Formulation Development and Bioavailability Evaluation of a Self-nanoemulsifying Drug Delivery System (SNEDDS) of Atorvastatin Calcium

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ABSTRACT

The main purpose of the present investigation is to enhance the solubility and bioavailability of poorly water-soluble atorvastatin calcium (ATR) through the self-nanoemulsifying drug delivery system (SNEDDS). The components for self-nanoemulsion were identified by solubility studies and tendency for self-emulsification in various excipients. Sol gb 218, Cremophor RH 40 and Propylene glycol were selected as oil, surfactant and cosurfactant respectively. Pseudoternary phase diagrams were plotted to identify the efficient self-emulsification regions. Prepared SNEDDS formulations were tested for nanoemulsifying properties including robustness to dilution, visual assessment of self-emulsification, viscosity, drug content, droplet size and zeta potential. The SNEDDS formulation F3 consists of 20% (w/w) Sol gb 218, 40% (w/w) Cremophor RH40 and 40% (w/w) Propylene glycol of each excipient showed the highest drug content (96.58%) and a small mean droplet size (12.7±0.3 nm), was selected as optimized SNEDDS formulation. For in vitro dissolution studies, the ATR SNEDDS was then formulated into soft gelatin capsules. It showed a faster rate of drug release into the aqueous phase. The optimized formulation ATR soft gelatin capsules were then subjected to stability studies at 30 ± 2°C/65 ± 5% RH for a period of three months. The ATR-loaded SNEDDS was then formulated into soft gelatin capsules. It was concluded that the potential use of the SNEDDS can improve the dissolution and oral bioavailability of atorvastatin calcium (ATR).

Keywords: Self-nanoemulsifying drug delivery system (SNEDDS), atorvastatin calcium, ternary phase diagrams, droplet size, dissolution, oral bioavailability.

1. INTRODUCTION

Improving oral bioavailability of drugs such as solid dosage forms remains a challenge for the formulation scientists due to a number of problems. Poor bioavailability can be due to poor solubility, degradation in GI lumen, poor membrane penetration and pre-systemic elimination [1,2]. Different approaches have been used for avoiding these problems. One of the most popular methods is lipid based formulation such as oils, surfactant dispersions, self-emulsifying formulations, emulsions, and liposomes [3]. Lipid based formulations can highly improve the delivery of poorly soluble compounds. A typical lipid dosage form mostly contains one or more drugs that are dissolved in a mixture of lipophilic excipients such as triglycerides, partial glycerides, surfactants or co-surfactants [4]. There are a number of potential advantages of self-emulsifying lipid formulations including physicochemical stability, greater oral bioavailability, dose reduction, consistent drug absorption profiles, selective targeting of drug in GIT (Gastrointestinal Tract), control of drug delivery profiles, ability to enhance Cmax and AUC, reduce Tmax, linear AUC-dose relationship, reduced variations due to effect of food, protecting sensitive drug substances, high drug payloads and flexibility of designing liquid or solid dosage forms. Lipid based drug delivery systems, chiefly self-nanoemulsifying drug delivery system (SNEDDS) [5,6] due to its ability to improve oral bioavailability of poorly water soluble drugs as it has high solvent capacity, ease of dispersion and forms very fine droplet size, it has gained great attention. Basically, SNEDDS are isotropic mixtures of oil, surfactant, co-surfactant/co-solvent and drug that form fine oil-in-water nanoemulsion when added to aqueous phases under gentle agitation [5]. There is one limitation for most self-emulsifying systems as they have to be administered in lipid-filled soft or hard-shelled gelatin capsules because of the liquid nature of the product. It is important to avoid interaction between the capsule shell and the emulsion so as to inhibit the hydroscopic contents from dehydrating or entering into the capsule shell. Atorvastatin calcium (ATR) is an Anti-hyperlipidemic agent and it is used in the treatment of obesity. As per its mechanism of action, it is an inhibitor of 3-hydroxy-3- methylglutaryl-coenzyme A reductase (HMG-CoA reductase), an enzyme which catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis.
limiting step in sterol biosynthesis [41]. Thus ATR is used to lower cholesterol and triglycerides in patients with hypercholesterolemia and mixed dyslipidemia and in the treatment of homozygous familial hypercholesterolemia [9]. It is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, while slightly soluble in ethanol, and freely soluble in methanol [8]. The absolute bioavailability of ATR is approximately 14%, main reasons are thought to be the low solubility of it, presystemic clearance in gastrointestinal mucosa and/or first-pass metabolism in liver [7,10]. Food decreases the rate and extent of drug absorption by approximately 25% and 9%, respectively, as assessed by Cmax and area under the curve (AUC) [40]. The main objective of this research is to formulate, optimize and evaluate the performance of SNEDDS containing ATR with suitable excipients, in order to enhance its oral bioavailability.

2. MATERIALS AND METHODS

2.1 Materials

Atorvastatin calcium was kindly supplied by AFM pharm, Batch No: AFM-101202. Capryol 90, Labrafil M 1944 CS, Labrafﬁl CC, Pecelol, and Maisine 35-1 were received as gifts from Gattefosse Co. (Saint Priest Cedex, France). Cremophor RH40 and Cremophor EL were obtained from BASF Co. (Germany). Capmul MCM C8 and Capmul PG 8 were generous gifts from Abitec Corporation (USA).

Propylene glycol mono caprylic ester (Sefsol 218®) was a gift sample form Nikko Chemicals (Tokyo, Japan). Isopropyl myristate (IPM), Caster oil, Tween 80, Propylene glycol were purchased from Sinopharm Chemical Reagent Co., Ltd, (Shanghai, China). Polyethylene Glycol400 (PEG400) was purchased from XiLong Chemical Plant (Wuxi, China). All other chemicals were analytical grade.

2.2 Methods

2.2.1 Solubility studies

Shake flask method is used to determine Equilibrium solubility of Atorvastatin calcium in various excipients. Briefly, an excess amount of ATR was added to each vial containing 2ml of each excipient, and mixed by vortexing in order to facilitate proper mixing of ATR with the vehicles. Vials were then shaken for 48 h in a Thermostatically controlled shaking water bath at 37 ± 1°C followed by equilibrium for 24 h.

In order to separate the undissolved drug, the supersaturated sample was centrifuged at 3000 rpm for 10 min. The supernatant was then filtered using a membrane filter (0.45 μm, Whatman) and suitably diluted with methanol. The drug concentration was obtained via UV validated method at 246 nm (model 752; Exact Science Apparatus Ltd., Shanghai, China).

2.2.2 Pseudoternary phase diagram study

Regarding the results of solubility studies of drug and the self emulsification tendency of excipients, we used Sefsol 218 as the oil phase. Cremophor RH 40 was selected as surfactant and Propylene glycol as cosurfactant. The water titration method was used to obtain the pseud-ternary phase diagrams consisting of oil, surfactant, cosurfactant and water.

The surfactant/cosurfactant ratio used was 1:1.2:1:3:1.1:2. For each phase diagram, in different glass vials oil (Sefsol 218) and specific Smix ratio were mixed thoroughly in various volume ratios from 1:9 to 9:1. Sixteen different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.33, 1:2, 1:1.5, 1:1, 1:0.66, 1:0.43, 1:0.25, and 1:0.11) were blended and titrated with water by dropwise addition under gentle agitation. The suitable ratio of one excipient to another in the SNEDDS formulations was examined and according to the data, pseudoternary diagrams were mapped with Origin 8. The nanoemulsion area in each phase diagram was plotted and the wider region showed the better self nanoemulsifying efficiency.

2.2.3 Preparation of SNEDDS formulations

Once the nanoemulsion region was identified, SNEDDS formulations with desired component ratios were prepared (Table 1). Firstly Atorvastatin calcium (10mg) was dissolved into cosurfactant or Smix in a beaker, heated at 37°C in a water-bath or if necessary a vortex mixer is used to facilitate the solubilization. The required weight of oil was added into the beaker and mixed until the drug was perfectly dissolved. This mixture was then stored at room temperature for further study.

Table 1. Composition of various SNEDDS formulations of ATR

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sefsol 218 (mg)</th>
<th>Cremophor RH40 (mg)</th>
<th>Propylene glycol (mg)</th>
<th>ATR (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100</td>
<td>450</td>
<td>450</td>
<td>10</td>
</tr>
<tr>
<td>F2</td>
<td>150</td>
<td>425</td>
<td>425</td>
<td>10</td>
</tr>
<tr>
<td>F3</td>
<td>200</td>
<td>400</td>
<td>400</td>
<td>10</td>
</tr>
<tr>
<td>F4</td>
<td>250</td>
<td>375</td>
<td>375</td>
<td>10</td>
</tr>
<tr>
<td>F5</td>
<td>300</td>
<td>350</td>
<td>350</td>
<td>10</td>
</tr>
<tr>
<td>F6</td>
<td>350</td>
<td>325</td>
<td>325</td>
<td>10</td>
</tr>
</tbody>
</table>

2.2.4 Robustness to Dilution

The effect of dilution on SNEDDS preconcentrate is determined by the dilution study. Robustness of ATR SNEDDS to dilution was done by diluting it 100,250 and 1000 times of distilled water, 0.1 N HCl and Phosphate buffer of pH 6.8. These diluted nanoemulsions were kept for 24h and observed for any signs of phase separation or drug precipitation.
2.2.5 Drug content
For determining the ATR content, SNEDDS containing Atorvastatin Calcium was added in volumetric flask (VF) containing Methanol and mixed it well by shaking or inverting the VF two to three times [9,12]. By using a validated UV method at 246 nm after suitable dilution, this solution was analyzed spectrophotometrically for the ATR content (model 752; Exact Science Apparatus Ltd., Shanghai, China).

2.2.6 Visual assessment
Various compositions were categorized according to the speed of emulsification, clarity, and apparent stability of the obtained emulsion [13, 11]. Visual assessment was done by drop wise addition of the preconcentrate (SNEDDS) into 100 ml of distilled water. After equilibrium, following aspects were observed: self-emulsification time, dispersibility, appearance, then scored according to the five grading systems shown in Table 2[17,16,15].

Table 2. Grading System for Visual assessment of Self-nanoemulsifying Formulations

<table>
<thead>
<tr>
<th>Grade</th>
<th>Dispersibility</th>
<th>Appearance</th>
<th>Self-Emulsification Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Rapid emulsification</td>
<td>Clear or slightly bluish</td>
<td>&lt;1min</td>
</tr>
<tr>
<td>II</td>
<td>Rapid emulsification</td>
<td>Slightly less clear and bluish white</td>
<td>&lt;2min</td>
</tr>
<tr>
<td>III</td>
<td>Slow emulsification</td>
<td>Bright white emulsion</td>
<td>&lt;3min</td>
</tr>
<tr>
<td>IV</td>
<td>Slow emulsification</td>
<td>Dull, grayish white emulsion slightly oily</td>
<td>&gt;3min</td>
</tr>
<tr>
<td>V</td>
<td>Poor or minimal emulsification</td>
<td>Large oil droplets present on the surface</td>
<td>&gt;3min</td>
</tr>
</tbody>
</table>

2.2.7 Determination of droplet size, polydispersity index and zeta-potential
For determining the droplet size and zeta-potential, approximately 0.2mL concentration of Atorvastatin SNEDDS was diluted with purified water (20mL) or 0.1M HCl (20mL) and moderately shaken in a volumetric flask at 37°C [19]. The average droplet size and zeta-potential were determined by dynamic light scattering (DLS) using a photon correlation spectrometer (Zetasizer 3000 HSA, Malvern Ltd, UK). The droplet size, PDI and zeta-potential of various formulations are shown in Table 4.

2.2.8 Viscosity determination
The viscosity of the prepared SNEDDS formulations as such without being diluted was measured by Brookfield viscometer (Brookfield DV-III Ultra Rheometer ) using spindle C 16-1 at 25±0.5 °C [21,20]. The results are shown in Table 4.

2.3 Transmission electron microscopy (TEM) of SNEDDS
The morphology of the optimized ATR loaded SNEDDS was observed by TEM (H-7650, Hitachi, Japan). Briefly, nanoemulsion was formed by dilution of SNEDDS with distilled water then , one drop of diluted sample was deposited on a film-coated copper grid and negatively stained with 2% (w/v) phosphotungstic solution. After drying the sample was photographed by transmission electron microscopy.

2.3.1 In vitro dissolution studies
The optimized ATR SNEDDS F3 was filled into soft gelatin capsules and was stored at room temperature for 24 h to allow complete solidification of the systems before use [23]. The in vitro drug release of ATR from the capsules was developed using USP dissolution apparatus II (ZRS-8G, Tianjin, China). The dissolution medium consisted of 900 mL of freshly prepared pH 6.8 phosphate buffer maintained at 37 ± 0.5°C and the speed of the paddle was set at 100 rpm. Capsules were held to the bottom of the vessel using copper sinkers. At regular time intervals, 5 ml samples were withdrawn and replaced with equal volumes of fresh medium to maintain the volume and sink conditions. Samples were then filtered using a membrane filter (0.45 μm, Whatman) and drug concentration was obtained via UV validated method at 246 nm. All measurements were done in triplicate.

2.3.2 In vitro drug diffusion study
The diffusion of atorvastatin calcium from the SNEDDS was investigated by a dialysis membrane method. One end of pretreated cellulose dialysis bag (MWCO 12,000 Da) was sealed firmly with clamp and 0.5 mL of self-nanoemulsifying formulation F3 (equivalent to 10 mg ATR) was introduced in it along with 0.5 mL of dialyzing medium (phosphate buffer pH 6.8) [9]. The other end of bag was also secured with clamp and was allowed to rotate freely. The bags were incubated in beakers containing 500 ml phosphate buffer (pH 6.8) at 37±0.5 °C and shaken at a speed of 100 rpm [14]. Samples were withdrawn individually at 0.5, 1, 2, 4, 6, 8,10,12 and 24 h.
respectively and replaced with equal volumes of fresh medium at same time. The diffusion of drug from optimized formulation was compared with the pure drug. The drug content was determined spectrophotometrically at 246 nm.

2.3.3 Pharmacokinetic Study
The in vivo study was conducted in two groups consisting of six male albino rats weighing 150–200 g. Animals were purchased from the Animal Center of China Pharmaceutical University (Nanjing, China) and approved by the Ethics Committee of the university. They were housed and handled according to National Institutes of Health guidelines. The rats were starved for 24 h prior to the experiment with free access to water ad libitum. The formulations (optimized SNEDDS, conventional Tablet suspension) were given orally using oral feeding needle (25 mg/kg body weight). Blood samples (0.5ml) were collected from the retro-orbital vein into heparin-rinsed vials according to a programmed schedule just before dosing (0 h) and after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h. The blood samples were immediately centrifuged at 10000 rpm for 10 min and plasma samples were collected and stored at -20°C until drug analysis. Frozen plasma samples were thawed at room temperature just prior to extraction. Briefly, 150μL of internal standard (Indomethacin) was added into 150μL of plasma containing ATR and mixed for 1 min. Then 150 μL of ammonium acetate buffer pH 4 was added and vortexed for 1 min. The drug was extracted with 2 ml of Di-ethyl ether by mixing vigorously for 3 min. After centrifuging at 4000 rpm for 15 min, the organic layer was transferred to a clean tube and evaporated under a stream of nitrogen at 40 °C. The residue was dissolved in 150 μL of methanol and injected into the HPLC system.

The chromatographic column used was Kromasil C18 (150mm× 4.6 mm, 5 μm) and the mobile phase was 0.1M ammonium acetate buffer, pH 4.0–5.6. It was run at a flow rate of 1.0 ml/min. Eluents were monitored using UV detection at a wavelength of 246 nm.

3. PHARMACOKINETIC ANALYSIS
The plasma concentrations versus time profiles were analyzed using Kinetica™ software (version 4.4.1, Thermoelectron Corporation, Philadelphia, USA). Data from the plasma concentration–time curve within 24 h after drug intake were employed to estimate the following pharmacokinetic parameters for individual rat in each group, peak plasma concentration (Cmax), the time to reach Cmax (Tmax), area under the plasma concentration versus time curve from zero to last sampling time 24 h (AUCO–24h).

Student’s t-test was performed to evaluate the significant differences between the two formulations. Values are reported as mean±S.D. and the data were considered statistically significant at P < 0.05.

3.1 Stability studies
The optimized SNEDDS formulations were filled into soft gelatin capsules kept in glass bottles and subjected to stability studies at 30 ±2°C/65±5% RH for a period of three months. Samples were charged in stability chambers with humidity and temperature control [18]. The SNEDDS was evaluated at 0, 30, 60 and 90 days for drug content, disintegration time and in vitro dissolution profile [9].

4. RESULTS AND DISCUSSION
4.1 Solubility studies
Solubility studies were aimed at identifying suitable vehicles that possess good solubilizing capacity on ATR. Oil can solubilize the lipophilic drug in a specific amount [35]. It is the main excipient because it can facilitate self-emulsification and increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract [22]. Sefsol 218 was selected as oil phase due to its highest solubilization (43.78±1.6 mg/ml) of ATR compared to other oils (Table 3). Various non-ionic surfactants such as the polysorbates and polyoxyls, which cover the HLB range from 2 to 18, may be used in combination with lipid excipients to promote self-emulsification or micro-emulsification [36,37]. Surfactants are amphiphilic in nature and they can dissolve or solubilize relatively high amounts of hydrophobic drugs. This can prevent precipitation of the drug within the GI lumen and for prolonged existence of drug molecules [24].

Table 3. Solubility profile of Atorvastatin calcium in different Excipients (Oils, Surfactants and Co-Surfactants).

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene glycol</td>
<td>152.77±2.68</td>
</tr>
<tr>
<td>Tween 80</td>
<td>24.49±1.47</td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>13.25±1.22</td>
</tr>
<tr>
<td>Labrafil M 1944 CS</td>
<td>1.78±1.32</td>
</tr>
<tr>
<td>Cremophor RH40</td>
<td>35.03±1.95</td>
</tr>
<tr>
<td>Capmul PG8</td>
<td>16.35±0.99</td>
</tr>
<tr>
<td>Capmul MCM C8</td>
<td>39.20±1.03</td>
</tr>
<tr>
<td>PEG400</td>
<td>43.54±2.37</td>
</tr>
<tr>
<td>Castor oil</td>
<td>3.72±0.48</td>
</tr>
<tr>
<td>Labrafil CC</td>
<td>0.27±0.035</td>
</tr>
<tr>
<td>Sefsol 218</td>
<td>43.78±1.6</td>
</tr>
<tr>
<td>Capryol 90</td>
<td>17.87±1.17</td>
</tr>
<tr>
<td>Peccele</td>
<td>12.83±1.29</td>
</tr>
<tr>
<td>Maisine 35-1</td>
<td>1.7±0.65</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>16.52±0.43</td>
</tr>
</tbody>
</table>

Data are mean ± S.D., n = 3.

As per the observed solubility, Capmul MCM C8 and Cremophor RH 40 could be the most appropriate surfactants for the development of the SNEDDS. However, Capmul MCM8 formed a coarse emulsion with a white appearance, when it was mixed with other lipids and...
cosurfactants. Thus, Cremophor RH40 was chosen as surfactant for its better nano emulsification efficiency. Furthermore, co-surfactant increases interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules [26]. Hence use of co-surfactant stabilizes the formed nanoemulsion. Solubility studies of ATR in different excipients indicated that it was more soluble in Sefsol 218, Cremophor RH40 and Propylene glycol, and these excipients were short listed for the preparation of SNEDDS as oil, surfactant and cosurfactant respectively.

4.2 Pseudo ternary phase diagram study

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 [25]. Therefore, Pseudo-ternary phase diagrams were constructed to identify self nano-emulsifying regions and to select suitable concentrations of oil, surfactant and co-surfactant for the formulation of SNEDDS. SNEDDS form fine oil-water emulsion with only gentle agitation when introduced into aqueous media, since the free energy required to form a emulsion is very low, the formulation is thermodynamically spontaneous [27]. The phase diagrams were mapped at surfactant/co-surfactant ratios of 1:1, 1:2, 1:3:1, 1:2. The size of the nanoemulsion region in the diagrams was compared, the larger the size the greater the self-nanoemulsification efficiency. The area of the nanoemulsion was larger at a ratio of 1:1 of surfactant/co-surfactant and therefore, surfactant/co-surfactant ratio (1:1) was selected as optimum for ATR SNEDDS (Fig. 1(a)–(d)). From each phase diagram different concentrations of oil, at which nanoemulsions formed, were selected at a difference of 5% (10, 15, 20 a 25, 30 and 35%) so that maximum formulations could be selected for optimizing the best formulation.

Fig. 1. Pseudo-ternary phase diagrams of the formulation composed of sfsol 218:Cremophor RH 40:Propylene glycol dispersed with water at 37 ºC. Smix ratio = 1:1 (a), 1:2 (b), 2:1 (c), 3:1(d). The dotted area represents o/w nanoemulsion region

4.2.1 Robustness to Dilution

Physical integrity of nanoemulsion formed and drug solubilization capacity after dilution of SNEDDS must be assessed and ensured as it gives an idea about its performance in vivo [29]. In view of this, robustness of ATR SNEDDS to dilution was studied by diluting it
100, 250 and 1000 times with various dissolution media. The diluted nanoemulsions showed no visible signs of phase separation or drug precipitation after storage for 24h, which implies formation of stable nanoemulsions.

4.2.2 Drug content

Drug from pre-weighed SNEDDS is extracted by dissolving in methanol. Drug content in the solvent extract was analyzed spectrophotometrically against the standard solvent solution of the drug. The results are reported in Table 4. In all the six selected formulations, drug content was found highest in 20% of oil and there was no significant difference in drug content among the various formulations.

<table>
<thead>
<tr>
<th>Table 4. Characterization of SNEDDS formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Drug content* (%)</td>
</tr>
<tr>
<td>Droplet size (nm)</td>
</tr>
<tr>
<td>PDI a,b</td>
</tr>
<tr>
<td>Zeta-potential (mV)</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
</tr>
</tbody>
</table>

* Data represent mean±SD, n=3.

b Polydispersity index

4.2.3 Visual assessment

In SNEDDS, the primary means of self-emulsification assessment is visual estimation [42]. ATR SNEDDS concentration was diluted with purified water (100 ml) and gently stirred with magnetic stirrer. Temperature should be 37°C. Formulations F1, F2 and F3 were clear, slightly bluish appearance and graded I, formulations F4, F5 and F6 were slightly less clear emulsions, which have a white bluish appearance and graded II. The assessment of time of emulsification showed that with the increase in the proportion of Sefsol 218 in the composition the time of emulsification increases.

4.2.4 Determination of droplet size, polydispersity index and zeta-potential

Droplet size after nanoemulsification was the most essential criterion of SNEDDS. It may affect the release and absorption of drug in GI tract [28, 30]. The smaller the globule size, larger the surface area provided for drug absorption [28]. In the present study, the mean emulsion droplet size formed upon drop wise addition to distilled water decreased as the surfactant percentage in the liquid SNEDDS formulations increased up to 60%. The droplet size increased from 9.8±0.46 nm to 26.97±5.54 nm when the concentration of lipid added increased from 10% to 35%.

Polydispersity PDI is the ratio of standard deviation to the mean droplet size. This signifies the uniformity of droplet size within the formulation. The higher the value of polydispersity, the lower is the uniformity of the droplet size in the formulation [34]. The polydispersity values of Formulations F1, F2, and F3 are 0.24±0.025, 0.22±0.035 and 0.26±0.025, respectively, which indicates uniformity of droplet size within the formulation. These values are contrary increased for F4, F5, and F6 to 0.31±0.021, 0.38±0.0026 and 0.43±0.0058, respectively, which indicates nonuniformity of particles in nanoemulsion.

The charge of oil droplets of SNEDDS is another property that should be assessed for increased absorption [43]. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions [39]. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability then the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized. Negative values of zeta potential of the selected formulations indicated that the formulations were negatively charged and therefore give indication of stable system [38]. The results are shown in Table 4. Among the various formulations, F3 showed the highest drug content and a small mean droplet size. Hence it was decided to consider F3 as optimized formulation.

4.2.5 Viscosity determination

Viscosity studies are necessary for SNEDDS to characterize the system physically and to control its stability [46]. The viscosity of the ATR SNEDDS is crucial in determining its ability to be filled in hard or soft gelatin capsules. If the system has very low viscosity, it may enhance the probability of leakage from the capsule and the system with very high viscosity may create problem in pourability [31].

From viscosity determination, it was observed that as the concentration of oil increased viscosity of formulations
decreased (Table 4). Overall, the viscosity of the undiluted liquid ATR SNEDDS was found less than 10,000 cps, it implied that the developed SNEDDS can be filled in soft gelatin capsules.

4.2.6 Transmission electron microscopy (TEM) of SNEDDS
The photograph of the transmission electron microscopy (TEM) of the optimized formulation was shown in Fig 2. It could be seen that spherical and homogeneous droplets were formed in the size range of less than 100 nm, which is consistent with the distribution data obtained from particle sizing apparatus.

![Fig.2. TEM of reconstituted ATR SNEDDS.](image)

4.2.7 In vitro dissolution studies
Fig.3 demonstrates the drug dissolution from ATR loaded SNEDDS formulation. Dissolution results showed that 97.24% of ATR dissolved from the self-nanoemulsifying formulation, demonstrating that the self-nanoemulsifying systems could significantly increase ATR dissolution rate.

![Fig.3. Dissolution profiles of ATR from optimized SNEDDS](image)

From diffusion profile of optimized SNEDDS, it was evident that the fraction of ATR dissolved in the fine oil droplets is slowly released and provides the sustained effect. The sustained effects of SNEDDS have also been reported before [13], but there is no detailed explanation for this effect and the one possible mechanism is that the surfactant (Cremophor RH40) at high concentration may lead to sequestration of the drug into the surfactant micelles or emulsified oil droplet and delay/reduce the permeation of the drug across the dialysis bag [44]. A more detailed study is needed to provide an appropriate justification of this observed phenomenon.

4.2.8 In vitro drug diffusion study
Diffusion study was carried out to study the release behavior of formulation from liquid crystalline phase around the droplet using dialysis technique. In vitro diffusion of ATR from optimized SNEDDS in phosphate buffer (pH 6.8) is depicted in Fig 4. It was observed that at the end of 24 h, formulation F3 showed about 95.34% diffusion due to its nano range globule size and presence of surfactant/co-surfactant. In contrast, the pure drug showed about 58.76% diffusion of the drug in 24 hrs due to low aqueous solubility.

![Fig.4. In vitro diffusion study profile of optimized ATR SNEDDS and pure drug.](image)

It could be suggested that the SNEDDS formulation resulted in spontaneous formation of a nanoemulsion with a small droplet size, which allowed a faster rate of drug dissolution into the aqueous phase. Thus this greater availability of dissolved ATR from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.

4.2.9 Pharmacokinetic study
The plasma concentration versus time profiles of ATR after oral administration of suspension of commercial tablet (Lipitor®) and optimized SNEDDS formulation are presented in Fig.5 and the pharmacokinetic parameters are given in Table 5.
surfactant. The oily solution of the drug is then emulsified into nanometer size droplets when exposed to water or gastrointestinal media. It can be envisaged that following oral administration in rats, the ATR SNEDDS will disperse spontaneously to form a nanoemulsion in the gastrointestinal fluid where the active components are present in a solubilized form (i.e. free molecular form incorporated into micelles or dispersed in the microemulsion droplets), and the small droplet size provides a large surface area for drug absorption [32,22]. Such an ultraltrafine dispersion of the oil will afford rapid and extensive absorption. In addition, high concentration of surfactant in SNEDDS may increase the permeability of the oil across the cell membrane [33], and the lymphatic transport through the transcellular pathway may also contribute to the increased bioavailability [28].

4.2.10 Stability studies

Stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions [45]. The optimized ATR loaded SNEDDS was stable when stored at 30 ± 2°C/65 ± 5% RH for three months where there was no apparent change in the physical parameters such as homogeneity and clarity, reflecting the excellent stability of the developed SNEDDS formulation. Furthermore, there was no significant difference in the drug content, dissolution time, and in vitro dissolution profile. It was also seen that the formulation was compatible with the soft gelatin capsule shells, as there was no sign of capsule shell deformation, drug precipitation or capsule leaks. Thus, these studies confirmed the stability of the developed formulation and its compatibility with soft gelatin capsules [18] (Table 6).

Table 6. Stability study of ATR SNEDDS at 30±2°C/65±5% RH.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Disintegration time (min)</th>
<th>Drug content (%)</th>
<th>Drug release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.19±1.3</td>
<td>96.58±2.9</td>
<td>97.24±2.24</td>
</tr>
<tr>
<td>30</td>
<td>3.35±0.38</td>
<td>96.18±1.75</td>
<td>95.62±1.06</td>
</tr>
<tr>
<td>60</td>
<td>4.19±1.7</td>
<td>95.99±1.24</td>
<td>94.24±1.37</td>
</tr>
<tr>
<td>90</td>
<td>4.31±0.9</td>
<td>95.59±1.55</td>
<td>93.04±1.64</td>
</tr>
</tbody>
</table>

*a Data expressed as mean ± SD, n=3.

5. CONCLUSION

The present study has clearly demonstrated the potential use of SNEDDS for the oral delivery of atorvastatin Calcium. The optimized formulation consisting of Atorvastatin calcium (10mg), Sefsol 218 20%(w/w), Cremophor RH40 40%(w/w) and propylene glycol 40%(w/w) exhibited a small droplet size (12.7±0.3nm) and a high drug content(96.58%). In vitro dissolution studies revealed a faster rate of drug release from SNEDDS. From the results of in vivo study, we may conclude that the SNEDDS formulation has approved to be superior in almost all the pharmacokinetic parameters
of comparison over the marketed product (Lipitor®) Thus the development of SNEDDDS is promising approach for improving the oral bioavailability of poorly soluble drugs.

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