. Amrutkar, K. Salunkhe, S. Chaudhary. *W. J. Pharma. Res.* **2014**, 3(4), 2137-51.
15. N. S. Kulkarni, N. S. Ranpise, G. Mohan. *Trop. J. Pharma. Res.* **2015**, 14(4), 575-82.
16. P. Nehe, K. S. Salunkhe, S. R. Chaudhari, P. B. Gadge, G. S. Dighe, A. Asati A. *W. J. Pharma. Res.* **2014**, 4(1), 1812-32.
17. K. Meena. *Res. J. Pharma. Bio. Chem. Sci.* **2015**, 6(2), 220-227.