NOVEL ORAL SOLID SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (S-SNEDDS) OF ROSUVASTATIN CALCIUM: FORMULATION, CHARACTERIZATION, BIOAVAILABILITY AND PHARMACOKINETIC STUDY

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ABSTRACT

Background and Objective: rosuvastatin calcium is an anti-hyperlipidemic drug. It is generally employed to treat hypercholesterolemia. This drug is a class II drug in the biopharmaceutical classification system (BCS) that shows low dissolution because of its crystalline nature and, thus, the poor oral bioavailability of 20%. The main purpose of this study is to develop a solid self-nanoemulsifying drug delivery system (S-SNEDDS) of rosuvastatin calcium for enhancement of its oral bioavailability.

Methods: In this study, liquid SNEDDS (L-SNEDDS) containing rosuvastatin along with garlic oil was formulated and further developed into a solid form by the spray drying technique using Aerosil 200 as a solid carrier. Ternary phase diagrams were constructed based on rosuvastatin calcium solubility analysis for optimizing the system. A mixture of garlic oil and Stepan-Mild® GCC (1:1) used as oil phase, tween 80 and PEG 400 were used as a surfactant and co-surfactant respectively. The prepared S-SNEDDS formulas were evaluated for flow properties, reconstitution properties, FTIR study, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), drug content and *in-vitro* drug release profile. To clarify the possible improvement in pharmacokinetic behavior of rosuvastatin S-SNEDDS, plasma concentration-time curve profiles in rats after the oral administration of optimized S-SNEDDS formula (S-B4) were compared to marketed product and pure drug in suspension.

Results: results showed that S-SNEDDS formulas has good flow properties and high drug content. Reconstitution properties of S-SNEDDS showed spontaneous self-nanoemulsification and no sign of phase separation. SEM photographs showed a smooth uniform surface of S-SNEDDS with less aggregation. Results of the *in-vitro* drug release showed that there was a great enhancement in the dissolution rate of rosuvastatin. At all-time points, it was observed that rosuvastatin plasma concentrations in rats treated with S-SNEDDS were significantly higher than those treated with the drug in suspension and marketed product.

Conclusion: in conclusion, the relative bioavailability of solid self-nano emulsified formulation S-B4 was about 2.38-fold compared to the marketed product and about 3.42-fold compared to the pure drug suspension. S-SNEDDS appeared to be an interesting approach to improving problems associated with oral delivery of rosuvastatin.

Keywords: Rosuvastatin, Solid Self-Nano Emulsifying Drug Delivery System (S-SNEDDS), Bioavailability and Biopharmaceutical Classification Systems (BCS).

1. INTRODUCTION

Lipid-based formulation approaches, particularly the self-emulsifying drug delivery system (SEDDS), are well known for their potential as alternative approach for delivery of hydrophobic drugs (Pouton, 2000), which are associated with poor water solubility and low oral bioavailability (Kim et al.,

2000). SEDDSs are isotropic and thermodynamically stable solutions consisting of oil, surfactant, co-surfactant and drug mixtures that spontaneously form oil-in-water (O/W) emulsion when mixed with water under gentle stirring. The motility of stomach and intestine provides the agitation required for self-emulsification in-vivo (Shah et al., 1994).

This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption (Kommuru et al., 2001). Apart from solubilization, the presence of lipid in the formulation further helps to improve bioavailability by enhancing the drug absorption (Constantinides, 1995).

Self-nano emulsifying drug delivery system (SNEDDS), upon dilution typically produces droplet sizes between 20 and 200 nm. These nano-sized droplets may offer an improvement in dissolution rates as well as bioavailability which results in more reproducible blood–time profiles. SNEDDS is a physically more stable formulation when compared to emulsions, and easier to manufacture in a large scale. The rationale to use SNEDDS for the delivery of poorly soluble drugs is that, they are presented in the form of pre concentrated solution. Hence, the dissolution step required for solid crystalline compounds shall be avoided. In addition, the formation of a variety of colloidal species on dispersion and subsequent digestion of SNEDDS facilitates drug absorption (Chakraborty et al., 2009 and Fatouros et al., 2007).

In recent years, much attention has been paid to solid self-nanoemulsifying drug delivery systems (S-SNEDDS), which have shown reasonable successes in improving oral bioavailability of poorly soluble drugs (Nasr et al., 2016). This drug delivery system combines the advantages of liquid SNEDDS with those of a solid dosage form and overcomes the limitations associated with liquid formulations. S-SNEDDS also exhibited more commercial potential and patient acceptability. Many techniques are offered to convert conventional liquid SNEDDS to solid form such as spray drying, adsorptions to solid carriers, spray cooling, melt extrusion, melt granulation, supercritical fluid based methods and high-pressure homogenization. The resulting powder may then be filled directly into hard gelatin capsules or mixed with suitable excipients before compression into tablets.

Rosuvastatin calcium is a synthetic lipid-lowering agent, it is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. The oral bioavailability of rosuvastatin is 20% because of low aqueous solubility due to its crystalline nature and is extensively metabolized by liver via oxidation, lactonisation, and glucuronidation. The oral bioavailability of rosuvastatin is low due to its low solubility in water (Ahsana et al., 2013). To overcome the problems concerning rosuvastatin, there was a need to develop S-SNEDDS which improves the oral bioavailability of rosuvastatin. Hence, the present study aimed towards the development of S-SNEDDS of rosuvastatin by a spray drying technique using Aerosil 200 as solid carrier for enhanced oral bioavailability.

2. MATERIALS AND METHODS
2.1. Materials
Rosuvastatin was provided as a gift sample from Jubilant Life Science, Noida (India). The oils (garlic oil, grape seed oil, olive oil and almond oil) were purchased from Falcon, Bengaluru (India). Sefsol 218® was provided as gift sample from Nikko Chemicals (Tokyo, Japan). Stepan-Mild® GCC was provede as gift sample from Stepan Company, Northfield, IL 60093, USA. Sesame oil was purchased from Falcon, Bengaluru (India). Linseed oil and soy bean oil was purchased from Sigma Aldrich, Mumbai, India. The oil vitamin E, polyethylene glycol 400 (PEG 400), tween 80, tween 60 and tween 20 were purchased from Merck, Mumbai, India. Lauroglycol 90 and PlurolOleique were provided as gift samples from Gattefosse (Saint Priest, Cedex, France). Solutol HS 15 and Unitop FFT 40 were provided as gift samples from Signet Chemicals Corporation Pvt. Ltd, Mumbai, India and Unitop Chemical Pvt Ltd (Mumbai, India), respectively. Propylene glycol was procured from Thomas Baker Chemicals, Mumbai, India. The HPLC-grade water was obtained from Milli-Q Water Purification System (Millipoire, MA). All other chemicals used during the experiment were of analytical grade.

2.2. Methods:
2.2.1. Bioanalytical Method
Plasma samples were assayed for rosuvastatin with a validated procedure using HPLC (Tripathi et al., 2017). Atorvastatin was used as internal standard. LiChrospher®100 RP-18 (5µm) column (Merck, Germany) was used for chromatographic separation with a mobile phase consisting of a 70:30 (v/v) 0.2% formic acid and methanol at a flow rate of 1 ml/min. Total run time was 8 min. The samples were detected at 240 nm (Kumar et al., 2006).

2.2.2. solubility study of rosuvastatin in different oils, surfactants and co-surfactants
In order to estimate the right SNEDDS excipients with good solubilizing capacity for rosuvastatin, saturation solubility was performed in different oils (sesame oil, garlic oil, Sefsol 218, grape seed oil, Stepan-Mild® GCC, olive oil, almond oil, linseed oil, vitamin E and soya bean oil), surfactants (Unitop FFT 40, tween 60, tween 80, tween 20 and Solutol HS 15) and co-surfactants (Plurol Oleque, propylene glycol, lauroglycol 90 and PEG 400) using the shake flask method. The solubility was estimated by adding an excess quantity of rosuvastatin in 1 ml of excipient (oils, surfactants and co-surfactants). These samples were kept at 26 ± 0.5 ºC in an isothermal shaker. After 72 h, the samples were collected and centrifuged in order to separate out the undissolved drug (Kumar et al., 2016). Supernatant was followed by filtration through a 0.45 -µm Millipore filter membrane and the concentration of drug was determined using HPLC method. All measurements were done in triplicate.

2.2.3. Phase diagram construction
In order to estimate the concentration of components for the existing range of the SNEDDS, a pseudo ternary phase diagram was constructed at ambient temperature using aqueous titration method. Oils, surfactant and cosurfactant were grouped in different combinations for phase studies. Diagrams were plotted according to the procedure explained by Kumar and associates (Kumar et al., 2016). Compositions containing different proportions of surfactant/co-surfactant mixtures (S_{mix}), i.e. 1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1 and 5:1 were tried to emulsify the selected oil. These S_{mix} ratios were chosen in increasing concentration of surfactant with respect of cosurfactant and increasing concentration of cosurfactant with respect of surfactant. For the preparation of each phase diagram, different volume ratios of oil and S_{mix} (1:9, 2:8, 3:7, 4:6, 5:5, 6:4,7:3, 8:2, 9:1, 1:2, 1:3, 1:3.5, 1:5, 1:6, 1:7 and 1:8) in glass vial were vortexed to form a clear and homogenous system; followed by titration with the aqueous phase. During titration with water, the mixture of lipid and S_{mix} were subjected to phase clarity evaluation by visual examination. The volume of water required to bring phase transition from transparency to turbidity was noted, and pseudoternary plots were drawn using CHEMIX School software ver 3.60 (Arne Standnes, USA).

2.2.4. Preparation of rosuvastatin loaded SNEDDS

Once the self-nanoemulsifying area was identified, SNEDDS formulas with desired component ratios were prepared. The ratio of surfactant to co-surfactant (S_{mix}) was also optimized using pseudoternary phase diagrams. Based on the optimization of of surfactant to co-surfactant and Oil to Smix ratio, two successful liquid SNEDDS formulas were prepared presented in table 2.1. In all formulas, the content of rosuvastatin was kept constant. Briefly oil, surfactant and co-surfactant were properly weighed and mixed in stoppered glass vial using a vortex mixer in order to obtain a complete mixture. An amount of rosuvastatin was dispersed into the mixture of oil and S_{mix} with continuous mixing until drug was completely dissolved. Prepared formulas were then stored at room temperature until further use (Ehab et al., 2004).

2.2.5. Preparation of rosuvastatin loaded Solid-SNEDDS

Based on the rank order performed for all conventional rosuvastatin SNEDDS formulas depending on their characterization and evaluation tests, two optimized SNEDDS formulas were selected to be solidified by spray drying technique using Aerosil 200 as solid carrier, the ratio of SNEDDS: Aerosil 200 (1:1.5) was found to be optimized. Briefly, SNEDDS formula (666mg) and Aerosil 200 (1000 mg) were suspended in 200 mL ethanol with continuous stirring until forming an isotropic mixture. The mixture was then kept at room temperature 25 ± 2 °C and equilibrated for 24 h. The obtained mixture was then spray dried using a Buchi mini spray dryer (Buchi, Switzerland) under the following conditions: inlet temperature: 60 °C; outlet temperature: 35 °C; aspiration: 85%; feeding rate of the suspension: 5 mL/min and atomization air pressure: 5 kPa. (Dong et al., 2011).

2.3. Characterization of rosuvastatin loaded S-SNEDDS
2.3.1. Reconstitution properties of S-SNEDDS

In order to examine the reconstitution capability of prepared S-SNEDDS, a 200 mg S-SNEDDS was totally dispersed in water (100 mL) for one hour. The resulted dispersion was centrifuged at 4000 rpm for 10 min. Supernatant was collected and by using UV spectrophotometer, transmittance was determined (by taking water as blank) at 630 nm. Moreover, reconstitution efficiency was determined in terms of polydispersity index, globule size and globule surface charge. After 12 h of formulation post dispersion, samples were examined visually for physical appearance, phase separation, variations in globule size, zeta potential and percentage transmittance. Studies were performed thrice (Nasr et al., 2016).

2.3.2. Fourier Transform Infrared Spectroscopy (FTIR)

All experiments were recorded at room temperature (25 ± 0.5 °C) and dry conditions. FTIR was used to study the interaction between rosuvastatin, excipients, physical mixture and drug-loaded formulation. FTIR spectroscopy instrument Shimadzu (FTIR 8400S), Japan was used for the study. FTIR spectra of samples were obtained by scanning on spectral range from 4000 to 400 cm^{-1} by using the potassium bromide pellet technique (Tang et al., 2007).

2.3.3. Scanning Electron Microscopy (SEM)

Scanning electron micrographs for rosuvastatin, Aerosil 200 and developed S-SNEDDS (B1 and B4) were taken using Scanning electron microscope (LEO 435VP model, Cambridge, UK). Sample was deposited onto stubs using one side of a double-sided adhesive dried carbon tape. Images were captured at an acceleration voltage of 15 kV with the Secondary Electron Image (SEI) as a detector (Surender et al., 2011).

2.3.4. Differential Scanning Calorimetry (DSC)

DSC thermograms of pure rosuvastatin, Aerosil 200, physical mixture of both, prepared optimized L-SNEDDS and S-SNEDDS were measured using differential scanning calorimeter (Perkin Elmer, Pyris 1, India). Samples of 3 mg weight were heated in hermetically sealed aluminium pans over temperature range of 40–400 °C at a constant rate of 10 °C/min. An empty aluminium pan was used as reference (Reddy et al., 2004).

2.3.5. Drug loading efficiency

For determining the rosuvastatin content, an amount of solid SNEDDS formulas (equivalent to 40 mg of rosuvastatin) was diluted with methanol in volumetric flask and mixed well by shaking or inverting the volumetric flask two to three times. Samples were prepared in triplicate and drug content was measured after suitable dilutions by HPLC method (Odeberg et al., 2003). Drug loading efficiency was calculated using the given formula:

$$Drug\ loading\ efficiency = \frac{Rosuvastatin\ conent\ in\ 100\ mg\ of\ solidified\ SNEDDS}{Actual\ rosuvastatin\ added\ in\ solidified\ SNEDDS} \times 100$$

2.3.6. *In-vitro* drug release profile

The *in-vitro* drug release of rosuvastatin from the optimized S-SNEDDS was performed using the procedure explained by Alaa and associates. In this study rosuvastatin release from the optimized S-SNEDDS, pure rosuvastatin and marketed product was carried out using USP dissolution apparatus type II (Erweka, DT 600). The dissolution medium consisted of 900 ml of freshly prepared phosphate buffer pH 6.8 maintained at 37 ± 0.5°C and the paddle speed was set at 50 rpm. Hard gelatin capsules, size "000" filled with preconcentrate (equivalent to 40 mg rosuvastatin) were put inside spiral capsule sinker. About 5 mL samples were withdrawn using filter syringe (0.45µm) at regular time intervals (0, 5, 15, 30, 60, 90, 120, 240, 300 and 360 min) and aliquot amount of buffer was replaced in order to maintain sink condition. The samples were analyzed for the drug content using HPLC method (Alaa et al., 2010).

2.3.7. Pharmacokinetic studies

The final attainment of a test for a developed formulation relies on its *in-vivo* performance. Pharmacokinetic studies were carried out using Wistar albino rats to evaluate plasma levels of rosuvastatin. Animals were divided into three groups (*n = 6*). All animal studies were performed out after approval of the protocol by HMU-College of Pharmacy-Ethics Committee with reference No. (160620-106). Formulations were given orally by using oral feeding cannula. Group A was orally administered with rosuvastatin (at 10 mg/kg body weight to albino Wistar rats) (Tripathi et al., 2017) suspended in 0.5% sodium carboxy methylcellulose (as suspending agent), group B received marketed conventional tablet (dose equivalent to 10 mg/kg body weight of albino Wistar rats) suspended in 0.5% sodium carboxy methylcellulose, whereas group C received S-SNEDDS B4 (dose equivalent to 10 mg/kg body weight of albino Wistar rats) redispersed in about one milliliter of distilled water.

The rats were anesthetized using diethyl ether and blood samples (0.2 mL) were withdrawn from the retro-orbital eye

vein of a rat at 0 (predose), 1, 2, 4, 6, 8 and 24 h. The samples were collected in EDTA coated micro centrifuge tubes. After collection, blood was centrifuged at 10,000 rpm for 10 min, and plasma was separated and kept at −20°C for further processing through HPLC (Tripathi et al., 2017). Frozen plasma samples were thawed at room temperature. Plasma samples (100 µL) were separated and 0.9 mL of acetonitrile was added to each of plasma samples to precipitate the protein. The samples were then centrifuged again at 5000 rpm for 5 min and the supernatant (20 µL) were filtered and directly injected into the HPLC column and peak area values were recorded (Surender et al., 2010). Rosuvastatin concentration-time profile in plasma after oral delivery was determined by pharmacokinetic software (Pharsight Corporation, CA). Pharmacokinetic parameters (C_{max}: maximum concentration of drug, AUC_{0-24}: area under the curve between 0 and 24 h and $t_{1/2}$: half-life) in plasma for rosuvastatin suspension, marketed formulation and optimized S-SNEDDS were evaluated by using software for each group. All the data were statistically analyzed using paired t test (p<0.05).

2.3. Statistics
Results were presented as a mean ± SD for three times repeated tests. One-way analysis of variance (ANOVA) method was employed to identify insignificant factors. Data were analyzed using a design of expert developed for response variables by omitting the insignificant term with P > 0.05 and significant term when P < 0.05.

3. RESULTS AND DISCUSSION:
3.1. Bioanalytical Method
The standard plots were constructed with seven concentrations in triplicate ranging from 0.25 to 10 ng/mL for plasma. The peak area ratio of the drug to internal standard (IS) was plotted against the concentration and the regression equation obtained was y = 0.1422x + 0.0025 with the correlation coefficient of 0.999. The results showed excellent correlation between the peak area ratio and concentration of rosuvastatin.

3.2. Study of Rosuvastatin solubility in different oils, surfactants and co-surfactants
Adequate selection of excipients is very essential for the development of SNEDDS as drug loading and absorption is chiefly affected by the excipients. All excipients should belong to GRAS (generally regarded as safe) category. Since the present work aimed at preparation of an oral formulation therefore adequate solubility of drug in the selected oil, surfactant and cosurfactant is very important. The solubility of rosuvastatin in different oils, surfactant and cosurfactant is shown in table 3.1. All measurements were done in triplicate and then solubility is expressed as the mean value of (mg/mL) ± S.D.

Oil phase plays an important role in maintaining the drug in solubilized form in formulation and prevents the drug from precipitation in GIT (Wooster et al., 2008). Rosuvastatin possessed highest solubility in a mixture of garlic oil and Stepan-Mild® GCC (1:1) (68.56 ± 3.02 mg/mL) amongst the various oils tried. Stepan-Mild® GCC is a medium chain mono- and diglyceride emulsifier and has lipophilic characteristics (Prajapati et al., 2012). It has been extensively used in the preparation of microemulsions and nanoemulsions, as its solubilization capacity for lipophilic drugs is better than that of fixed oils. It acts as solubilizer due to its medium chain glyceride capacity to facilitate absorption and improve bioavailability. It provides

protection against enzymatic hydrolysis (Sindhu et al., 2018). It find application in dietary supplements and pharmaceutical products as vehicle, bioavailability enhancer, viscosity modifier, emulsifier and stabilizer.

Surfactants are another important ingredient of SNEDDS as it helps in decreasing the interfacial tension to assist dispersion mechanism and to provide a flexible film around the globules. Non-ionic hydrophilic surfactants are considered to be more suitable than the ionic ones for the formulation of nanoemulsions. The reason might be that they are less toxic than ionic surfactants (Nagi et al., 2017). Moreover, they facilitate better emulsion stabilization over a wider range of pH and ionic strength in comparison to the anionic and cationic ones. Rosuvastatin showed maximum solubility in tween 80 (40.34 ± 3.82 mg/mL). Tween 80 was also found to be miscible with mixture of garlic oil and Stepan-Mild® GCC therefore; it was selected as surfactant for the development of SNEDDS.

Co-surfactant reduces the bending stress of O/W interface. In this way they assist interfacial film to form various curvatures required for nanoemulsion production. PEG 400 was selected as co-surfactant on the basis of solubility and miscibility studies (table 3.3) and it also showed good miscibility with mixture of garlic oil and Stepan-Mild® GCC.

3.3. Phase diagram construction
One of the most important characteristics of SNEDDS is the change that occurs when the system is diluted (since it will be diluted by body fluids after administration), which may cause drug precipitation due to the loss of solvent capacity (Pouton 2000). Phase diagrams were constructed by using different ratios of S_{mix} and it was found the different nanoemulsion areas (isotropic region) were obtained with different S_{mix} (figure 3.1). An increasing order of nanoemulsion region was found with S_{mix} ratio of 3:1> 2:1> 1:1> 4:1> 5:1> 1:0> 1:2 > 1:3. The ratio of 3:1 of S_{mix} showed more nanoemulsion region due to its capability to solubilize the oil phase and its potential to decrease the free energy of system to very low level which was required to formulate nanoemulsion. Increased amount of tween 80 resulted in decrease in interfacial tension as well as increment in oil-water interface fluidity resulting in higher system entropy. It was also observed that S_{mix} having ratio of 4:1 and 5:1 showed decreased region implying that on further tween 80 addition, the S_{mix} was unable to result in any more emulsification. Influence of increased amount of co-surfactant with respect to surfactant (1:2 and 1:3) was also examined and it was observed that on increasing PEG 400 content there was reduction in the nanoemulsion region as in case of 1:2 and 1:3 ratios, due to incapability of PEG 400 to solubilize oil that could also have been due to decrease capacity of PEG 400 to reduce free energy and interfacial tension, which is needed for emulsification mechanism. Pseudoternary phase diagrams showed that nanoemulsion area was more in formulas prepared with tween 80- PEG 400 mixture (S_{mix}) at 3:1 ratio as shown in figure 3.5 Thus, fixing the surfactant/cosurfactant ratio at 3:1 is a better choice from a stability point of view. At S_{mix} 3:1, a higher nanoemulsion region was observed, perhaps because of the further reduction of the interfacial tension and increased fluidity of the interface at S_{mix} 3:1.

3.4. Characterization of Rosuvastatin loaded S-SNEDDS
3.4.1 Reconstitution properties of S-SNEDDS
Reconstitution is required for the self-emulsification of S-SNEDDS to a nanoemulsion during gastric/aqueous dilution. The two formulas showed spontaneous nanoemulsification

and there was no sign of phase separation or phase inversion of nanoemulsion after storage of 24 h. A transmittance value higher than 98% suggests efficient self-emulsification via aqueous dispersion as shown in table 3.2. Marginal differences in percent transmittance for S-SNEDDS B1 and S-SNEDDS B4 were found after 1 h and 12 h (table 3.2).

In addition, the no decline in globule size after 12 h compared to 1 h indicated complete self-nanoemulsification by the surfactant and co-surfactant for both formulations. There was negligible difference between the percentage transmittance and zeta potential at 1 h and 12 h as shown in table 3.2. All formulations showed mono-dispersed globules (as per PDI value, table 3.2) without signs of precipitation. Moreover, the absence of globule coalescence and drug precipitation may be affirmed by the zeta potential values. This finding corroborates the enhanced stability of the nanoemulsion after reconstitution.

3.4.2. Fourier Transform Infrared Spectroscopy (FTIR) study

FTIR study was carried out to find out any possible interaction taking place between rosuvastatin and excipients in SNEDDS preparation. Spectra of rosuvastatin, aerosol 200, physical mixture (comprised of drug, mixture of garlic oil and Stepan-Mild® GCC (1:1), tween 80 and PEG 400), optimized drug loaded L-SNEDDS and S-SNEDDS are shown in Figure 3.2 (a, b, c, d and e) respectively. Pure Rosuvastatin, as reported in previous work, exhibited major characteristic peaks at 3230 cm^{-1} (O-H group); 2969.21 cm^{-1} (C-H stretch); 1604.66 cm^{-1} (carbonyl group) and 1335.61 cm^{-1}, 1382.87 cm^{-1}, 1435.90 cm^{-1} (aromatic trio) respectively (Ahsan et al., 2017). It was observed that all major peaks in FTIR of pure drug were retained in physical mixture of drug and excipients suggesting that there was no interaction between rosuvastatin and excipients. Moreover, in rosuvastatin loaded L-SNEDDS and S-SNEDDS the vibration bands were not affected by the excipients of SNEDDS as the entire drug peaks were present in the SNEDDS which showed intactness of drug in formulation and absence of any possible interaction between drug and formulation excipients.

3.4.3. Scanning Electron Microscopy (SEM)

The surface morphology of pure rosuvastatin powder, Aerosil 200 and S-SNEDDS B1 and B4 was evaluated using scanning electron microscope as shown in figure 3.3. The drug powder appeared with an irregular crystalline shape as irregular and plate-shaped crystals having rough surfaces. Aerosil 200 appears to be spherical porous particles. The image of the solid SNEDDS formulas (S-B1 and S-B4) containing rosuvastatin however, illustrate that the particles had the same outer macroscopic morphology consisting of well separated spherical particles with relatively deep dents and similar diameters. Following spray drying, the crystalline rosuvastatin changed to highly amorphous in nature.

3.4.4. Differential Scanning Calorimetry (DSC) study:

Thermograms of pure rosuvastatin, Aerosil 200, physical mixture of both, prepared optimized L-SNEDDS and S-SNEDDS were obtained using differential scanning calorimeter as shown in Figures 3.4 (a, b, c, d and e) respectively. The Thermograms of pure rosuvastatin exhibited a sharp endothermic peak at about 132.575 °C, corresponding to its melting point as shown in figure 3.4.a. Aerosil 200 showed no specific peaks from 40 to 400 °C as presented in Figure 3.4 b. However, a melting endotherm having the characteristic peak of rosuvastatin was observed in the physical mixture of rosuvastatin and Aerosil 200. In case of rosuvastatin L-SNEDDS and rosuvastatin S-SNEDDS, the endothermic peak of rosuvastatin were absent as shown in figure 3.4 d, and figure 3.4 e. The change in melting behavior of drug can be attributed to the inhibition of its crystallization and solubilization of rosuvastatin in L-SNEDDS and S-SNEDDS. Therefore, it could be concluded that rosuvastatin in the solid SNEDDS was in the amorphous form. It is known that transforming the physical state of a drug to the amorphous or partially amorphous state leads to a high-energy state and high disorder, resulting in enhanced solubility. As a result, it was expected that the solid particles would also have enhanced solubility.

3.4.5. Drug loading efficiency

The amount of rosuvastatin present in the optimized S-SNEDDS (S-B1 and S-B4) formulas was found to be within the USP limit. The drug loading efficiency was found to be 93.05 ± 0.23 % for S-SNEDDS B1 and 97.34 ± 0.53 % for S-SNEDDS B4. Drug content in S-SNEDDS was almost identical with the results obtained in liquid SNEDDS, so there was no change of percentage drug content after conversion of liquid to solid SNEDDS using spray drying technique.

3.4.6. *In-vitro* drug release profile

Release of rosuvastatin from S-SNEDDS was higher in comparison to pure rosuvastatin and marketed product as presented in figure 3.5. In initial 30 min only 8.43 ± 0.75 % and 24.74 ± 1.70 % of rosuvastatin was released from pure drug and marketed tablets, respectively. On the other hand, S-SNEDDS B1 and S-SNEDDS B4 showed improved release within the same time period. Rosuvastatin release from S-SNEDDS B1 reached 70.55 ± 2.11% and 75.04 ± 2.65% for S-SNEDDS B4 within 30 min. The drug release study also indicates that the self-nanoemulsifying property of the formulation remains unaffected by the conversion of the liquid SNEDDS to the solid form as shown in figure 3.5. It was also noticed that the release of rosuvastatin from S-SNEDDS was slightly lower than liquid SNEDDS which might be due to presence of adsorbent material which may delay the dissolution rate for a small extent (Nasr et al., 2016).

3.4.7. Pharmacokinetic studies

To clarify the possible improvement in pharmacokinetic behavior of rosuvastatin, the plasma concentration-time curve profiles of rosuvastatin after the oral administration of optimized S-SNEDDS formulas were compared to marketed products and drug in suspension as depicted in figure 3.6. S-SNEDDS (B4) was selected for pharmacokinetic study due to its smallest globule size, great drug release profile as well as its maximum efficiency of rosuvastatin loading. As represented in table 3.6, the maximum concentration C_{max} of S-SNEDDS B4 was 850.41± 5.11 ng/mL, compared with marketed product which was 296.31± 2.72 ng/ml and pure drug suspension which was 230.23 ± 2.11 ng/ml. The C_{max} was enhanced 2.87 and 3.69-fold as compared with marketed product and pure drug, respectively. It was also observed that S-SNEDDS B4 showed high area under the curve value (AUC_{0-24}) which was 4094.62 ± 22.34 ng.h/mL in comparison with marketed product (1715.04 ± 10.52 ng.h/mL) and pure drug suspension (1196.38 ± 6.01 ng.h/mL) indicating rapid absorption and higher bioavailability of drug from S-SNEDDS. The relative bioavailability of S-SNEDDS B4 was about 2.38-fold compared with marketed product and about 3.42-fold compared to pure drug suspension.

4. CONCLUSION

It is concluded that S-SNEDDS preserved the self-emulsification performance of the liquid SNEDDS and gave a faster *in-vitro* dissolution rate than the pure drug and marketed product.

The relative bioavailability of self-nano emulsified formula B4 was about 2.38-fold compared to the marketed product and about 3.42-fold compared to the pure drug suspension. S-SNEDDS appeared to be an interesting approach to improving problems associated with oral delivery of rosuvastatin. Finally, the oral delivery of hydrophobic drugs can be made possible by S-SNEDDS, which have been shown to substantially improve the oral bioavailability.

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Table 2.1. Compositions of excipients of optimized rosuvastatin loaded SNEDDS formulas

Formula	Rosuvastatin (mg)	Oil (% w/w)	S_{mix}(% w/w)	Total % of Oil (% w/w)	Ratio Oil: Smix	S/Cos Ratios
B1	40	5	30	16.6	1:6	3:1
B4	40	6.41	38.46	16.6	1:6	

Table 3.1. Solubility of Rosuvastatin in various excipients and miscibility of selected oil with surfactants and co-surfactants

Solubility of Rosuvastatin in oils		Solubility of Rosuvastatin in surfactants and co-surfactants		Miscibility of Garlic oil: Stepan-Mild® GCC (1:1) with surfactant/co-surfactant
Oil	Solubility (mg/mL) ± S.D. (n=3)	Surfactant (S) and co-surfactant (C)	Solubility (mg/mL) ± S.D. (n=3)	Observation
Sesame oil	1.12 ± 0.23	Tween 60 (S)	8.54 ± 1.88	Clear
Sefsol 218	10.00 ± 1.56	Tween 80 (S)	40.34 ± 3.82	Clear
Grape seed oil	1.32 ± 0.31	Tween 20 (S)	18.31 ± 2.04	Clear
Stepan-Mild® GCC	20.32 ± 2.70	Unitop FFT 40 (S)	4.32 ± 0.87	Phase separation
Olive oil	12.92 ± 1.80	Solutol HS 15 (S)	5.24 ± 0.54	Phase separation
Almond oil	13.30 ± 2.04	Plurol Oleque (C)	3.73 ± 0.76	Turbid
Vitamin E	7.22 ± 1.64	Propylene glycol (C)	20.58 ± 2.57	Clear
Linseed oil	8.34 ± 1.72	Lauroglycol 90 (C)	2.50 ± 0.14	Phase separation
Soya bean oil	6.23 ± 1.34	PEG 400 (C)	30.11 ± 3.06	Clear
Garlic oil	13.41 ± 1.95			
Garlic oil: Stepan-Mild® GCC (1:1)	68.56 ± 3.02			

Table. 3.2. Reconstitution properties of S-SNEDDS

Parameter	S-SNEDDS B1		S-SNEDDS B4	
	1 h	12 h	1 h	12 h
Globule size (nm)	92.13 ± 2.30	92.16 ± 2.42	93.32 ± 2.55	95.01 ± 2.63
PDI	0.221 ± 0.004	0.226 ± 0.005	0.230 ± 0.007	0.236 ± 0.008
Percentage transmittance	99.02 ± 0.07	98.92 ± 0.08	98.71 ± 0.13	98.68 ± 0.17
Zeta potential (mV)	-30.07 ± 0.19	-30.05 ± 0.20	-29.83 ± 0.29	-29.12 ± 0.32

Table. 3.3. Pharmacokinetic parameters of Rosuvastatin after oral administration of optimized S-SNEDDS (B4), pure drug and marketed product in rat.

Formulation	Cmax (ng/ml)	Tmax (h)	Ke(h-1)	AUC0-24(ng.h/ml)	AUC0-∞(ng.h/ml)	t1/2 (h)
Drug suspension	230.23 ± 2.11	4	0.098 ± 0.001	1196.38 ± 6.01	1334.97 ± 7.15	7.06
S-SNEDDS B4	850.41± 5.11	2	0.101 ± 0.001	4094.62 ± 22.34	4559.12 ± 3.11	6.83
Market product	296.31± 2.72	4	0.092 ± 0.001	1715.04 ± 10.52	1948.94 ± 12.52	7.56

Figure: 3.1. Phase diagrams showing existence of o/w nanoemulsion region for different surfactant: co-surfactant ratios (or S$_{mix}$). (a)

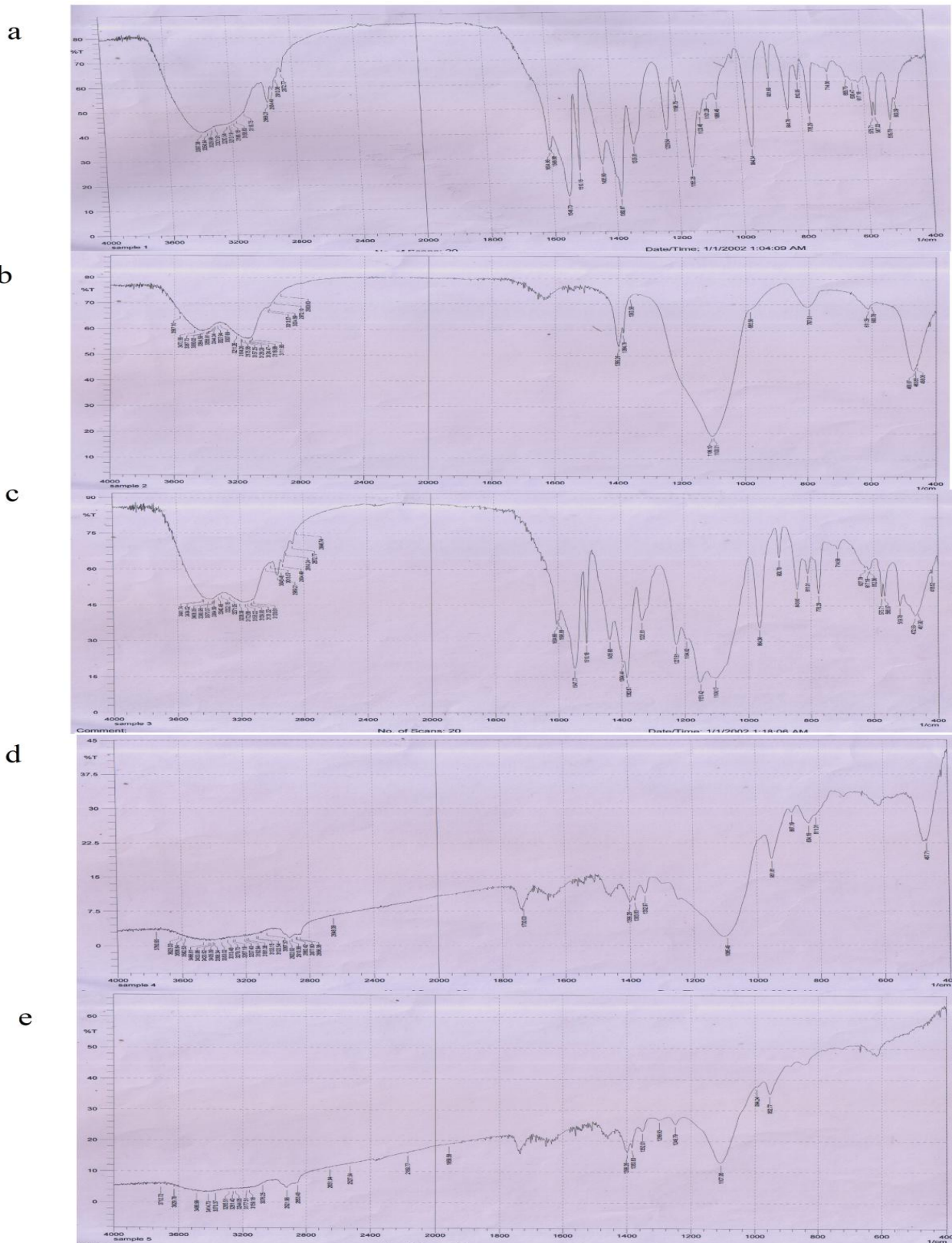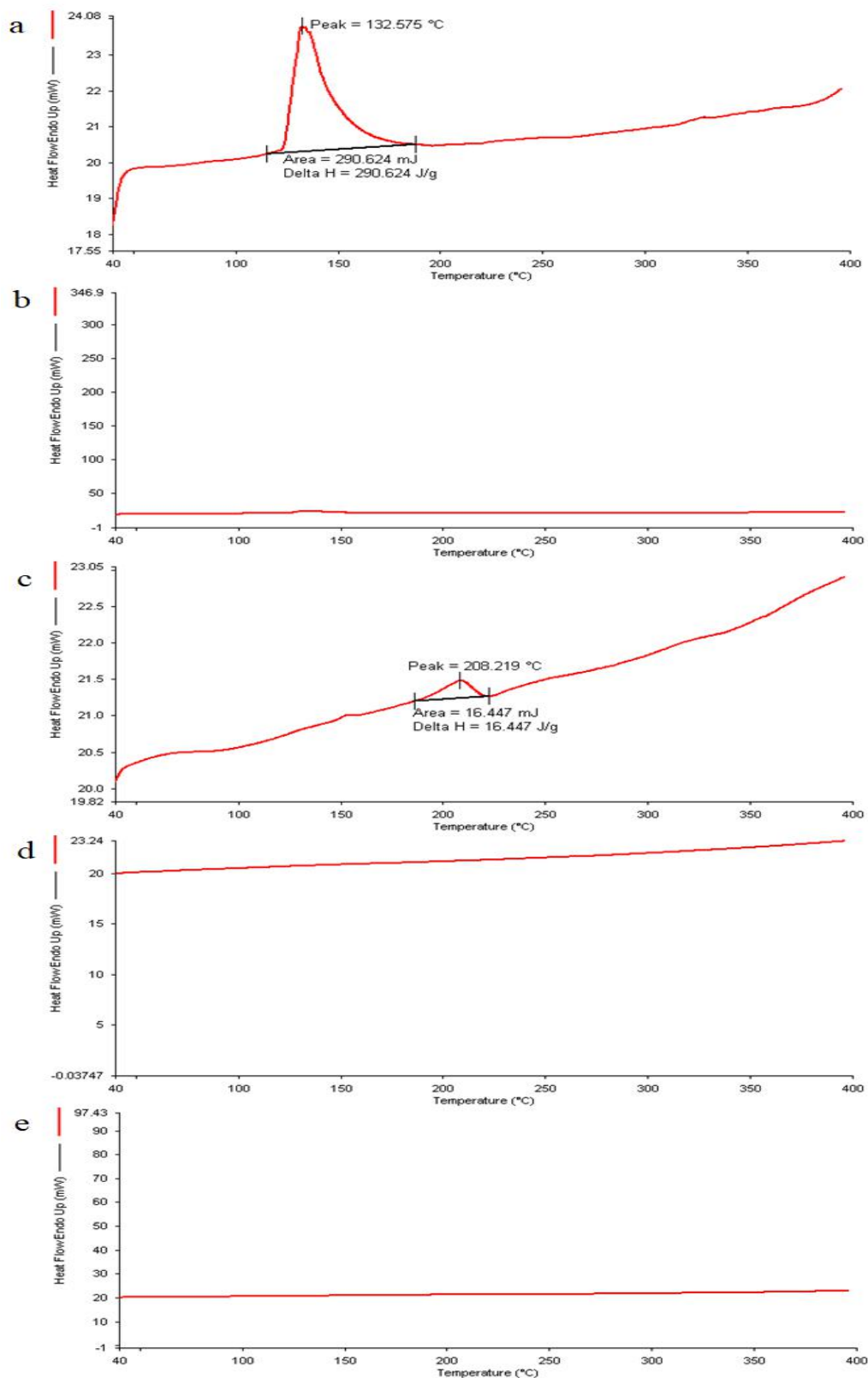1:0; (b) 1:1; (c) 2:1; (d) 3:1; (e) 4:1; (f) 5:1; (g) 1:2 and (h) 1:3.

Figure: 3.2. FTIR spectra of (a) pure rosuvastatin, (b) Aerosil 200, physical mixture of rosuvastatin and Aerosil 200, (d) rosuvastatin loaded L-SNEDDS, (e) rosuvastatin loaded S-SNEDDS.
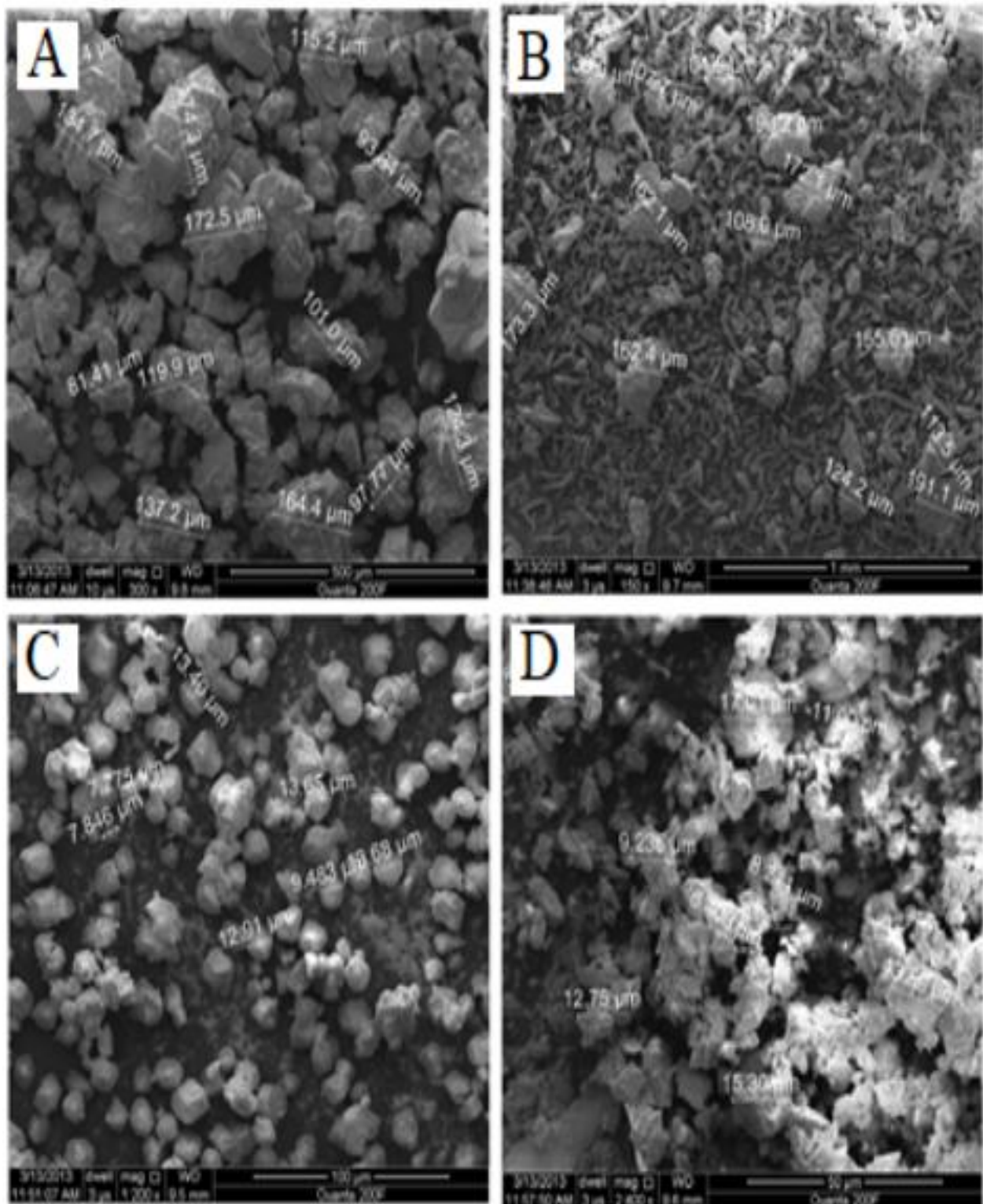
Figure: 3.3. SEM photograph of pure (a) rosuvastatin; (b) Aerosil 200; (c) S-SNEEDS B1; (d) S-SNEDDS B4.

Figure: 3.4. DSC Thermograms of (a) pure rosuvastatin, (b) Aerosil 200, (c) physical mixture of rosuvastatin and Aerosil200, (d) rosuvastatin L-SNEDDS formula (B4), (e) rosuvastatin S-SNEDDS formula (S-B4)
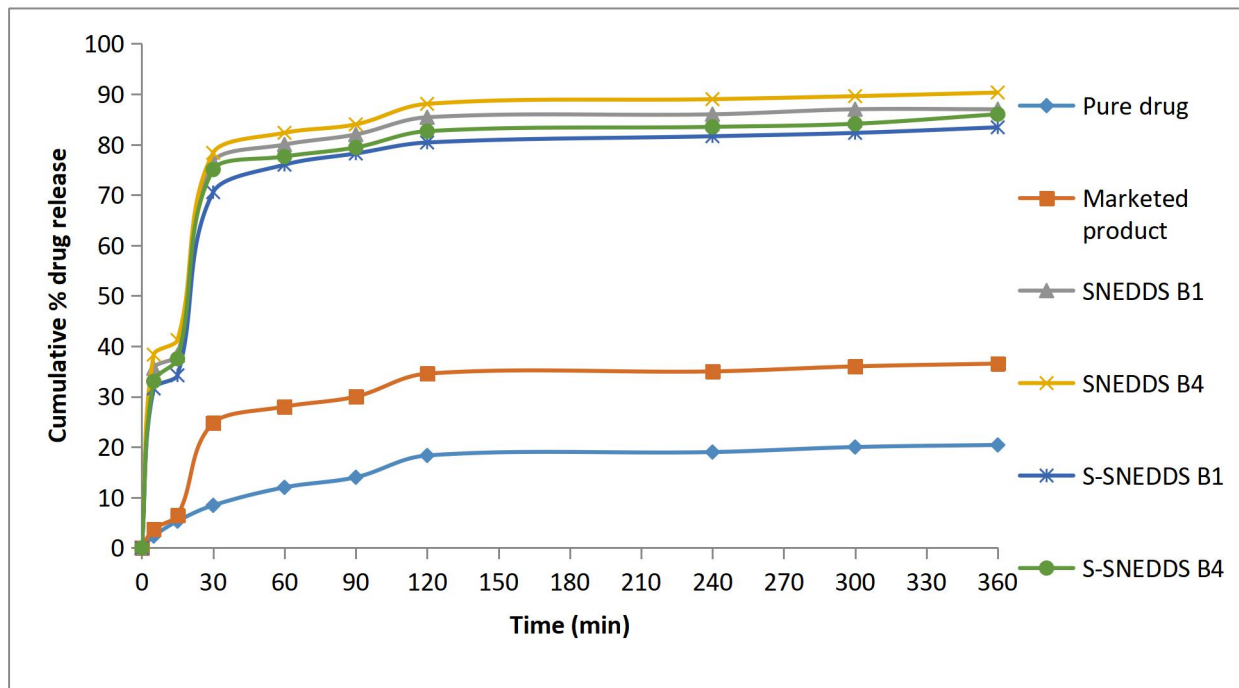
Figure: 3.5. Comparison study of *in-vitro* release profiles of rosuvastatin SNEDDS formulations (B1 and B4) and S-SNEDDS formulations (S-B1 and S-B4)
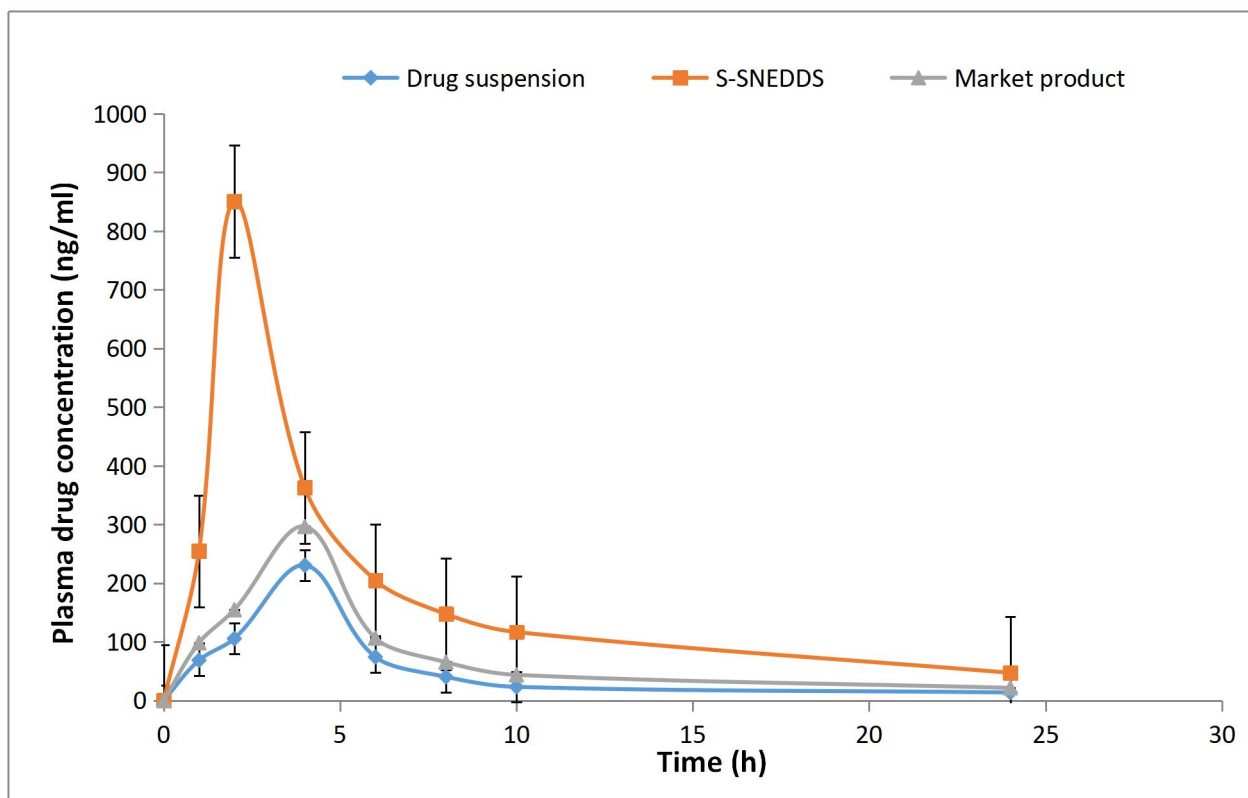

Figure: 3.6. Comparative *in-vivo* absorption profile of S-SNEDDS B4, market product and drug suspension.