SOLUBILITY ENHANCEMENT OF BCS CLASS II ANTIHYPERTENSIVE DRUG USING SOLID SELF EMULSIFICATION TECHNIQUE

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ABSTRACT
Self emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oil, surfactant and co-surfactant which are generally present in the form of liquid or semi-solid. Solid- SEDDS (S-SEDDS) are solid forms of liquid SEDDS converted into solid by suitable means. Candesartan cilexetil a Biopharmaceutical classification system (BCS) Class II drug is a nonpeptide angiotensin II type 1(AT1) receptor antagonist used in the treatment of hypertension. It has an oral bioavailability of 15% because to its low solubility across the physiological pH. The main objective of the study was to formulate S-SEDDS of candesartan cilexetil by adsorption process using a solid inert carrier. Various formulations were prepared at concentrations of oleic acid 20%, 40%, Poly ethylene glycol (PEG) 400 and Tween 80 at 1:2 and 1:4 ratios, and liquid SEDDS to Microcrystalline cellulose (MCC) at 1:1 and 1:2 ratios. The results of micromeritic properties showed that S-SEDDS had good powder flow properties. A visual test was carried out to assess self emulsification of S-SEDDS which showed spontaneous emulsification and there was no sign of phase separation. A fine milky emulsion was formed within 2 min. The formulations containing 1:4 surfactant to co-surfactant ratio showed faster drug release when compared to 1:2 ratio. The effect of adsorbent in drug release characteristics of Liquid SEDDS was assessed and its concentration in the formulation was optimized. Thus S-SEDDS of candesartan showed better solubility enhancement and dissolution rate in contrast to pure drug formulation which would further prove to enhance the oral bioavailability.
Key words- Candesartan cilexetil, Solid self emulsifying drug delivery system, solid carriers, solubility enhancement.

INTRODUCTION
In drug discovery, about 40% of new drug candidates display low solubility in water, which leads to poor bioavailability.\textsuperscript{1} Increasing the aqueous solubility of insoluble and slightly soluble drugs is major importance, because most of the newly developed drugs are highly lipophilic in nature and its analysis are mainly carried out using organic solvents like methanol, chloroform, ethanol, benzene, acetone, toluene, carbon tetrachloride, diethyl ether and acetonitrile. Most of these organic solvents are toxic, volatile and costlier.\textsuperscript{2} Various techniques have been employed to enhance the aqueous solubility of poorly water- soluble drugs.\textsuperscript{3} Self emulsifying drug delivery systems (SEDDS) or self micro emulsifying drug delivery systems (SMEDDS) are the familiar approaches for their potential as an optional scheme for delivery of hydrophobic drugs which are associated with poor water solubility and low oral bioavailability.\textsuperscript{4} SEDDS or SMEDDS are defined as isotropic mixtures of oils, surfactants and co-solvents.\textsuperscript{5} The digestive motility of stomach and intestine provides the agitation required for self- emulsification \textit{in-vivo}. The advantages of these systems include not only improved drug solubilization but also enhanced release and absorption properties, due to the already dissolved form of drug in formulation and the resulting small droplet size thus providing a large interfacial surface area.\textsuperscript{6} The systems can form fine oil in water (o/w) emulsions (SEDDS) with a droplet size between 100 and 1000 nm or in microemulsions (SMEDDS) with a droplet size of less than 100 nm when meeting with aqueous media.\textsuperscript{5}

SEDDS and SMEDDS are normally prepared either as liquids or encapsulated in soft gelatin capsules, which have some shortcomings especially in the manufacturing process, leading to high production costs. Moreover, these dosage forms may be inconvenient to use and incompatibility problems with the shells of the soft gelatin are usual. Incorporation of a liquid self-emulsifying formulation into a solid dosage form may combine the advantages of SEDDS with those of a solid dosage form and overcome the disadvantages of liquid formulations described above.\textsuperscript{7} Many techniques are offered to convert conventional liquid SEDDS to solid such as adsorptions to solid carriers, spray drying, spray cooling, melt
extrusion, nanoparticles technology, supercritical fluid based methods, etc. But among these, the adsorption technique is simple and just involves addition of liquid formulation onto carriers by mixing in a blender. The resulting powder may then be filled directly into capsules or alternatively, mixed with suitable excipients before compression into tablets. A significant benefit of the adsorption technique is good content uniformity. The SEDDS can be adsorbed at high levels up to 70% w/w on to suitable carrier.\textsuperscript{8} Thus S-SEDDS will have combined advantages of SEDDS such as enhanced solubility and bioavailability and with those of solid dosage forms, such as low production cost, convenience of process control, high stability and reproducibility, better patient compliance.\textsuperscript{9} Candesartan cilexetil is an esterified prodrug of candesartan, a nonpeptide angiotensin II type 1(AT1) receptor antagonist used in the treatment of hypertension.\textsuperscript{6} Candesartan cilexetil is a white crystalline powder with melting point 157-160$^\circ$C, insoluble in water, soluble in methanol with a biological half-life of 5.1 h.\textsuperscript{10,11} Based on its solubility across physiological relevant pH conditions and absorption characteristic, candesartan cilexetil is classified in the Biopharmaceutical Classification System (BCS) as a class II drug.\textsuperscript{6} Low solubility of candesartan cilexetil across the physiological pH range is reported to result in incomplete absorption from the gastrointestinal (GI) tract and hence is reported to have an oral bioavailability of about 15%. Candesartan cilexetil is a highly lipophilic compound and has good solubility in tri- and diglyceride oils. These factors, may contribute toward absorption via the lymphatic route.\textsuperscript{12}

The aim of the present study was to investigate the use of S-SEDDS technique in improving the dissolution profile of the candesartan cilexetil using micro crystalline cellulose (MCC) as an inert solid carrier which can be filled into hard gelatin capsules.

**EXPERIMENTAL MATERIALS**

Candesartan cilexetil was received as a gift sample from Matrix laboratories Ltd., Secunderabad. Oleic acid obtained from Ranbaxy, Fine chemicals Ltd., New Delhi, Tween 80 obtained from Merck Ltd., Mumbai, Polyethylene glycol 400 obtained from Himedia Laboratories Pvt. Ltd., Mumbai, MCC obtained from Yarrow chemicals, methanol from RFCL Ltd., New Delhi.
METHODS

Preliminary solubility studies\textsuperscript{13,14,15}

The solubility of Candesartan cilexetil in various vehicles, including oils, surfactants, and co-surfactant was determined by the shake flask method. An excess amount of Candesartan cilexetil was added to each centrifuge tube containing 2 ml of the selected vehicle. The mixture was vortexed using a cyclomixer at a maximum speed for 10 min, in order to facilitate proper mixing of Candesartan cilexetil with the vehicle. Mixtures were kept at ambient temperature for 48h. to attain equilibrium. The equilibrated samples were centrifuged at 5,000 rpm for 15 min and the resulting supernatant was filtered through the whatmann filter paper. The concentration of Candesartan cilexetil dissolved was determined by UV-VIS spectrophotometer (UV-1601, Shimadzu Ltd, Japan) at $\lambda$ max 257 nm.

Saturation solubility of drug in mixture of surfactants and co-surfactants\textsuperscript{6,16}

The saturation solubility of candesartan cilexetil was evaluated in predetermined ratios of selected surfactant and co-surfactants using the procedure as described earlier in the text.

Preparation of liquid SEDDS\textsuperscript{17}

A series of Liquid SEDDS formulations was prepared using Oleic acid, tween 80 and PEG 400 as oil, surfactant and co-surfactant. Formulations were prepared using various concentrations of oleic acid (20%, 40%), PEG 400 and Tween 80 ratios (1:2 and 1:4), and liquid SEDDS to MCC ratios (1:1 and 1:2) as shown in Table 1. In all the formulations, the amount of candesartan cilexetil was kept constant (8 mg). The volume of liquid phase containing oil, surfactant and co-surfactant was kept constant (10ml). Required amount of candesartan cilexetil was weighed accurately and added into a volumetric flask containing oil, surfactant and co surfactant. The components were mixed by gentle stirring followed by vortex mixing and finally heated at 40°C on a magnetic stirrer, until candesartan cilexetil was perfectly dissolved. The mixture was stored at room temperature until further use.

Preparation of Solid-SEDDS\textsuperscript{13,8,16}

S-SEDDS was prepared by mixing liquid SEDDS containing candesartan cilexetil with MCC in various proportions. The liquid SEDDS of candesartan cilexetil was adsorbed onto MCC carrier at 1:1, 1:2 ratio by physical mixing process. After each addition, mixture
was homogenized by trituration using mortar and pestle to ensure uniform distribution of the formulation. Resultant damp mass was passed through sieve no. 120 and dried at ambient temperature and stored until further use.

Table 1. Formulations of candesartan S-SEDDS

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug (in mg)</th>
<th>Oleic acid</th>
<th>PEG 400 : Tween 8</th>
<th>Liq. SEDDS : MCC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>8</td>
<td>20%</td>
<td>80% (1:2)</td>
<td>1:1</td>
</tr>
<tr>
<td>F2</td>
<td>8</td>
<td>40%</td>
<td>60% (1:2)</td>
<td>1:1</td>
</tr>
<tr>
<td>F3</td>
<td>8</td>
<td>20%</td>
<td>80% (1:4)</td>
<td>1:1</td>
</tr>
<tr>
<td>F4</td>
<td>8</td>
<td>40%</td>
<td>60% (1:4)</td>
<td>1:1</td>
</tr>
<tr>
<td>F5</td>
<td>8</td>
<td>20%</td>
<td>80% (1:2)</td>
<td>1:2</td>
</tr>
<tr>
<td>F6</td>
<td>8</td>
<td>20%</td>
<td>80% (1:4)</td>
<td>1:2</td>
</tr>
</tbody>
</table>

EVALUATION OF S-SEDDS

Micromeritic properties of S-SEDDS\textsuperscript{8,18,19,20,21}

A] Bulk density
It is the ratio of total mass of powder to the bulk volume of powder. It was measured by filling the weighed powder into a measuring cylinder and the volume noted. It can be expressed in g/cc :

\[
\text{Bulk density} = \frac{\text{Mass of powder}}{\text{Volume of powder}}
\]

B] Tapped density
It is the ratio of total mass of powder to the tapped volume of powder. The tapped volume was measured by tapping the powder to constant volume. The equation is represented with the unit g/cc :

\[
\text{Tapped density} = \frac{\text{Total mass of powder}}{\text{Tapped volume of powder}}
\]

C] Angle of repose
The angle of repose of S-SEDDS was determined by funnel method. Accurately weighed sample were taken in a funnel. Height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of S-SEDDS powder. The powder samples were
allowed to flow through funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose calculated using the following equation:

\[ \tan \theta = \frac{h}{r} \quad \text{(or)} \quad \theta = \tan^{-1} \frac{h}{r} \]

**D) Carr’s Compressibility Index**

Carr’s Compressibility Index is a measure of powder flow properties and was calculated using the following equation:

\[ \text{Carr’s Compressibility Index (\%) = } \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \]

**E) Hausner ratio**

A similar index like compressibility index has been defined by Hausner. Hausner’s ratio is the ratio of tapped density to bulk density and can be calculated by using the following equation:

\[ \text{Hausner’s Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \]

**Drug content**

The percent drug content of candesartan cilexetil in S-SEDDS was estimated by dissolving appropriate quantity of individual S-SEDDS formulation equivalent to 8mg of drug in sufficient quantity of aqueous ethanol solution and further diluted with distilled water. The samples were sonicated using ultrasonicator for 15 min. to thoroughly dissolve the drug and filtered. The absorbance of the filtrate was analyzed using UV-visible spectrophotometer and the percentage drug content was determined.

**Assessment of Efficiency of self emulsification**

The USP 24 rotating paddle apparatus was used to evaluate the efficiency of self-emulsification of different mixtures. One gram of each formulated mixture was added to 200ml of distilled water with gentle agitation condition provided by a rotating paddle at 70 rpm and at a temperature of 37°C. The process of self-emulsification was visually monitored for the rate of emulsification and for the appearance of the produced emulsion. On the basis of dispersibility, appearance and time required to emulsify SEDDS were categorize into different grades.
Reconstitution properties of S-SEDDS\textsuperscript{7,8}

Dilution study was done to study the effect of dilution on S-SEDDS, because dilution may better mimic the condition of stomach after oral administration. In this method, S-SEDDS (100 mg) was introduced into 100 ml of double distilled water in a glass beaker that was maintained at 37°C and the contents mixed gently using a magnetic stirrer. The tendency to emulsify spontaneously and progress of emulsion droplets were observed with respect to time.

\textit{In-vitro} drug release studies\textsuperscript{8,16,24}

Drug release studies from S-SEDDS were performed using USPXXIII type II dissolution apparatus with 900 ml of distilled water separately as a medium at 37 ± 0.5°C. The speed of the paddle was adjusted to 50 rpm. Candesartan cilexetil loaded S-SEDDS (equivalent to 8 mg of candesartan cilexetil) and 8 mg of pure candesartan cilexetil were placed in a dissolution tester. At predetermined time intervals of 5, 10, 20, 30, 45 and 60 min., an aliquot (5 ml) of the sample was collected and replaced with the same volume with the fresh media to maintain the sink conditions. The withdrawn sample was filtered, diluted suitably and analyzed spectrophotometrically at 257nm using UV-visible spectrophotometer.

FTIR Studies\textsuperscript{25,26}

FTIR spectra of Candesartan Cilexetil pure drug and S-SEDDS formulations were recorded using a Fourier Transform Infrared Spectrophotometer (Shimadzu 8400, Japan). Samples were prepared as KBr disks using a hydraulic press and scanned from 4000 to 400 cm\textsuperscript{-1} to study the possible interaction between drug and excipients.

RESULTS AND DISCUSSION

Solubility studies\textsuperscript{13,14,15}

The self-emulsifying formulations consisting of oil, surfactant, co-surfactant and drug should be clear with monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution. The solubility of candesartan cilexetil in various vehicles was determined and among them a combination of surfactant and co-surfactant and an oil phase were selected. One important consideration when formulating a self-emulsifying formulation is avoiding precipitation of the drug. Therefore, the components used in the system should have high solubilization
capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion. In order to prevent precipitation of drug on dilution, it is necessary to select oil which shows higher solubility. Amongst the various oily phases that were screened, oleic acid provided the good solubility of candesartan cilexetil so were chosen for further investigations. Tween 80 showed higher solubility compare to Tween 20. Drug showed highest solubility in co-surfactant PEG 400. Thus, after preliminary trials, oleic acid as oil, Tween 80 and PEG 400 as surfactant and co-surfactant were selected respectively.

Saturation solubility of drug in mixture of surfactants and co-surfactants\textsuperscript{6,16} The solubility of candesartan cilexetil in different binary combinations of selected surfactants and co-surfactant is summarized in Table 2. The solubility data were used to estimate the drug loading capacity to develop a dosage form for capsule filling. The results were compared with pure candesartan cilexetil which showed aqueous solubility of 0.0012mg/ml.

Table 2. Solubility of drug in various ratios of surfactant to co-surfactant mixture

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
<th>Ratio</th>
<th>Aqueous Sol (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>1:1</td>
<td>150.28</td>
</tr>
<tr>
<td>2</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>2:1</td>
<td>157.11</td>
</tr>
<tr>
<td>3</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>3:1</td>
<td>166.51</td>
</tr>
<tr>
<td>4</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>4:1</td>
<td>172.24</td>
</tr>
<tr>
<td>5</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>1:2</td>
<td>110.14</td>
</tr>
<tr>
<td>6</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>1:3</td>
<td>85.18</td>
</tr>
<tr>
<td>7</td>
<td>Tween 80</td>
<td>PEG 400</td>
<td>1:4</td>
<td>74.24</td>
</tr>
</tbody>
</table>

EVALUATION OF S-SEDDS

Micromeritic properties and drug content of S-SEDDS\textsuperscript{19,20,21,22} Micromeritic properties such as bulk and tapped density, angle of repose, Carr’s compressibility index, Hausner’s ratio and drug content are shown in Table 3. The results showed that prepared S-SEDDS had good flow properties and drug content. The content of drug in various S-SEDDS formulation containing candesartan cilexetil varies from 97.21 to 100.37%.
Table 3. Evaluation parameters of the S-SEDDS containing candesartan cilexetil.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>% yield</th>
<th>Bulk Density (g/cc)</th>
<th>Tapped density (g/cc)</th>
<th>Angle of repose (°)</th>
<th>Carr's Index (%)</th>
<th>Hausner's ratio (%)</th>
<th>% Drug Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>91.1</td>
<td>0.4</td>
<td>0.454</td>
<td>35.35</td>
<td>12</td>
<td>1.13</td>
<td>99.62</td>
</tr>
<tr>
<td>F2</td>
<td>90.8</td>
<td>0.4</td>
<td>0.465</td>
<td>34.56</td>
<td>14</td>
<td>1.16</td>
<td>98.93</td>
</tr>
<tr>
<td>F3</td>
<td>97.4</td>
<td>0.434</td>
<td>0.5</td>
<td>35.75</td>
<td>13.04</td>
<td>1.15</td>
<td>100.37</td>
</tr>
<tr>
<td>F4</td>
<td>88.6</td>
<td>0.416</td>
<td>0.476</td>
<td>35.95</td>
<td>12.5</td>
<td>1.14</td>
<td>97.72</td>
</tr>
<tr>
<td>F5</td>
<td>95.6</td>
<td>0.285</td>
<td>0.384</td>
<td>31.55</td>
<td>25.71</td>
<td>1.34</td>
<td>98.56</td>
</tr>
<tr>
<td>F6</td>
<td>98.2</td>
<td>0.294</td>
<td>0.392</td>
<td>29.65</td>
<td>25</td>
<td>1.33</td>
<td>97.21</td>
</tr>
</tbody>
</table>

Assessment of Efficiency of self emulsification\textsuperscript{14,23}

The \textit{in-vitro} performance of SEDDS was visually assessed using the grading system and it was found that, all S-SEDDS formulation showed spontaneous emulsification and there was no sign of phase separation. The formed emulsion was milky white in appearance. It was observed that an increase in the proportion of oil and surfactant in the composition resulted in increasing self-emulsification time. The increase in self-emulsification time can be assumed to be due the relative increase in oil and surfactant concentration, leading to increased viscosity of the formulation. A fine milky emulsion was formed within 2 minutes.

Reconstitution properties of S-SEDDS\textsuperscript{7,8}

A visual test was carried out to assess self-emulsification of S-SEDDS in 100 ml double distilled water at 37°C under gentle agitation. S-SEDDS showed spontaneous micro emulsification and there was no sign of phase separation or phase inversion of micro emulsion even after storage for 2 h. and the process of self emulsification is shown the Fig 1.

![a] Before emulsification

![b] During emulsification
c) After emulsification

d) After storage for 2 hours

Fig 1: Reconstitution properties of S-SEDDS

In-vitro drug release studies \textsuperscript{8,16,24}

The \textit{in-vitro} dissolution profile of S-SEDDS formulations in comparison to the pure drug are indicated in Fig 2. The S-SEDDS showed better drug release characteristics than the formulation containing pure drug. The formulation F3 showed complete drug release within 80 minutes in comparison to pure drug over a period of 160 minutes. The formulations containing surfactant to co-surfactant 1: 4 ratio showed faster drug release when compared to 1: 2 ratios. The formulation containing Liquid SEDDS: MCC (1:2) showed delayed release of drug compared to 1:1 of Liquid SEDDS: MCC. The faster dissolution from S-SEDDS when compared to pure drug may be attributed to the fact that in this formulation, the drug is in solubilised form and upon exposure to dissolution medium, results in small droplets that can mix and dissolve rapidly in the dissolution medium.

![Fig 2: Comparative in-vitro drug release profile of S-SEDDS formulations (F1 –F6) with pure drug.](image)

\textsuperscript{8}
FTIR studies\textsuperscript{25,26}

The IR spectral studies performed are shown in Fig. 3 for the pure drug and excipients to detect any sign of interaction which would be reflected by a change in the position or disappearance of any characteristic peaks of the compound. The IR spectrum of the pure candesartan cilexetil (Fig 3a), pure MCC (Fig 3b) and S-SEDDS formulation (3c) are presented. The spectrum of pure candesartan cilexetil showed its characteristic peaks at 1753.17 cm\textsuperscript{-1}, 1714.60 cm\textsuperscript{-1}, 1548.73 cm\textsuperscript{-1} (Ketonic C=O Stretch), 1114.78 cm\textsuperscript{-1} (Aliphatic ether C — O — C Stretch), 3298.05 cm\textsuperscript{-1} (Secondary NH Stretch), 1473.51 cm\textsuperscript{-1}, 1438.60 cm\textsuperscript{-1} (Aromatic C — C Stretch), 1360.08 cm\textsuperscript{-1}, 1315.36 cm\textsuperscript{-1} (C — N Stretch). The pure MCC showed its characteristic peaks at 2879.52 cm\textsuperscript{-1} (alkane CH symmetrical stretch), 3278.7 cm\textsuperscript{-1} (OH symmetrical stretch), 1035.7 cm\textsuperscript{-1} (aliphatic ethers C — O — C stretch), 1321.15 cm\textsuperscript{-1} (CH\textsubscript{2} bending). Majority of principle characteristic peaks of the drug and the excipients indicated above are weakened, shifted or disappeared in S-SEDDS formulation confirming the solubilization of the drug within the formulation system.

![Fig 3: IR spectrum of pure Candesartan Cilexetil (3a), pure MCC (3b), S-SEDDS formulation (3c)](image_url)
CONCLUSION
S-SEDDS formulations of a poorly water-soluble drug, candesartan cilexetil were formulated for direct filling into hard gelatin capsules for oral administration. The study concluded that, S-SEDDS of candesartan prepared using MCC by adsorption process showed good flow properties and drug content. The formulations after reconstitution formed good emulsion with drug solubilization. Drug releases from S-SEDDS formulations were found to be significantly higher as compared with that of pure drug. Thus the solubility and the dissolution rate of BCS Class – II drug Candesartan cilexetil was enhanced which would prove a promising result of increased absorption and increased oral bioavailability.

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REFERENCE
soluble drugs. European Journal of Pharmaceutics and Biopharmaceutics, 2001; 70: 439-44.


