Development of Dispersible Self-microemulsifying Tablet of Atorvastatin

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ABSTRACT

Aim: The aim of this study was to develop dispersible self-microemulsifying (SMEDDS) tablet of atorvastatin for promoting its solubility and thus its oral bioavailability. Materials and Methods: The liquid SMEDDS were prepared by water titration method using oil, surfactant and co-surfactant and converted into solid- SMEDDS (S-SMEDDS) by adsorption on solid carriers (Neusilin US2). The S-SMEDDS were blended with sodium starch glycolate (disintegrant) and tablet excipients and compressed into tablets that were dispersible and self-microemulsifying in nature. All these formulations were assessed for various physicochemical parameters viz. weight variation, hardness, friability, disintegration test. In vitro studies of pure drug, SMEDDS, S-SMEDDS and dispersible SME-tablets were carried out. Results: Pure drug released only 29.84 ± 0.16% upto 60 minutes and all the SMEDDS formulations (i.e. SMEDDS, S-SMEDDS and dispersible SME-tablets) released 100% of drug in comparatively lesser time. Formulations containing atorvastatin, 30% oleic acid, 65% tween 80 and 5% co-surfactant came out to show the best results in in vitro studies. But, FB1 (tablet) was considered to be the best since it released 100% drug in 35 min and also has advantages over SMEDDS and S-SMEDDS in terms of stability and patient compliance. Conclusion: The study revealed the potential use of dispersible SMEDDS tablet for the oral delivery of hydrophobic drugs, such as atorvastatin.

Key words: Atorvastatin, Bioavailability, Phase diagram, SMEDDS.

INTRODUCTION

Atorvastatin (ATV), a selective inhibitor of HMG-CoA reductase and a widely prescribed drug in case of hyperlipidemia. Nevertheless, poor solubility of ATV has been the major constrain in the attainment of good absorption property. The solubility of ATV in aqueous solution of pH 2.1 is about 20.4 mg mL⁻¹, while the solubility is only 1.23 mg mL⁻¹ in aqueous solution of pH 6.0. Although the T_max of ATV is quite rapid, 1-3 h, it suffers from very poor bioavailability and this is only 14%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and hepatic first-pass metabolism. To improve the solubility of ATV, some solubilization strategies have been explored, such as solid dispersion, microsphere, emulsion, nanosuspension, self-...
microemulsion formation etc., which decrease the particle size of the drug, thereby increase in the large surface area for drug absorption in the gastrointestinal tract (GIT) which ultimately enhances the oral bioavailability of drug.

SMEDDS have gained importance recently in the field of pharmaceutical technology for solubility and bioavailability enhancement of various poorly water soluble drugs. SMEDDS formulations are isotropic mixtures of an oil, a surfactant, a co-surfactant (or solubilizer) and a drug. The basic principle of this system is its ability to form fine oil in water (o/w) microemulsions under gentle agitation following dilution by aqueous phases i.e., the digestive motility of the stomach and intestine provide the agitation required for self-emulsification in vivo in the lumen of the gut. 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. Apart from solubilization, the presence of lipid in the formulation further helps improve bioavailability by affecting the drug absorption. Therefore SMEDDS is a potential technology to enhance the oral bioavailability of hydrophobic drugs.

However, SMEDDS are liquid formulations, which have several disadvantages, such as few choices of dosage forms, low stability and portability during the manufacturing process and the interaction between the filling and the capsule shell. To overcome these problems, lipid formulations could be transformed to solid dosage forms by using suitable adsorbents. Solid-SMEDDS (S-SMEDDS) combine the advantages of SMEDDS with those of solid dosage form e.g. low production cost, convenience of process control, high stability and better patient compliance.

However, the dissolution rate of such S-SMEDDS preparations is inclined to delay because of the strong adhesion interaction of lipid SMEDDS with adsorbents, which also lead to poor disintegration in case of tablets. Compared to conventional tablets, dispersible tablets present an advantage in administration of drug, with a fast disintegration (disintegration time <3 min). In this study, we developed dispersible SME-tablets of ATV, which can disintegrate rapidly and spontaneously emulsify in the mixed aqueous gastrointestinal environment. Since ATV is encapsulated in the lipid SMEDDS, an isotropic oil mixture of poor fluidity, it is necessary to select a suitable adsorbent and disintegrating agent to develop the dispersible tablets.

In the present study, SMEDDS were formulated using screened oil, surfactant and co-surfactant to improve the solubility of ATV and decrease the first pass metabolism which in turn will improve the oral bioavailability of the drug. The prepared SMEDDS were converted into S-SMEDDS by adsorption technique. S-SMEDDS were further formulated into tablet dosage forms (which are self-microemulsifying in nature) as tablets (dispersible) are more patient compliant and are of great use in pediatrics, geriatrics and psychologically ill patients.

**MATERIAL AND METHODS**

**MATERIALS**

ATV was obtained as a gift sample from Dr. Reddy’s Laboratories, India. Oleic acid was purchased from Himalaya Agro Company, India. Tween 80 was purchased from Gattefosse, Mumbai, India. Other chemicals used were of analytical grade.

**METHODS**

**Solubility of atorvastatin**

The solubility of ATV was determined in various oils, surfactants and co-surfactants by dissolving an excess amount of ATV in 500 mg of each of selected oils, surfactants and co-surfactants in vials and the mixtures were continuously stirred for 10 min using vortex mixer and kept at 37 ± 0.5°C in orbital shaker for 72 h to attain equilibrium. The equilibrated samples were then centrifuged at 3000 rpm for 15 min, supernatant was filtered and analyzed using UV-VIS spectrophotometer (Shimadzu-1700, Japan) at 296 nm.

**Screening of components**

Screening of surfactant and co-surfactant was done on the basis of percent transmittance. Emulsification ability of surfactants was assessed by adding each of the surfactant as well as co-surfactant (300 mg) to selected oil (300 mg). The mixture was gently heated for few sec to achieve homogenization. 50 mg of the mixture was weighed and diluted up to 50 ml with water to yield fine emulsion. The resulting mixture was then observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and transmittance was assessed by UV-VIS spectrophotometer (Shimadzu-1700, Japan) at 638 nm, using distilled water as blank.

**Construction of ternary phase diagrams**

Ternary phase diagram was constructed by dilution method. The mixtures of oil, surfactant and co-surfactant were prepared in which concentration of oil varied from 30 to
70% w/w; surfactant from 15 to 60% w/w and co-surfactant varied from 0 to 35% w/w. But, the total concentration of the mixture containing oil, surfactant and co-surfactant was always added to 100%. First mixture consisted of 30% of oil, 70% of surfactant and 0% of co-surfactant. Subsequently, in further mixtures, oil concentration was kept constant, co-surfactant concentration was increased by 5% for each composition and the surfactant concentration was adjusted to obtain a total of 100%.

50 mg of each of the compositions was then diluted to 50 ml with double distilled water to evaluate the % transparency, globule size and polydispersity index of the resulting dispersion with help of zeta sizer. Formulations with desired particle size were used to obtain the microemulsion region.

**Preparation of SMEDDS**

The amount of the components (oil, surfactant and co-surfactant) to be taken was decided on the basis of microemulsification region in the ternary phase diagram. ATV was accurately weighed and dissolved in oil. Surfactant and co-surfactant were added to the mixture, stirred for 10 minutes and further sonicated at 45°C for 15 minutes. All the 14 formulations with different concentrations were loaded with 40 mg of drug.

**Physicochemical characterization of SMEDDS**

**Drug content**

40 mg equivalent of SMEDDS was added to 50 mL methanol and sonicated for 10-15 min. 0.1 mL of this solution was diluted with 25 mL with methanol and the drug content was determined using UV-spectrophotometer at 296 nm.

**Globule size determination**

Analysis of globule size and polydispersity index (PDI) measurement is the measurement of droplet size homogeneity that varies from 0.0 to 1.0. It is the ratio of standard deviation to mean droplet size in the formulation. Higher the polydispersity, lower the uniformity of the droplet size in the formulation. The closer to zero the polydispersity value the more homogenous are the droplets. It was carried out by Zeta sizer HSA 3000 (Malvern Instruments Ltd, UK). All the samples were subjected to sonication prior to globule size and PDI determination.

**Viscosity determination**

20 g formulation of SMEDDS was transferred to beaker and the viscosity of formulation was determined with the help of Brookfield Viscometer (Model DV-E spindle- 6) at 10 rpm for 5 min and the corresponding dial reading was noted.

**Cloud point measurement**

SMEDDS were diluted with distilled water in the ratio of 1:250, placed in a water bath and its temperature was increased gradually. Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness visually.

**Robustness to dilution**

Robustness to dilution was studied by diluting SMEDDS to 50, 100 and 1000 times with water, phosphate buffer pH 6.8 and PBS 7.4. The diluted SMEDDS were stored for 12 h and observed for any signs of phase separation or drug precipitation.

**Thermodynamic stability studies**

It was determined by carrying heating cooling cycle, centrifugation test and freeze thaw cycle.

**Heating cooling cycle:** Six cycles between refrigerator temperatures 4°C and 45°C with storage at each temperature for not less than 48 hours was studied. If SMEDDS were stable at these temperatures, they were subjected to centrifugation test.

**Centrifugation test:** Passed SMEDDS were centrifuged at 3500 rpm for 30 min using digital centrifuge (Remi motors Ltd.). If SMEDDS did not show any phase separation was taken for freeze thaw stress test.

**Freeze thaw cycle:** Three freeze thaw cycles between -21°C and +25°C with storage at each temperature for not less than 48 hours was done for SMEDDS.

**Conversion of SMEDDS into S-SMEDDS**

Solid SMEDDS were prepared by mixing liquid SMEDDS containing specified amount of ATV with Neusilin US2. Liquid SMEDDS was added dropwise over Neusilin US2 contained in broad porcelain dish. After each addition, mixture was homogenized using glass rod to ensure uniform distribution of formulation.

**Physiochemical characterization of S-SMEDDS**

**Drug content:** S-SMEDDS were dissolved in sufficient quantity of methanol and was sonicated for 10-15 mins.
and filtered. The absorbance of filtrate was noted using UV spectrophotometer.

**Micromeritic properties:** Prepared S-SMEDDS were evaluated for various micromeritic properties such as angle of repose, bulk and tapped density, compressibility index and Hausner ratio.**20,27** Globule size, PDI and zeta potential for S-SMEDDS were also determined.

**Scanning electron microscopy (SEM):** The surface morphology of Solid SMEDDS was investigated by scanning electron microscope (SEM) (JEOL, USA). Samples were fixed on a brass stub using double sided adhesive tape and were made electrically conductive by coating with a thin layer of gold and SEM images were recorded at different accelerating voltage.**28**

**X-ray Diffraction study (XRD):** Change in crystalline structure of the drug when loaded into SMEDDS was measured using X-Ray diffractometer (X’Pert Pro, India) having Ni-filtered Cu radiation.**22**

**Preparation of dispersible SMEDDS tablets**

Solid-SMEDDS powder containing drug was compressed into tablet dosage forms (150 mg weight) by direct compression method. All the ingredients were sieved first and then blended together. Powder blend was then compressed using tablet press. The composition of three different batches is shown in Table 1.

**Physicochemical characterization of dispersible SMEDDS tablets**

All the tablet formulations were evaluated for physicochemical parameters viz appearance, dimensions, hardness, friability, weight variation, content uniformity, disintegration test and in vitro dissolution studies as specified in IP. The optimized batch of tablets was further evaluated for reconstitution test and TEM studies.

**Reconstitution Test:** A dispersible SME-tablet was dispersed in 50 mL distilled water by vortex mixing for 30 s. The resulting microemulsion was incubated for 30 min at room temperature, and then the supernatant was withdrawn for PDI determination.**27**

**Transmission electron microscopy:** Examining the surface of a polymeric drug delivery system can provide vital information on the porosity and microstructure of this system. The distribution and morphology of the surface and the encapsulated matrix can also be directly observed. The most common technique used for characterizing the surface morphology of drug delivery systems is transmission electron microscopy (TEM). The sample sizes, which can be analyzed using this method, range from nanometers to micrometers to centimeters.**29**

**In vitro release studies**

**In vitro** release of prepared SMEDDS, S-SMEDDS and dispersible SME-tablet were assessed in triplicate using United States Pharmacopoeia (USP) Dissolution Type II apparatus (Paddle Type) at 37±0.5ºC. SMEDDS containing 10mg equivalent of drug was placed in 900 mL of dissolution medium (phosphate buffer pH 6.8 with methanol in 9:1 ratio). The revolution speed of the paddle was maintained at 100 rpm. At predetermined time intervals, 5 mL of dissolution medium was collected, filtered using 0.45 μm filter and the same volume of fresh dissolution medium was replenished to maintain the sink conditions. The samples were analyzed for the drug concentration using UV-VIS spectrophotometer (Shimadzu, Japan) at 246 nm.**30**

**Release kinetics**

To study the release kinetics, data obtained from in-vitro dissolution study was fitted in various kinetic models: zero order as cumulative percent of drug released vs. time, first order as log cumulative percentage of drug remaining vs. time and Higuchi’s model as cumulative percent drug released vs. square root of time, Hixon crowel describes the release from systems when there is a change in a surface area and diameter of particles. To determine the mechanism of drug release, the data was fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released vs. log time and the exponent n was calculated from slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is fickian; if 0.5<n <1.0, then it is anomalous transport. If n is 1.0, it is case II transport and if n>1.0, then it is super case II transport.**31**

**Accelerated stability studies**

Stability studies of these formulations were studied at different temperature conditions according to ICH guidelines at 25ºC.
± 2°C/60% ± 5% RH and at 40°C ± 2°C/75% RH ± 5%. The samples were withdrawn at different time intervals as 0, 30, 60, 90 days. Formulation equivalent to 40 mg of the drug was dissolved in methanol, diluted approximately and estimated for the drug content spectrophotometrically at 246 nm using methanol as blank. Effect of storage conditions on drug release was also studied.\\(^{32}\)

**In vivo Pharmacodynamic Studies**

24 Male Wistar rat weighing 200-250 g obtained from Department of Livestock Management, Guru Angad Dev University, Ludhiana, Punjab, India, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water were employed in the present study. They were housed in the departmental animal house and were exposed to a regular 12 hr cycle of light and dark. The experiments were conducted in a semi-sound proof laboratory. The observer was blind to the treatment group assignment. The experimental protocol was approved by the institutional animal ethical committee and care of the animals was done as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 1181/ab/08/CPCSEA).

For *in vivo* estimation of LDL (Low density lipoproteins), HDL (High density lipoproteins) and triglycerides, the study was carried out in 24 healthy male wistar rats weighing 200-250 g. The rats were randomly divided into 6 groups, containing 6 rats in each group. All the animals were fed high fat diet (HFD) and water *ad libitum* for 11 weeks except Control group (Group I). HFD consists of powdered normal pellet diet (NPD) (300 gm/kg), lard (275 gm/kg), casein (200 gm/kg), cholesterol (10 gm/kg), vitamin and mineral mix (60 gm/kg), dl-methionine (3 gm/kg), sodium chloride (2 gm/kg) and sucrose (150 gm/kg). The rats of Group I were fed NPD (commercial rat pellets from Kisan Feeds Ltd., Mumbai, India) for 11 weeks and water *ad libitum*. Group II served as disease control group. Standard group (Group III) of rats received pure drug Atorvastatin calcium (3 mg/kg/day, *P.o*) and Test group (Group IV) received S-SMEDDS of Atorvastatin (3 mg/kg/day, *P.o*) for 3 weeks as suspension, started at the end of 8th week. Weekly body weight and daily food intake were measured. At the end of 11th week, blood samples were collected under ether anaesthesia by retro-orbital puncture from overnight fasted rats. Serum was separated by centrifugation and was used to estimate serum total cholesterol, HDL (High density lipoproteins) cholesterol, and Triglycerides. Estimation of total cholesterol, HDL was done by using Bayer Diagnostic kit (Bayer Diagnostic India Ltd) and estimation of Triglycerides was done by using Erba Diagnostics Manheim, Germany kit.\\(^{33,34}\)

**Statistical Analysis**

Graph pad prism 5 was used for statistical analysis. All studies were done in triplicates unless specified and data represent the mean ± SD. The statistical analysis was performed using student’s *t*-test. A difference below the probability level was considered statistical significant (p<0.05).

**RESULT AND DISCUSSION**

**Screening of components**

To develop SMEDDS of ATV, it should possess good solubility in the oil, surfactants and co-surfactants of system. The solubility of ATV in various oils, surfactants and co-surfactants were investigated (Table 2). ATV had significantly higher (p<0.05, *t*-test) solubility in oleic acid (49.23 ± 2.93 µg/ml) than castor oil, soy bean oil, arachis oil. Among surfactants and co-surfactants, tween 80 (38.32 ± 3.41 µg/ml) and PEG 400 (40.11 ± 5.7 µg/ml) respectively showed highest solubilities. Therefore, oleic acid was screened as oil phase based on solubility studies. Surfactant and co-surfactant were selected on the basis of percent transmittance\\(^{21}\) shown in Table 3.

Out of various surfactants and co-surfactants screened, tween 80 revealed 96.34 ± 0.24% transmittance, which was the highest amongst all (Table 3). As shown by outcomes, tweens are showing higher transmittance values. Hence this

<table>
<thead>
<tr>
<th>Component</th>
<th>Solubility (mg/mL)</th>
<th>Component</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>49.23 ± 2.93</td>
<td>Tween 80</td>
<td>38.32 ± 3.841</td>
</tr>
<tr>
<td>Castor oil</td>
<td>9.2 ± 3.10</td>
<td>Tween 20</td>
<td>32.85 ± 6.69</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>8.9 ± 5.13</td>
<td>PEG 400</td>
<td>40.11 ± 5.7</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>8.8 ± 3.42</td>
<td>Glycerol</td>
<td>5.91 ± 5.11</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>5.097 ± 0.86</td>
<td>PEG 200</td>
<td>39.09 ± 3.52</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>19.43 ± 6.17</td>
<td>Cremophor RH 60</td>
<td>21.34 ± 3.85</td>
</tr>
</tbody>
</table>

*Significant, p<0.05, Student unpaired t-test was used to compare solubility studies in between oils.*
Table 3: Percent transmittance of surfactants and co-surfactants

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Percent transmittance (%)</th>
<th>Co-surfactant</th>
<th>Percent transmittance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 80</td>
<td>96.34 ± 0.24</td>
<td>PEG 400</td>
<td>88.58 ± 0.27</td>
</tr>
<tr>
<td>Tween 20</td>
<td>80.41 ± 0.66</td>
<td>Glycerol</td>
<td>81.21 ± 0.63</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>50.66 ± 0.69</td>
<td>PEG 200</td>
<td>74.98 ± 0.57</td>
</tr>
<tr>
<td>Cremophor RH 60</td>
<td>37.09 ± 1.09</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Significant, p<0.05, Student unpaired t-test was used to compare percent transmittance in between surfactants;
**Significant, p<0.05, Student unpaired t-test was used to compare percent transmittance in between co-surfactants.

Co-Surfactant

Figure 1: Ternary Phase Diagram

surfactant was selected for development of the formulation. This is also compliant with the purpose of SMEDDS which has to form oil-in-water emulsion in situ.

Similarly, in case with co-surfactants, PEG 400 resulted in higher percent transmittance (88.58 ± 0.27%) followed by glycerol (81.21 ± 0.63%) and PEG 200 (74.98 ± 0.57%). Therefore, tween 80 and PEG 400 were selected as surfactant and co-surfactant, respectively, for the phase study. Moreover, tween 80 is a nonionic surfactant which is nontoxic compared to ionic surfactants and has appropriate blend of low and high hydrophilic lipophilic balance (HLB), (HLB=15) which can result in stable emulsion.

Construction of Phase Diagram

The aim for constructing ternary phase diagram was to explore the microemulsion region. Oleic acid was used as oil phase. Surfactant co-surfactant mixture was composed of tween 80 as surfactant and PEG 400 as co-surfactant. The phase diagram was constructed in the absence of drug, ATV. Initially, thirty formulations were made (Table 4) and diluted with 100 ml of water and on the basis of opaqueness observed visually only fourteen formulations were selected, rest were turbid and rejected. The selected formulations were further carried for zeta sizer and PDI. Formulations F1-F14 without drug was selected for constructing the ternary diagram F12-F14 were rejected as they don’t lie in the size range. Different ratios for these final eleven formulations were placed in the pseudo ternary phase diagram software and diagram was plotted. The microemulsion region was demarcated using particle size studies and showed that the formulations lie in this region (Figure 1). The rest of the region on the phase diagram represents the turbid and conventional emulsions.

Development of formulation

Eleven formulations (F1-F11) with different concentrations of oil, surfactant and co-surfactant, each containing ATV at a final loading of 40 mg of drug were prepared by ultrasonication method and were evaluated.
Table 4: Data for construction of ternary phase diagram without drug

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% oil (w/w)</th>
<th>% surfactant (w/w)</th>
<th>% co-surfactant (w/w)</th>
<th>Zeta size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>30</td>
<td>70</td>
<td>0</td>
<td>85.10</td>
<td>0.512</td>
</tr>
<tr>
<td>F2</td>
<td>30</td>
<td>65</td>
<td>05</td>
<td>35.21</td>
<td>0.542</td>
</tr>
<tr>
<td>F3</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td>74.21</td>
<td>0.438</td>
</tr>
<tr>
<td>F4</td>
<td>40</td>
<td>35</td>
<td>25</td>
<td>82.1</td>
<td>0.719</td>
</tr>
<tr>
<td>F5</td>
<td>40</td>
<td>50</td>
<td>10</td>
<td>62.68</td>
<td>0.361</td>
</tr>
<tr>
<td>F6</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>124.5</td>
<td>0.849</td>
</tr>
<tr>
<td>F7</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>146.5</td>
<td>0.541</td>
</tr>
<tr>
<td>F8</td>
<td>60</td>
<td>25</td>
<td>15</td>
<td>84.63</td>
<td>0.251</td>
</tr>
<tr>
<td>F9</td>
<td>50</td>
<td>45</td>
<td>05</td>
<td>182.6</td>
<td>0.301</td>
</tr>
<tr>
<td>F10</td>
<td>60</td>
<td>35</td>
<td>05</td>
<td>221.7</td>
<td>0.629</td>
</tr>
<tr>
<td>F11</td>
<td>70</td>
<td>30</td>
<td>0</td>
<td>214.4</td>
<td>0.919</td>
</tr>
<tr>
<td>F12</td>
<td>30</td>
<td>40</td>
<td>30</td>
<td>299.6</td>
<td>0.885</td>
</tr>
<tr>
<td>F13</td>
<td>70</td>
<td>15</td>
<td>15</td>
<td>329.8</td>
<td>0.743</td>
</tr>
<tr>
<td>F14</td>
<td>40</td>
<td>60</td>
<td>0</td>
<td>355</td>
<td>0.891</td>
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</table>

Table 5: Physicochemical characterization of SMEDDS

<table>
<thead>
<tr>
<th>Batch</th>
<th>% Drug content</th>
<th>Droplet size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
<th>Viscosity (mPas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>101.92 ± 0.215</td>
<td>148.5</td>
<td>0.300</td>
<td>-13.6</td>
<td>2239 ± 0.01</td>
</tr>
<tr>
<td>F2</td>
<td>102.1 ± 0.092</td>
<td>139.5</td>
<td>0.291</td>
<td>-42.5</td>
<td>2021 ± 0.02</td>
</tr>
<tr>
<td>F3</td>
<td>99.60 ± 0.023</td>
<td>180.4</td>
<td>0.321</td>
<td>-18.4</td>
<td>2042 ± 0.02</td>
</tr>
<tr>
<td>F4</td>
<td>99.16 ± 0.042</td>
<td>150.2</td>
<td>0.315</td>
<td>-11.5</td>
<td>2401 ± 0.03</td>
</tr>
<tr>
<td>F5</td>
<td>99.54 ± 0.083</td>
<td>179</td>
<td>0.349</td>
<td>-41.5</td>
<td>2519 ± 0.03</td>
</tr>
<tr>
<td>F6</td>
<td>98.75 ± 0.126</td>
<td>183.8</td>
<td>0.398</td>
<td>-15.4</td>
<td>2560 ± 0.02</td>
</tr>
<tr>
<td>F7</td>
<td>99.25 ± 0.043</td>
<td>181.4</td>
<td>0.412</td>
<td>-12.9</td>
<td>3012 ± 0.01</td>
</tr>
<tr>
<td>F8</td>
<td>99.69 ± 0.011</td>
<td>169.7</td>
<td>0.450</td>
<td>-15.4</td>
<td>2789 ± 0.01</td>
</tr>
<tr>
<td>F9</td>
<td>90.08 ± 0.124</td>
<td>212.4</td>
<td>0.530</td>
<td>-51.3</td>
<td>Unstable</td>
</tr>
<tr>
<td>F10</td>
<td>93.25 ± 0.452</td>
<td>220.1</td>
<td>0.598</td>
<td>-11.8</td>
<td>Unstable</td>
</tr>
<tr>
<td>F11</td>
<td>92.35 ± 0.824</td>
<td>223.5</td>
<td>0.750</td>
<td>-12.7</td>
<td>Unstable</td>
</tr>
</tbody>
</table>

Table 6: Micromeritic Properties of S-SMEDDS

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Angle of Repose (°)</th>
<th>Bulk Density (gm/cm³)</th>
<th>Tapped Density (gm/cm³)</th>
<th>Carr’s Index (%)</th>
<th>Hausner’s Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>38.6 ± 0.01</td>
<td>0.59 ± 0.02</td>
<td>0.65 ± 0.01</td>
<td>19.4 ± 0.02</td>
<td>1.33 ± 0.02</td>
</tr>
<tr>
<td>SF2</td>
<td>29.5 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>0.76 ± 0.03</td>
<td>17.5 ± 0.03</td>
<td>1.18 ± 0.01</td>
</tr>
<tr>
<td>SF3</td>
<td>29.9 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.69 ± 0.02</td>
<td>13.2 ± 0.01</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td>SF4</td>
<td>27.5 ± 0.02</td>
<td>0.52 ± 0.01</td>
<td>0.70 ± 0.01</td>
<td>22.6 ± 0.02</td>
<td>1.19 ± 0.01</td>
</tr>
<tr>
<td>SF5</td>
<td>30.0 ± 0.04</td>
<td>0.54 ± 0.02</td>
<td>0.67 ± 0.02</td>
<td>18.5 ± 0.01</td>
<td>1.28 ± 0.02</td>
</tr>
<tr>
<td>SF6</td>
<td>27.6 ± 0.02</td>
<td>0.56 ± 0.04</td>
<td>0.73 ± 0.01</td>
<td>27.3 ± 0.04</td>
<td>1.30 ± 0.01</td>
</tr>
<tr>
<td>SF7</td>
<td>28.4 ± 0.03</td>
<td>0.39 ± 0.01</td>
<td>0.35 ± 0.03</td>
<td>29.2 ± 0.03</td>
<td>1.26 ± 0.03</td>
</tr>
<tr>
<td>SF8</td>
<td>30.3 ± 0.05</td>
<td>0.58 ± 0.02</td>
<td>0.39 ± 0.02</td>
<td>12.6 ± 0.01</td>
<td>1.27 ± 0.02</td>
</tr>
</tbody>
</table>

**PHYSICOCHEMICAL CHARACTERIZATION OF SMEDDS**

**Drug content of SMEDDS**

Irrespective of ratios of oil and surfactant used, the drug content in the eleven formulations (F1-F11) was found in the range of 90.08–102.1%, indicating uniform dispersion of drug in formulations (Table 5).

**Globule size determination**

As it is clearly seen in Table 6, size of globule ranges from 139–223 nm of which the lowest PDI and Particle size was observed for F2 batch. Further in F4, ratio of co-surfactant is high in comparison to F2 due to which its droplet size is slightly increased therefore F2 was considered to be best among all with optimum ratio of surfactant (tween 80) and co-surfactant (PEG 400). An increase in the ratio of the oil phase (oleic acid) also resulted in a proportional increase in particle size. It is well known that the addition of surfactants to these systems causes the interfacial film to stabilize and condense, while the addition of co-surfactant causes the film to expand; thus, the relative proportion of surfactant to co-surfactant has varied effects on the globule size. Also, it has been reported that the smaller particle size of the emulsion globules may lead to more
rapid absorption and improve the bioavailability.\textsuperscript{37,38} The PDI obtained for all the formulations varied from 0.291-0.750. PDI below 0.3 indicates good uniformity in the droplet size distribution after dilution with water.\textsuperscript{39,40} PDI of F2 (0.291) was the lowest and found best (Figure 2). Data of SMEDDS formulations reveals that there is not much difference in the zeta potential of the formulations (Table 5). A dividing line between stable and unstable aqueous dispersions is generally taken at either ± 30 mV. Particles with zeta potentials more negative than -30 mV are normally considered stable.\textsuperscript{41} Zeta potential was found in the range between -11.5 to -51.3 mV. Most appropriate zeta potential was of F2 formulation (-42.5 mV) (Figure 3).

**Viscosity determination of SMEDDS**

The range of the viscosity lies between 2021-3012 mPas. As F9-F11 were rejected due to gel formation in the...
formulations. F1-F8 formulations were subjected to viscosity determination. From viscosity determination (Table 5) it was observed that as the concentration of co-surfactant increased viscosity of formulation also increased.24,36

It was expected that F1 and F6 would show least viscosity due to the absence of co-surfactant but it was not practically obtained as there surfactant concentration was high (70% and 50% surfactant respectively). Therefore, F2 (30% oil, 65% surfactant and 5% co-surfactant) due to optimum concentration of surfactant and co surfactant showed least viscosity.

Cloud point measurement of SMEDDS

Cloud point of prepared SMEDDS formulations F1-F8 was found to be higher than 85°C, which indicates that micro emulsion will be stable at physiological temperature without risk of phase separation. But F9-F11 formulations showed phase separation after 50°C. It may be due to precipitation of drug. So even if these formulations have good drug content, still they cannot be considered among the good formulations due to phase separation at higher temperature.

Robustness to dilution

After diluting SMEDDS to 50, 100 and 1000 times with water and buffer pH 7.4 and storing for 12 h, it was observed that there was no sign of phase separation or drug precipitation in F1-F8 formulations but F9-F11 formulations turned hazy after standing for long hours.

Thermodynamic stability studies

The formulations (F1–F11) were subjected to different thermodynamic stability by using heating cooling cycle; centrifugation and freeze thaw cycle stress tests which included freezing at -4°C for 24 hours followed by thawing at 40°C for 24 hours. Centrifugation was performed at 3500 rpm for 5 minutes. The formulations were then observed for phase separation.

F1-F8 formulations passed all the thermodynamic tests. F9 failed at freeze thaw. F10 was not able to clear centrifugation test. F11 did not passed any of the test due to absence of co-surfactant and large quantity of oil present in it. Thus, F1-F8 formulations that were stable to phase separation were selected for further studies.41,42

CONVERSION OF SMEDDS INTO S-SMEDDS

For converting a Liquid SMEDDS into the solid state, a highly porous powder with good oil adsorbing capacity is required. Neusilin US2 has such capacity to convert them into free flowing powder. 5 ml of Liquid SMEDDS were added drop wise over Neusilin US2 in fractions contained in broad porcelain dish. After each addition, mixture was homogenized using glass rod to ensure uniform distribution of formulation. 2.40 g of Neusilin US2 was completely adsorbed over the liquid SMEDDS to give final powder with free flowing characteristics. 10 mg of drug was equivalent to 60 mg of SMEDDS powdered formulation.

PHYSICOCHEMICAL CHARACTERIZATION OF SOLID-SMEDDS

Drug content

Drug content of all the formulations varied in the range 86.04 ± 0.12% to 101.3 ± 0.02% (Table 7) and were found to be within I.P. limits and there were no significant difference in the drug content of S-SMEDDS.

Micromeritic properties

Results showed that prepared S-SMEDDS showed good flow properties (Table 6) overall. All formulations (SF1-SF8) showed angle of repose (27.5 ± 0.02 to 30.3 ± 0.05) which showed that they had excellent flow properties. Bulk density (0.33 ± 0.01 to 0.59 ± 0.02) and tapped density
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(0.35 ± 0.03 to 0.76 ± 0.03) was evaluated to study Carr’s index (<25%) and Hausner ratio (1.15 ± 0.03 to 1.33 ± 0.02). High concentration of surfactant can also cause loss of flowability in S-SMEDDS.

Globule size ranged from 161-384 nm (Table 7). It was observed that globule size range of S-SMEDDS was higher as compared to SMEDDS. It might be due to presence of adsorbent (Neusilin US2) in solid-SMEDDS which may lead to increase in globule size during dispersion of formulation. The lowest globule size was observed of SF2-SF4 formulation.

The PDI obtained for all the formulations varied from 0.368-0.550 (Table 7). PDI of SF2 (0.368) again was the lowest and found best in comparison to all other formulations. Zeta potential was found in the range between -8.70 to -43.8 mV.

SEM

SEM Studies revealed the morphology of S-SMEDDS particles. S-SMEDDS appeared as smooth surfaced particles (Figure 4), indicating that the liquid SMEDDS is adsorbed onto the Neusilin US2 Powder with a lesser amount of aggregation.

X Ray Diffraction studies

XRD patterns of Pure ATV and its S-SMEDDS formulation (F2) is shown in Figure 5. The diffraction pattern of atorvastatin revealed several sharp high intensity peaks at diffraction angles 2θ suggesting that the drug existed as crystalline material.

The absence of characteristic peaks suggested that the SMEDDS are completely adsorbed over the surface of Neusilin US2. There were few characteristic peaks of atorvastatin with considerable reduction in the peak intensity (Figure 5). This diminished peak suggests conversion of drug into amorphous form.

Table 8: Physicochemical characterization of dispersible SMEDDS tablet

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulation Code (FB1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (mm)</td>
<td>3 ± 0.14</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>10 ± 0.12</td>
</tr>
<tr>
<td>Hardness (kg/cm²)</td>
<td>4.1 ± 0.18</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.52 ± 0.34</td>
</tr>
<tr>
<td>Weight variation (gm)</td>
<td>149 ± 0.05</td>
</tr>
<tr>
<td>Content Uniformity (%)</td>
<td>99.91 ± 0.03</td>
</tr>
<tr>
<td>Disintegration Time (sec)</td>
<td>60 ± 0.30</td>
</tr>
<tr>
<td>Particle Size (nm)</td>
<td>165.28</td>
</tr>
</tbody>
</table>

*All values are expressed as Mean ± SD, n=10 for weight variation and friability.
PREPARATION OF DISPERSEABLE SMEDDS TABLET

Three tablet batches were formulated FB1, FB2 and FB3. FB1 batch consisted of Neusilin US2 as additional disintegrant while FB2 consisted of pure S-SMEDDS and FB3 consisted of Crosspovidone as additional disintegrant. Only FB1 batch was considered as there was no problem of capping and chipping. Thus 20 tablets of FB1 batch was compressed using tablet press.

PHYSICOCHEMICAL CHARACTERIZATION OF DISPERSEABLE SMEDDS TABLET

SF2 solid SMEDDS formulation was compressed into tablet and evaluated. All the tablets showed weight variation, hardness, friability and disintegration time within the official limits (Table 8). All the tablets passed weight variation test as % weight variation was within Pharmacopoeial limits of (±5%) of weight. The tablets possessed good mechanical strength with sufficient hardness of 4.1 ± 0.18. Friability values below 1% are good indication of better mechanical resistance of tablets.

Reconstitution Test: The resultant microemulsion was formed due to high dispersibility. The particle size range was found in between 165.28 nm, which showed that microemulsion had been formed.

Transmission electron microscopy: The microemulsion droplets from dispersible SMEDDS tablet of atorvastatin were spherical with complete surface (Figure 6). The droplet size of microemulsions from dispersible SMEDDS tablet was slightly larger than that of liquid SMEDDS. This result showed that the preparation of SMEDDS dispersible tablet had no effect on the morphology of the microemulsion droplet of the SMEDDS system.

IN VITRO DISSOLUTION STUDY

The in vitro release studies were carried out for eight formulations of SMEDDS, S-SMEDDS and dispersible SMEDDS tablet of ATV.

In vitro release of SMEDDS

The results obtained from in vitro drug release studies of SMEDDS revealed that all the formulations showed 100% drug release within 60 min (Figure 7). The best formulation
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came out to be F2 (30% oleic acid, 65% tween 80 and 5% co-surfactant PEG 400). All formulations released 100% drug within a short duration but were slightly higher in globule size in comparison to F2 thus there dissolution got slightly reduced and also F2 showed 100% drug release in just 30 minutes. Hence, F2 was considered to be the finest formulation among all.

This behavior of drug release was due to their droplet size. As we know droplet size is inversely proportional to the surface area that means lesser the droplet size more is the surface area and surface area is directly proportional to the dissolution. Thus least droplet size with higher surface area formulation had the highest and fastest dissolution. Therefore, F2 (30% oleic acid, 65% tween 80 and 5% co-surfactant PEG 400) was found to release the 100% drug in 30 mins from the SMEDDS.

Also, small globule size of resultant microemulsion and solubilized form of drug in lipid and S_mic confirms that the solubility of drug increases several times which may result in higher absorption and improvement in oral bioavailability. Since the drug is present in solubilized form and in the center of lipid core in microemulsion globules, the gastric irritation potential of drug also got reduced.

In vitro release of S-SMEDDS

The results obtained from in vitro drug release studies of S-SMEDDS were also in the same pattern as of SMEDDS (Figure 8). The dissolution rate was observed to be quite low in contrast to SMEDDS because of increased droplet size of S-SMEDDS and as we know droplet size is inversely proportional to the dissolution rate therefore dissolution got reduced. Here still, all the formulations also showed 100% drug release and the dissolution rate of F2 was better among all as it released the drug in only 35 minutes.

This release pattern illustrated here was also due to the droplet size. Least the droplet size leads to increase in the surface area and more is the dissolution. In consideration with the smallest droplet size, SF2 (30% oleic acid, 65% tween 80 and 5% co-surfactant PEG 400; 161 nm) formulation showed the ideal and best release out of all eight formulations due to optimum concentration of oil and co-surfactant.

Effect of oil on release

The oil ratio in the system has an important role as many physiological parameters depend on it which eventually affects the dissolution of drug. Through results it was revealed that lesser the oil concentration more stable the formulation was as formulations F6-F8 showed delayed release amongst all and F11 was rejected even. This was due to high concentration of oil leads to coalescence or aggregation which leads to precipitation of the drug. F2 and SF2 (30% oleic acid, 65% tween 80 and 5% co-surfactant PEG 400) respectively showed the best dissolution due to less amount of oil and balanced ration amidst surfactant and co-surfactant.

Effect of co-surfactant on release

PEG 400 was employed as a co-surfactant in the system which is widely known for increasing solubility along with the surfactant. When the co-surfactant is added, the solubility of atorvastatin also increased which leads more

Figure 8: In vitro drug release profile of pure drug and SF1-SF8 S-SMEDDS
absorption of drug in the GI, ultimately enhancing its bioavailability. But it is also responsible for increasing the size of the globules hence the ratio can be increased up to optimal level only. The results revealed as PEG 400 in the formulation was increased from 10% w/w to 30% w/w, the dissolution was decreased.

**Effect of surfactant concentration on release**

*In vitro* release study in phosphate buffer 6.8 shows that the rate of drug release was faster in case of hydrophilic surfactant (tween 80). This is due to the hydrophilic nature of the surfactant. The broadness of the size distribution observed at higher surfactant concentrations could be due to the higher viscosity of the continuous phase which disperses the stirring energy. Thus, the PDI value increased with increasing surfactant concentrations. Zeta potential increased with increasing concentrations of surfactant.

**In vitro release of dispersible SMEDDS tablets**

*In vitro* dissolution profile of FB1 with pure drug is given in the Figure 9. It was found that cumulative percent drug release from the formulation was 100% within a short span of time which proved the solubility enhancement of atorvastatin by self microemulsifying properties within the tablet. FB1 showed 100% release within 35 minutes while that of pure drug was around 16% at the end of 35
minutes. The release was thus found to be faster than that of SMEDDS and S-SMEDDS

**Comparison of pure drug, SMEDDS, S-SMEDDS and dispersible SMEDDS tablet**

From the drug release studies it can be clearly seen that pure drug released only 29.84 ± 0.16% upto 60 minutes and all the SMEDDS, S-SMEDDS and dispersible SMEDDS tablets released 100% drug in comparatively lesser time (Figure 10). This clearly concludes that SMEDDS enhanced the release of the drug 7-8 folds which in turn can increases its bioavailability too.

All SMEDDS formulations were in the size range of 139-223 nm and S-SMEDDS were in the 161-384 nm size range which shows that when SMEDDS were converted into S-SMEDDS there size increased a little higher which only very slightly reduced the dissolution of S-SMEDDS and PDI, zeta sizer also varied accordingly. The tablet formed showed particle size of 165.28 nm, indicating that the microemulsion had been formed. However they formed microemulsions pretty instantaneously. Less particle size leads to increase in surface area. Increase in surface area leads to increase in the dissolution rate.

When the in vitro release study of SMEDDS, S-SMEDDS and dispersible SMEDDS tablets were compared with each other (Figure 10) it was found that in SMEDDS and S-SMEDDS, F2 and SF2 (30% oleic acid, 65% tween 80 and 5% PEG 400) formulation respectively were the best ones amongst all showing 100% release of drug in 35 minutes. However FB1 (dispersible SMEDDS tablet) batch also showed 100% drug release within 35 minutes. Thus F2, SF2 and FB1 are considered to be the best formulations due to the whole drug release within shortest span of time as compared to the pure drug.

**RELEASE KINETICS**

Kinetic analysis of the in vitro release data of SMEDDS, S-SMEDDS and dispersible SMEDDS tablet is shown in Table 9. To establish a relationship between the release kinetics of the dissolution study of ATV in these three formulations, data obtained from in vitro dissolution study was fitted into various kinetic models. The release data obtained were fitted to zero order, first order, Higuchi and Korsmeyer-Peppas kinetic models to determine the mechanism of drug release from the system.

The model that best fits the release data was evaluated by correlation coefficient ($r^2$). The correlation coefficient ($r^2$) value was used as criteria to choose the best model to describe drug release from SMEDDS, S-SMEDDS and SMEDDS tablet formulations. Models with the highest correlation coefficient ($r^2$) were judged to be the most appropriate model for the in vitro release study.

Pure drug showed Higuchi release and anomalous (i.e. non-fickian) mechanism. The formulations F2 of SMEDDS, SF2 of S-SMEDDS and FB1 of dispersible SMEDDS tablets were best fitted Higuchi model which indicated the drug release by diffusion in slow and sustained way.

The value of n in all these formulations was close to 0.5 suggesting that atorvastatin was released from the system by Fickian diffusion.

**SELECTION OF BEST FORMULATION**

Selection of batch was done on the basis of results obtained from in vitro drug release studies. It was observed that FB1 (30% oleic acid, 65% tween 80 and 5% PEG 400) formulation showed 100% drug release within 35 minutes. Also the solidified dosage forms are preferred over SMEDDS as solidified formulations are more ideal than liquid ones in terms of its stability.

The dispersible SMEDDS tablets (FB1) will be considered the best as they are solid plus they are more patient compliant since S-SMEDDS (powders) are difficult to intake. Also, in liquid dosage forms (SMEDDS) it is also sometimes necessary to add preservative so as to avoid its oxidation above room temperature but in case of solid dosage forms it is not obligatory.

**STABILITY STUDIES**

Since FB1 was selected as the best batch above hence the stability studies were performed on only this batch for three
months. It can be seen from Table 10 that the concentration of drug content in the tablet formulation at the end of 90 days was found to be 98 ± 0.41%.

At various time intervals of 1-3 months, sample was analyzed which revealed that there was no major change in the various physiochemical parameter. The tablet did not show any change in color and remain intact throughout the study period. Also, the friability and hardness of tablet was well within the range throughout the study period. No significant variation in drug content was observed with respect to time. Also the disintegration time remained almost the same.

Also the drug release was almost similar of that of freshly prepared and 3 month stored formulation (p<0.05, t-test), implying that it was stable.

**In vivo Pharmacodynamic Studies**

Suspension of atorvastatin calcium tablet, formed by S-SMEDDS was compared with pure atorvastatin calcium drug during *in vivo* studies. The results are depicted in Table 11 Significant decrease in total cholesterol, LDL and triglycerides level was observed in disease control group when treated with S-SMEDDS. Dose level (3 mg/kg/day) was employed and found there was a significance difference between drug and S-SMEDDS group at dose level of 3 mg/kg/day as compared to disease control group. The results indicated better performance of S-SMEDDS (3 mg/kg/day) formulation as compared to the standard pure drug (3 mg/kg/day). SMEDDS are vital tool in overcoming the formulation difficulties and improving the oral bioavailability of hydrophobic drugs. In this study, SMEDDS, S-SMEDDS and dispersible SMEDDS tablet formulations of atorvastatin were successfully prepared. Further they were assessed for *in vitro* performances. Among various formulations, F2 in SMEDDS, SF2 in S-SMEDDS and FB1 in dispersible SMEDDS tablets showed promising results in the terms of globule size analysis, self-emulsification time and *in vitro* drug release. FB1 (30% oleic acid, 65%, tween 80 and 5% PEG 400) was chosen as the best formulation. It not only released 100% drug in 35 minutes but also has advantages over liquid SMEDDS as solidified formulations are more ideal than liquid ones in terms of its stability. *In vivo* studies also showed significant decrease in total cholesterol, LDL and triglycerides level in disease control group when treated with S-SMEDDS tablets. Thus, the dispersible SMEDDS tablets will be preferred over S-SMEDDS and SMEDDS as they are unit dosage forms and highly patient compliant. The dispersible tablets are specifically useful for pediatrics, geriatrics and psychologically ill patients. This research work illustrates the potential utility of dispersible SMEDDS tablets for the delivery of poor water-soluble compounds.

**CONCLUSION**

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION

ATV: Atorvastatin
GIT: Gastrointestinal Tract
SMEDDS: Self-Micro-emulsifying Drug Delivery System
PDI: Polydispersity Index
SEM: Scanning Electron Microscopy
XRD: X-Ray Diffraction
PBS: Phosphate Buffer Saline
TEM: Transmission Electron
ICH: International Conference on Harmonization
RH: Relative Humidity
USP: United States Pharmacopoeia
CPCSEA: Committee for the purpose of control and supervision of experiments on animals
LDL: Low Density Lipoprotein
HDL: High Density Lipoprotein
HFD: High Fat Diet
NPD: Normal Pellet Diet
SD: Standard Deviation
PEG: Polyethylene Glycol
HLB: Hydrophilic Lophilic Balance

REFERENCES


