

# Formulation and *In Vitro* Characterization of Solid-self Nanoemulsifying Drug Delivery System of Atorvastatin Calcium

M. Sunitha Reddy, B. Sravanthi

Department of Pharmaceutics, Centre for Pharmaceutical Sciences, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana, India

## Abstract

**Aim:** The aim of present study is formulation and *in vitro* characterization of solid-self nanoemulsifying drug delivery system (S-SNEDDS) of atorvastatin calcium. Atorvastatin calcium is a BCS Class II HMG Co-A reductase inhibitor also called as statins used in the treatment of high cholesterol. The solubility and dissolution profile of atorvastatin calcium which has poor aqueous solubility and low bioavailability was improved using SNEDDS. **Materials and Methods:** The solubility of drug in various oils, surfactants and cosurfactants were analyzed and peceol as oily phase, labrasol as surfactant, polyethylene glycol 400 as cosurfactant were selected for the formulation. To obtain ideal self-emulsification region, pseudo-ternary phase plots were constructed. Liquid SNEDDS were prepared, evaluated and two formulations PL1PEG4 A1, PL1PEG4 A2 were found to be stable and optimum. **Results and Discussions:** The formulation consisting of peceol, labrasol, PEG400 and drug 20 mg was selected for converting into S-SNEDDS using crospovidone as a carrier. Particle size distribution, zeta potential, and polydispersity index were characterized and found to be 36.22 nm, -1.32 mV, and 0.164, respectively. Dissolution tests were performed, and percentage drug release was found to be 91.7%. Accelerated stability studies were performed RH 75% ± 5% and 40°C ± 2°C for 1 month and were found to be stable.

**Key words:** Atorvastatin calcium, oils, pseudo-ternary phase diagrams, self nanoemulsifying drug delivery system

## INTRODUCTION

Self nanoemulsifying drug delivery system (SNEDDS) is isotropic mixtures of drug, surfactants and lipids which on mild agitation in gastrointestinal tract generates ultrafine droplets of oil in water nanoemulsion. Self-nano emulsification process requires low free energy due to this the process occurs spontaneously.<sup>[1]</sup> Dissolution of the drug is enhanced by micronized globule's interfacial area and thereby improving the bioavailability and permeability through biological membranes due to the presence of surfactants and lipids.<sup>[2]</sup> Atorvastatin calcium is a BCS Class II drug. It is HMG Co-A reductase inhibitor also called as statins used in the treatment of high cholesterol. The solubility and dissolution profile of atorvastatin calcium which has poor aqueous solubility and low bioavailability was improved using SNEDDS. In the present study, attempts are made to formulate and

perform *in vitro* characterization of solid-SNEDDS (S-SNEDDS) of atorvastatin calcium.

## MATERIALS AND METHODS

### Materials

Atorvastatin calcium was obtained as gift sample from Aurobindo laboratories. Peceol, labrasol, PEG400, Tween 80 are obtained from Gattefosse, Mumbai.

### Address for correspondence:

Dr. M. Sunitha Reddy, Centre for Pharmaceutical Sciences, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad - 500 085, Telangana, India.  
Phone: +91-9849958604.  
E-mail: baddam\_sunitha@rediffmail.com

**Received:** 23-10-2017

**Revised:** 19-11-2017

**Accepted:** 28-11-2017

## Methods<sup>[3-5]</sup>

### Solubility studies

Solubility studies were conducted by addition of excess amount of drug to 1 g of excipient individually. This is then subjected to heat to improve solubilization at a temperature of 40°C. Uniform mixing is achieved using vortex mixer. For about 48 h continuous agitation of suspensions was done on a rotary shaker at room temperature. The supernatant was collected after centrifugation at 3000 rpm for about 15 min. The collected supernatant was filtered by passing through 0.45 µm membrane filter. These filtrates were then analyzed at 238 nm by UV spectrophotometer. Based on the results excipients were selected.

### Construction of pseudo-ternary phase diagrams<sup>[6,7]</sup>

To determine the ideal self-emulsification region pseudo-ternary phase diagrams were constructed for selected oil, surfactant, and cosurfactant with water at room temperature by water titration method. Smix was prepared in ratios of 4:1, 3:1, 2:1, and 1:1, respectively. Fractions of Smix were then mixed with oil (Peceol) at volume ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 in different vials. The vials were then vortexed for uniformity. Small amount of water in suitable increments was added and observed visually after each addition. Based on the visual assessment, the samples were classified as nanoemulsion, microemulsion, coarse dispersion and gel phases. Using Triplot software version 4.1.2 Ternary phase diagrams were constructed. Bluish (or) clear transparent in appearance samples is considered under nanoemulsions.

### Preparation of SNEDDS formulations

Varying ratios of oil, surfactant and cosurfactant were selected for the formulation systems. Based on this, different SNEDDS of atorvastatin calcium were prepared. Atorvastatin calcium was kept constant (20 mg) for all formulations. Smix was prepared by mixing suitable proportions of surfactant and cosurfactants, and they are cyclomixed for uniform mixing. Atorvastatin calcium was accurately weighed and dissolved in suitable proportions of oil/Smix mixtures. This is then cyclomixed for 1min and then heated in thermostatic water bath at 40°C to facilitate drug solubilization. Then, all formulations were cyclomixed until transparent preparations were obtained till entire drug gets dissolved. Finally, prepared SNEDDS of atorvastatin calcium was kept aside at room temperature and examined for signs of turbidity (or) phase separation by visual examination and the formulations were characterized for various parameters.

### Characterization of SNEDDS

#### Droplet size and zeta potential<sup>[7]</sup>

Prepared SNEDDS formulations were diluted with distilled water in ratio 1:100 in a test tube and cyclomixed for 1 min. Droplet size, polydispersity index (PDI) and zeta potential of the

preparations are determined by dynamic light scattering (DLS) technique at 90° angle using Malvern Zetasizer Nano Zs 90.

#### Determination of self-emulsification time<sup>[4]</sup>

By performing visual assessments self-emulsification properties of SNEDDS formulations were obtained

- Pre-concentrate of SNEDDS formulation was added dropwise to 250 ml of distilled water, 0.1N HCl and 6.8 pH phosphate buffer in separate glass beakers at 37°C
- Contents were gently stirred by magnetic stirrer at 100 rpm
- Time taken for the formation of nanoemulsion was determined
- Tendency to form an emulsion was determined as:
  - Good - if emulsification occurs in <1 min with clear (or) transparent emulsion.
  - Bad - if emulsion is less clear.

#### Phase separation and stability study<sup>[7]</sup>

The formulations were diluted, agitated with 20 ml of distilled water at 37°C and allowed to stand for 24 h. The extent of phase separation and precipitation was analyzed by visual assessment.

#### Percentage transmittance<sup>[5]</sup>

Clarity of the emulsions is analyzed by percentage transmittance. Each SNEDDS formulation (0.1 ml) was added to a vial containing 10 ml of distilled water, 0.1N HCl and 6.8 pH phosphate buffer and cyclomixed for 1 min. Each sample was observed for percentage transmittance at 238 nm by UV spectrophotometer (UV-SHIMADZU).

#### Robustness to dilution<sup>[8]</sup>

Formulations were diluted with an excess of water (100 times) distilled water, 0.1N HCl, 6.8 pH phosphate buffer and were stored for 24 h. No precipitation and phase separation indicates that the samples are stable on dilution.

#### Drug loading efficiency<sup>[8]</sup>

Drug content in formulations was determined UV spectrophotometrically. 50 mg of the formulation was taken and diluted to 100 ml with methanol. Resultant solution was analyzed by UV spectrophotometer at 238 nm following suitable dilution.

The drug loading efficiency was determined by the following formula:

Drug loading efficiency = (Amount of drug in known amount of formulation/Initial drug load)\*100

#### Dispersibility<sup>[9]</sup>

Each formulation (0.1 ml) was added to 500 ml of distilled water at 37°C ± 0.5°C.

A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation.

By visual assessment the formulations are graded as follows:  
Grade A: Rapidly forming (<1 min) nanoemulsion having a clear (or) bluish appearance.

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

Grade C: Fine milky emulsion that formed within 2 min

Grade D: Dull grayish white emulsion and slow to emulsify.

### **Thermodynamic stability studies**

The prepared SNEDDS formulations were subjected to thermodynamic stability studies to study the effect of centrifugation and temperature on the stability of nanoemulsions.

- Heating and cooling cycles: Six cycles between  $-20^{\circ}\text{C}$  and  $+25^{\circ}\text{C}$  with storage of not <48 h were studied those formulations which are stable are subjected to centrifugation test.
- Centrifugation: Formulations which pass heating-cooling cycles are centrifugated at 2500 rpm for 40 min. The formulations that are stable are taken for freeze-thaw cycle.
- Freeze-thaw cycle: Three freeze-thaw cycles among  $-21^{\circ}\text{C}$  and  $+25^{\circ}\text{C}$  with storage at each temperature for not <48 h were done for the formulations.

Formulations which pass these thermodynamic stress tests are only selected for further studies.

### ***In vitro* dissolution studies<sup>[10]</sup>**

*In vitro* dissolution test of atorvastatin calcium SNEDDS and pure drug was performed in 900 ml of 0.1N HCl media at pH 1.2 using USP II dissolution test apparatus. Bath temperature was maintained at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  paddle speed was maintained at 100 rpm. At regular intervals of 5, 10, 15, 30, 45, 60, 75, and 90 min samples are withdrawn, and subsequent replacement of media of equal amounts was done. The collected samples are then analyzed for atorvastatin calcium using UV visible spectrophotometer at 238 nm. Effect of pH on SNEDDS formulations was analyzed by changing the dissolution media with 6.8 pH phosphate buffer. To compare the percentage drug release pure drug dissolution studies was also done.

### **Formulation of S-SNEDDS**

S-SNEDDS was prepared by mixing PL1PEG4 (1:9) liquid SNEDDS containing atorvastatin calcium with crospovidone which is used as a carrier in 1:2 ratio. Measured amount of crospovidone was taken in porcelain dish to this liquid SNEDDS formulation was added in dropwise manner. Contents are mixed uniformly using glass rod after each addition of liquid SNEDDS. Resultant damp mass was

passed through sieve no 120 and dried at room temperature and stored for further use.

### **Evaluation of S-SNEDDS of atorvastatin calcium**

#### **Flow properties**

Angle of repose, tapped density, bulk density, compressibility index, Hausner's ratio, and drug content were evaluated.

#### **Reconstitution properties of S-SNEDDS**

##### **Effect of dilution on S-SNEDDS**

About 100 mg S-SNEDDS was accurately weighed and introduced into 100 ml distilled water in a beaker at  $37^{\circ}\text{C}$  and gently mixed using magnetic stirrer at 100 rpm. The property of rapid emulsification was observed.

- Tendency to form an emulsion was determined as:  
Good - if emulsification occurs in <1 min with clear (or) transparent emulsion.  
Bad - if emulsion is less clear.

##### **Droplet size determination**

About 100 mg of S-SNEDDS formulation was diluted with 100 ml distilled water in a test tube and cyclomixed for 1 min and filtered. The droplet size and polydispersity index of the emulsion were determined at  $25^{\circ}\text{C}$  by DLS technique using Zetasizer ZS90.

##### **Fourier transform infrared (FT-IR)**

FT-IR spectrum of pure drug, crospovidone and formulation were obtained by FT-IR spectrophotometer. The spectrums were taken with the accumulations 24 scans and a resolution of 4/cm over the range of 400–4000/cm. The spectrum of formulation so obtained was compared with the spectrum of the pure drug for any interactions.

##### ***In vitro* drug release**

The *in vitro* dissolution study of S-SNEDDS which was filled in 0 size capsule and marketed drug was carried out using USP II dissolution test apparatus in 900 ml buffer of pH 1.2 at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  with paddle speed of 100 rpm. Samples were withdrawn at 5 min intervals for about 90 min, and every withdrawal equal volume of media was replenished. The samples are filtered through 0.45  $\mu\text{m}$  filter and analyzed using UV spectrophotometer at 238 nm amount of drug release was calculated from the calibration curve.

##### **Accelerated stability studies**

Accelerated stability studies of S-SNEDDS of formulation was conducted by storing the formulation at  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and RH 75%  $\pm$  5% for 1 month in stability chamber and later after 1 month formulation was evaluated for parameters such as effect of dilution, droplet size, PDI, and *in vitro* drug release.

## RESULTS AND DISCUSSION

### Solubility studies

After performing solubility studies, the drug was found to be more soluble in peceol (oil), labrasol (surfactant), PEG400 (cosurfactant) results which are shown in Figures 1-3.

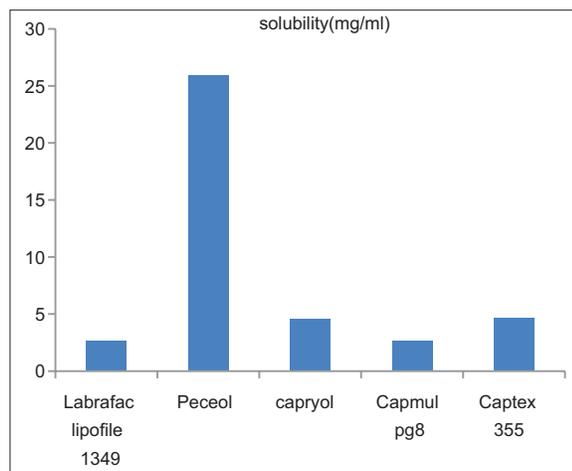


Figure 1: Solubility in various oils

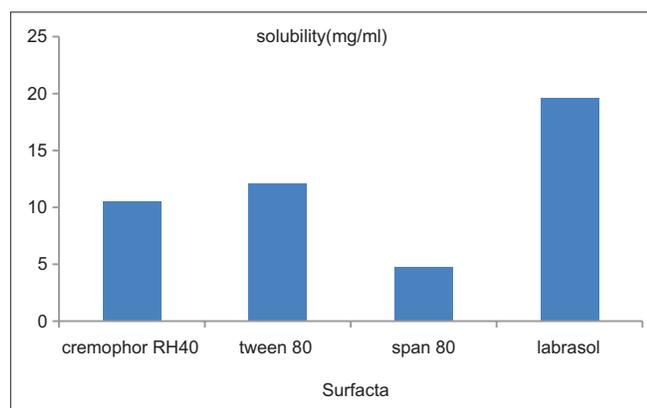


Figure 2: Solubility in various surfactants

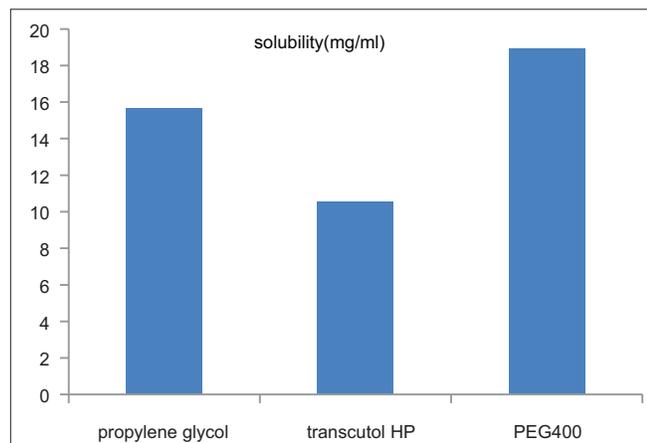


Figure 3: Solubility in various cosurfactants

### Construction of pseudo-ternary phase diagram

From pseudo-ternary phase diagrams, it has been found that the systems containing peceol (P) as oil phase, labrasol (L) as surfactant, and PEG400 (PEG4) as cosurfactant have showed good nanoemulsifying property. Four formulations are selected they are PLPEG400-A (L:PEG(1:4)), PLPEG400-B (L:PEG(1:3)), PTPG-C (T:PG (1:4)), and PTPEG-D (T:PEG (1:3)). Results are shown in Figures 4-8.

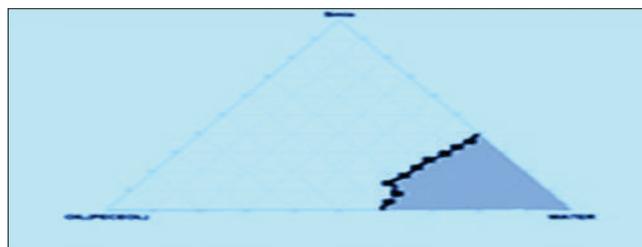


Figure 4: Pseudo-ternary diagram of PL1PEG4-A

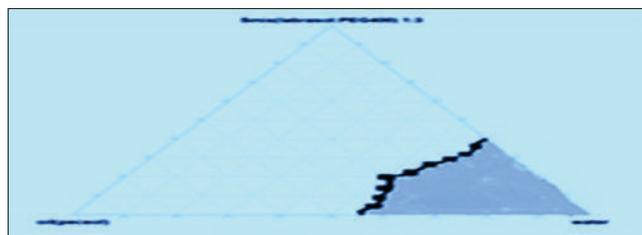


Figure 5: Pseudo-ternary diagram of PL1PEG3-B

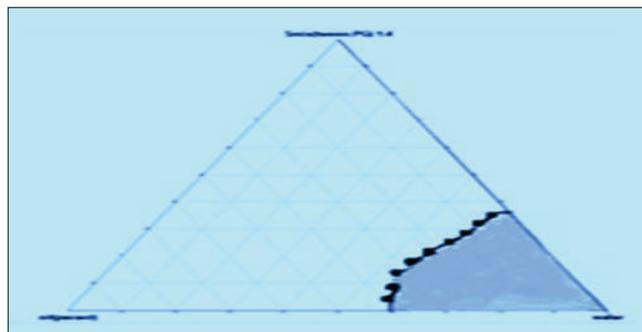


Figure 6: Pseudo-ternary diagram of PT1PG4-C

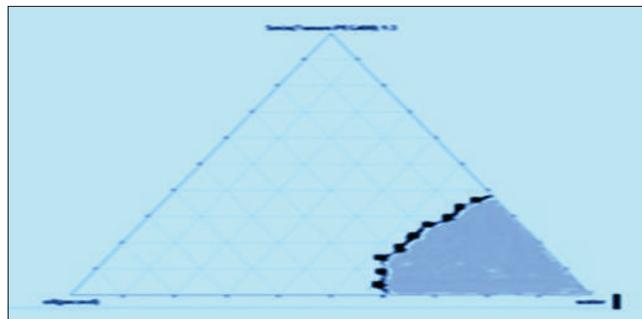


Figure 7: Pseudo-ternary diagram of PT1PEG3-D

### Size and zeta potential determination

Zetasizer is used to determine droplet size, PDI and zeta potential of the prepared formulations droplet size was found to be in nano range (14.26–75.15 nm). PDI was found to be above 100, zeta potential was found to be between –1.15 and –20.85 mV. Results are given in Tables 1-4 and Figures 9-12.



Figure 8: Formulation of PL1PEG4

### Dispersibility test

The two formulations showed GradeA emulsion when the test is performed in distilled water, 0.1 NHCl and phosphate buffer 6.8. Results are given in Table 2.

### Determination of self-emulsification time, phase separation and precipitation

The prepared SNEDDS of Atorvastatin Calcium were emulsified <1 min. Efficiency of all prepared emulsions was good. Results are given in Table 3.

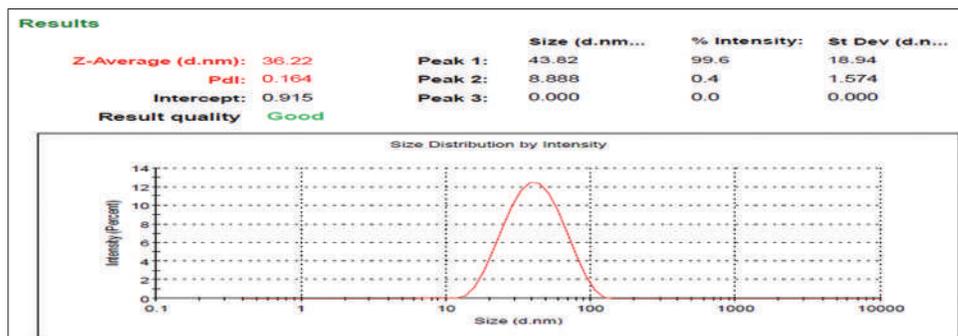


Figure 9: Particle size distribution of formulation PL1PEG4 A1

Table 1: Size, zeta potential and PDI of PL1PEG4

Formulation	Size (d-nm)	Region	Zeta potential	PDI	Result
PL1PEG4-A1	36.22	Nano	-1.32	0.164	Good
PL1PEG4-A2	42.36	Nano	-8.97	0.223	Good

PDI: Polydispersity index

Table 2: Results of dispersibility test for selected formulations

Formulation	Distilled water	0.1N HCl	pH 6.8 phosphate buffer
PL1PEG4-A1	Grade A	Grade A	Grade A
PL1PEG4-A2	Grade A	Grade A	Grade A

Table 3: Results of self-emulsification time, phase separation, and precipitation

Formulation	Emulsification time (s)	Precipitation	Phase separation
PL1PEG4-A1	32.62±0.54	No	No
PL1PEG4-A2	22.41±0.95	No	No

Table 4: Percentage transmittance of selected formulations

Formulation	Distilled water	0.1N HCl	6.8 pH phosphate buffer
PL1PEG4-A1	95.36±0.23	98.15±0.96	95.23±0.54
PL1PEG4-A2	96.45±0.31	97.23±0.12	96.76±0.34

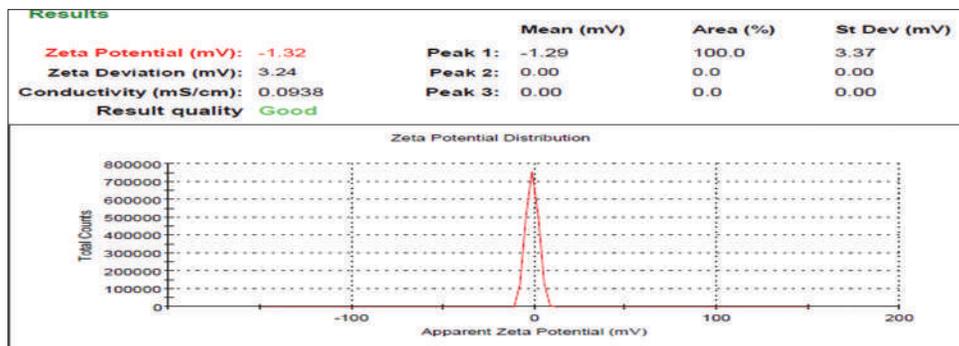


Figure 10: Zeta potential of formulation PL1PEG4 A1

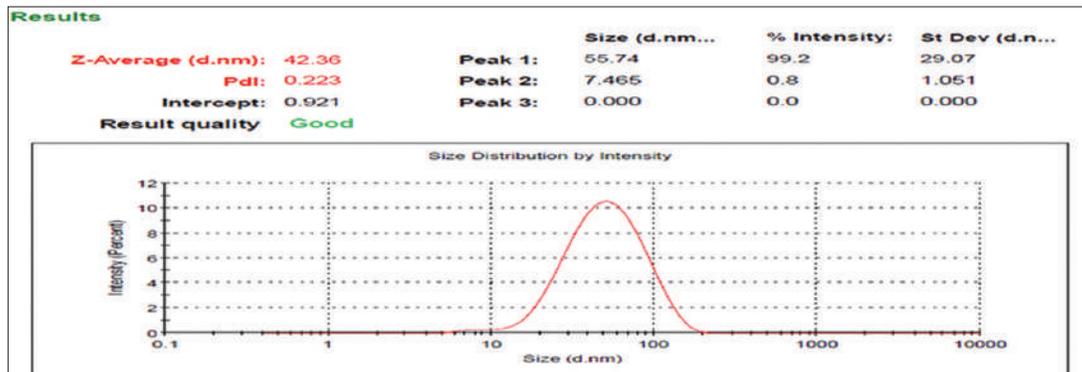


Figure 11: Particle size distribution of formulation PL1PEG4 A2

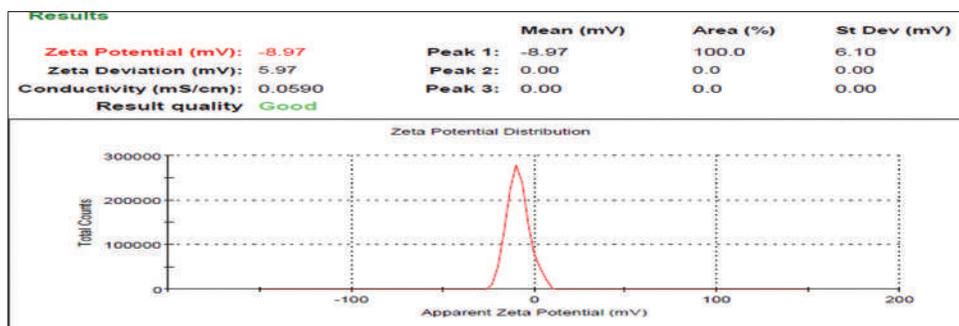


Figure 12: Zeta potential of formulation PL1PEG4 A2

### Percentage transmittance

Each diluted sample was observed for %transmittance at 238 nm. All formulations showed %transmittance more than 95% indicating clear emulsions. Results are given in Table 4.

### Robustness to dilution [Table 5]

Formulations selected were found to be stable without any precipitation to dilution in distilled water, 0.1 N HCl and phosphate phosphate buffer 6.8. Results are given in Table 5.

### FT-IR studies

The spectrum of drug excipient mixtures and final formulation is compared with the final formulation. It was found that the

Table 5: Results of robustness to dilution

Formulation	Distilled water	0.1N HCl	6.8 pH phosphate buffer
PL1PEG4-A1	Stable	Stable	Stable
PL1PEG4-A2	Stable	Stable	Stable

Table 6: Results of drug loading efficiency

Formulation	Drug loading efficiency(mg)
PL1PEG4-A1	97.54±0.96
PL1PEG4-A2	96.12±0.23

spectrum of pure drug and final formulation is almost similar, so it indicates that there is no interaction between atorvastatin calcium and final formulation [Figures 13 and 14].

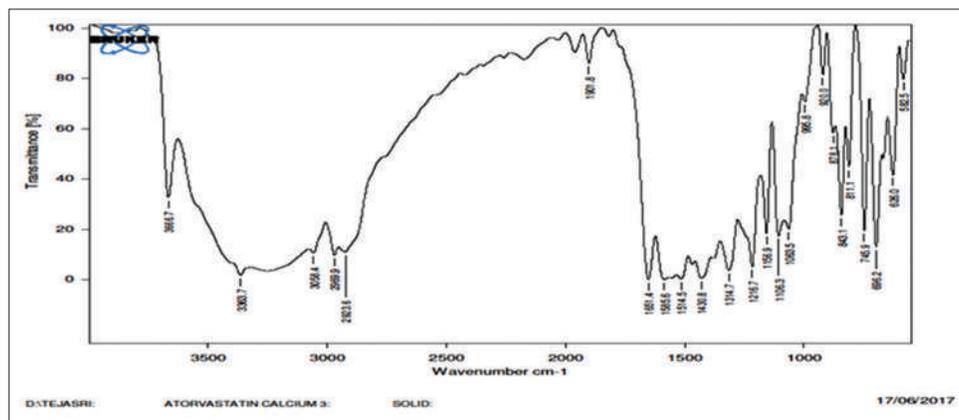


Figure 13: Infrared spectrum of pure drug

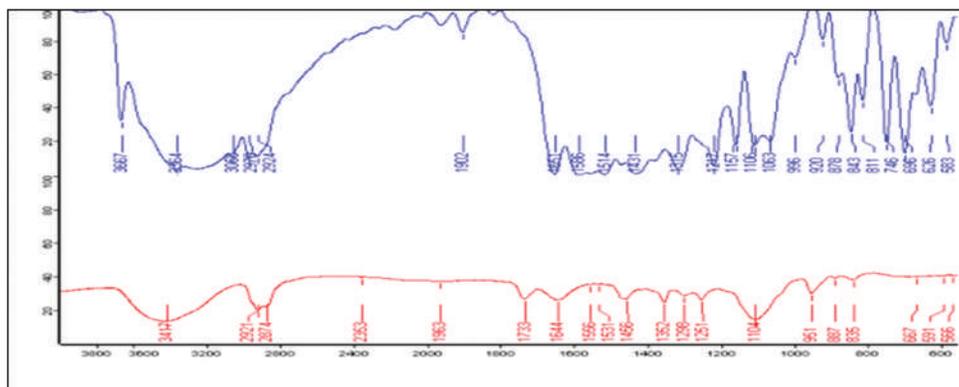


Figure 14: Infrared spectrum of pure drug and final formulation

■ Drug ■ Final formulation

### Drug loading efficiency [Table 6]

Drug loading efficiency for PL1PEG4-A1 was found to be  $97.54 \pm 0.96\%$  and for PL1PEG4-A2 was found to be  $96.12 \pm 0.23\%$ .

### Thermo dynamic stability studies

The formulations are found to be stable at the given conditions.

### Preparation of S-SNEDDS

Based on evaluation tests done for two liquid SNEDDS formulation, PL1PEG4 A-1 is selected for the preparation of S-SNEDDS as this formulation showed good self-emulsification property, particle size, PDI, and zeta potential.

### Evaluation of S-SNEDDS of atorvastatin calcium

#### Flow properties

Flow properties of S-SNEDDS such as angle of repose, bulk density, and tapped density; compressibility and Hausner's

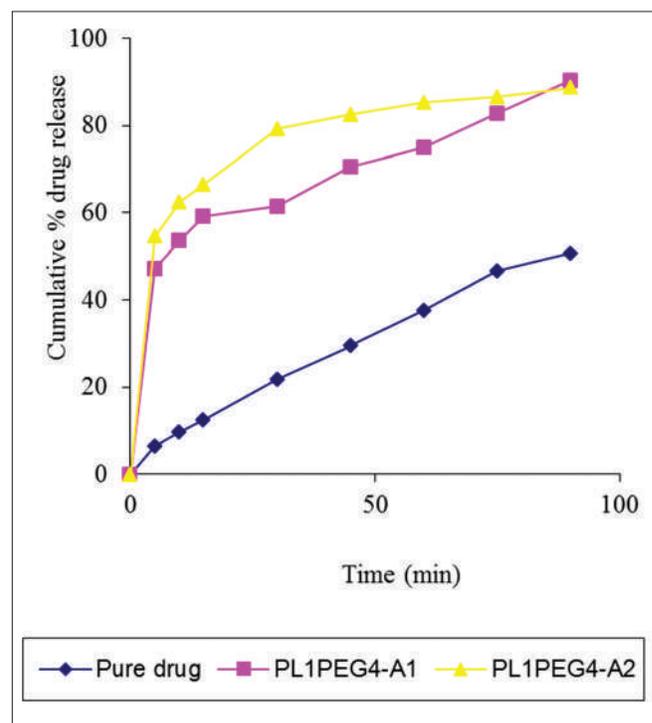


Figure 15: *In vitro* drug release study of self nanoemulsifying drug delivery system

ratio are determined and were found that the prepared S-SNEDDS showed “Good” flow properties.

### Drug content

Amount of drug present in prepared S-SNEDDS was determined. Drug content of S-SNEDDS was found to be  $95.23\% \pm 1.42\%$ .

### Reconstitution properties of S-SNEDDS

Effect of dilution on S SNEDDS: Effect of dilution on S -SNEDDS was studied, and it was found that prepared S- SNEDDS showed spontaneous emulsification, i.e., in <1 min and it was also found that there is no phase separation after 2 h of storage of diluted sample.

Droplet size determination: Mean droplet size and poly dispersibility index of reconstituted S-SNEDDS were found to be 35.70 nm and 0.352. Distribution of uniform sized particles was observed.

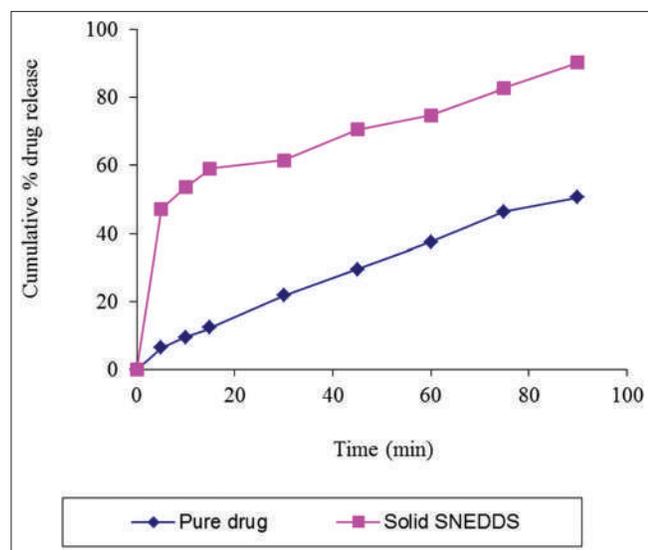


Figure 16: *In vitro* drug release study of solid-self nanoemulsifying drug delivery system

### Dissolution studies [Figures 15 and 16]

The formulation PL1PEG4-A1 is found to have better dissolution rates than the pure drug.

### FT-IR studies

The spectrums of drug-excipient mixtures and the formulations so obtained were compared with spectrum of pure drug for any interactions. FT-IR spectrum of pure drug and the formulation were almost similar due to same functional groups. It indicates that there was no interaction between atorvastatin calcium and crospovidone used in formulation [Figures 17-19].

### Accelerated stability studies

After 1 month storage formulation was evaluated for parameters such as effect of dilution, droplet size, PDI and *in vitro* drug release. S-SNEDDS passed the test of effect of dilution. Droplet size was found to be 35.70 nm with PDI 0.352 indicating no effect on droplet size after 1 month stability study. Cumulative percentage drug release from S-SNEDDS was 91% at the end of 1 month indicating no change in percentage drug release after 1 month stability study.

## CONCLUSION

Liquid SNEDDS of atorvastatin calcium with peceol as oil phase, labrasol as surfactant, PEG400 as cosurfactant was successfully developed. Based on thermodynamic stability studies, phase separation and precipitation, self-emulsification time, % transmittance, zeta potential and particle size studies, two formulations PL1PEG4-A1 and PL1PEG4-A2 were selected. From the above studies, it is concluded that the formulation PL1PEG4-A1 (1:9) is selected as the ideal formulation as it showed better drug release when compared to PL1PEG4-A2 (2:8). These were further converted to solid SNEDDS using crospovidone as a

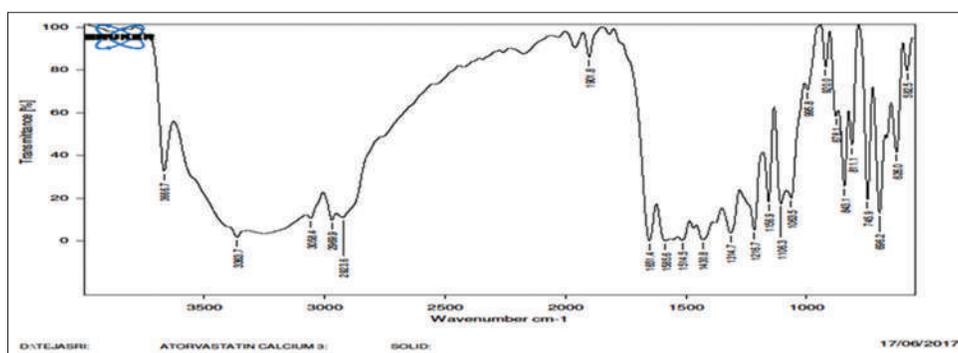


Figure 17: Infrared spectrum of pure drug

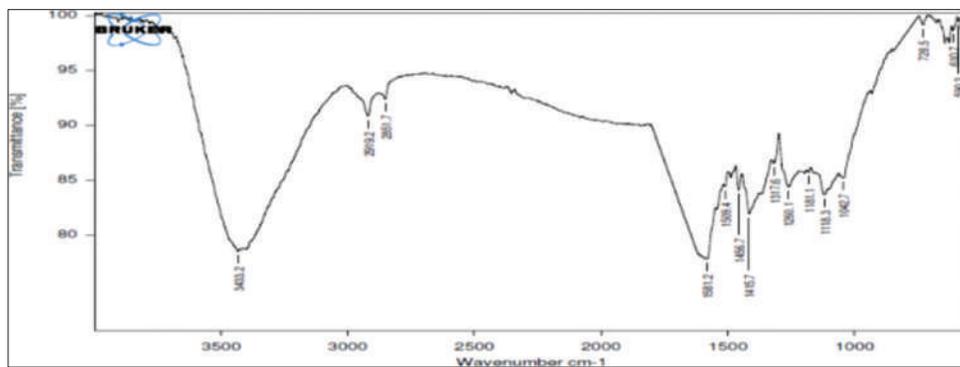


Figure 18: Infrared spectrum of crospovidone

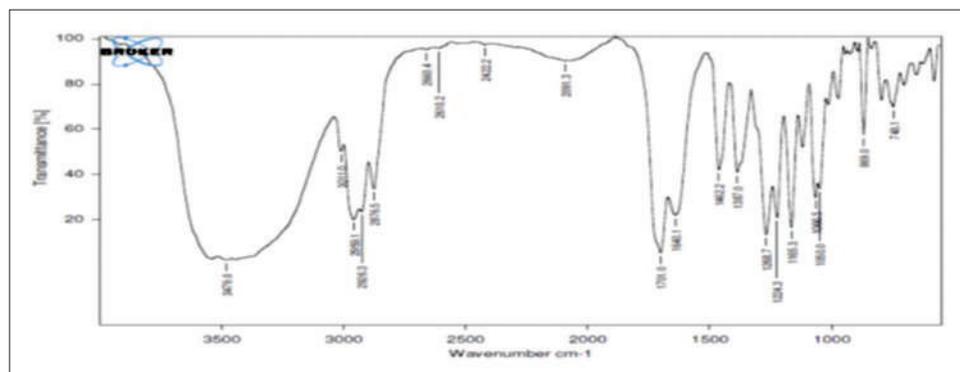


Figure 19: Infrared spectrum of solid-self nanoemulsifying drug delivery system formulation

carrier and evaluated for Flow properties and reconstitution properties of S- SNEDDS and showed good flow properties and reconstitution properties. Percentage drug release of the S-SNEDDS was almost similar to that of liquid SNEDDS.

## ACKNOWLEDGMENT

I would like to convey my sincere thanks to Centre for Pharmaceutical Sciences, IST, JNTUH, for excellent laboratory facilities necessary for carrying out this work. I would like to thank Gattefosse, Mumbai, for providing gift samples of vehicles. Gift sample of atorvastatin calcium was obtained from Aurobindo laboratories was duly acknowledged.

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Source of Support: Nil. Conflict of Interest: None declared.