Preparation, Physicochemical Characterization, Dissolution, Formulation and Spectroscopic studies of β-Cyclodextrins Inclusion Complex

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Abstract: The solubility of Candesartan by complexation using β-cyclodextrin. Physical mixture, freeze-drying methods have been utilized for complexation of Candesartan with β-cyclodextrin. The physicochemical characterization of Candesartan-β-cyclodextrin inclusion complex was performed using Ultraviolet (UV) spectroscopy, Fourier transform infrared spectroscopy (FTIR), 13C Nuclear Magnetic Resonance (NMR), Phase solubility analyses and in vitro permeation experiments through a synthetic membrane in both solid and solution phase. Moreover, interactions between Candesartan and β-Cyclodextrin were studied in DMSO by 13C nuclear magnetic resonance (NMR) spectroscopy. The effects of different preparation methods and drug-to-β-CD molar ratios were also evaluated. Phase solubility studies revealed 1:3 M complexation of candesartan when the freeze-drying method was used for the preparation of the inclusion complex. 1H NMR spectroscopy, FT-IR and UV studies confirmed the true inclusion for the freeze-dried inclusion complex. The dissolution study revealed that the drug dissolution rate was improved by the presence of CDs and the maximum and prompt release was obtained with the freeze-dried inclusion complex. Diffusion studies through a silicone membrane showed that Candesartan diffusion was higher from the saturated drug solution (control) than the freeze-dried inclusion complexes, prepared using different Candesartan -β-CD molar ratios. Comparison among the release profile of the pure drug, freeze dried complex and marketed preparation was performed in pH 3.5 and pH 6.1 buffer solutions.

Key words: Inclusion complex, angiotensin II, β-cyclodextrin, 13C NMR, FTIR.

Introduction: Various techniques for the improvement of the solubility of poorly water-soluble drugs include micronization1 formation of inclusion complexes with cyclodextrin2 formation of amorphous drug3 and formation of solid dispersions with hydrophilic carriers4-8 have been utilized. In the present work β-cyclodextrin (β-CD) has been used to increase the solubility and dissolution rate of Candesartan.

Figure 1. Structure of Candesartan Molecule
Candesartan (Figure 1) is a specific angiotensin II (AT_1), receptor antagonist. It is slightly soluble in alcohol, practically insoluble in water. Despite its hydrophobic nature, it is an orally active drug for treating hypertension. It is used in formulations that enhance solubility and therapeutic activity.

Materials and Methods
Materials
β-CD (Molec. Wt. 1135) was a gift from Gangwal Chemicals Pvt. Ltd. Mumbai. Candesartan was received as a gift sample from Sun Pharma Baroda Gujarat. Lactose monohydrate, microcrystalline cellulose, croscarmelose, silicon dioxide, magnesium stearate, and sodium lauryl sulphate were purchased. All chemicals and solvents used were of A.R. grade. Freshly prepared double distilled water was used throughout the work.

Methods
Preparations of Solid Binary Systems-
Candesartan-β-cyclodextrin binary systems were prepared (1:3 and 1:5 molar ratios) as described in detail below.

Physical Mixture-
Physical mixture (PM) of CD and Candesartan were prepared by simply mixing powders with a spatula for 15 min and then sieved through 60 #.

Co-evaporation method-
For preparation of complexes by co-evaporation, the CD and Candesartan were mixed in a molar ratio of 1:3, and then the mixture was evaporated at 45-50°C for 24 hrs. The resultant solids were then sieved through 60 #.

Kneading method-
For preparation of complexes by kneading method, the CD and Candesartan were taken in a 1:3 molar ratio. The CD was triturated in a mortar with small quantity of water to obtain a homogeneous paste. Candesartan was then added slowly while grinding; a small quantity of methanol was added to facilitate the dissolution of Candesartan. The mixtures were then grounded for 6 h.
hrs. During this process, an appropriate quantity of water was added to the mixture to maintain a desired consistency. The pastes were dried in an oven at 45-50°C for 24 hrs. The dried complexes were pulverized and then sieved through 60 #.

Characterization of Binary Systems
Ultraviolet spectroscopy
UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and The absorption spectra of the reference and test solutions were recorded in 1 cm quartz cells over the range of 200–400 nm. The Candesartan and Candesartan/β-cyclodextrin samples were prepared in situ in a 3ml cuvette. A 1μl aliquot of a 0.5 w/v Candesartan 95% v/v ethanol solution was pipetted into a cuvette containing a known volume of water (3ml). The cuvette was then stoppered and shaken. Further aliquots were then cumulatively added to the cuvette to give a final Candesartan concentration of 1.0 x 10^{-7} μg/ml and the absorbance measured as before. The procedure was repeated for various concentration of β-cyclodextrin. The reference solutions were similarly prepared except than an equivalent aliquot of 95% ethanol was substituted for the Candesartan 95% v/v ethanol aliquot.

Infrared Spectroscopy
Infrared spectroscopy (IR) spectra of pure Candesartan and β-CD, as well as their binary products, were obtained using a IR Magma IR750 by II instrument using NaCl disc method. Analysis was performed at room temperature.

13C NMR studies
NMR spectra were recorded with 500 MHz NMR machine Solutions of Candesartan, β-cyclodextrin and Candesartan/β-cyclodextrin (1:3 and 1:5 ratios) were prepared by filtering a saturated solution of the respective material in DMSO through a cotton wool plug in a 5 mm capillary tube. NMR measurements were then made using a 500MHz 13C NMR spectrometer operating at 500MHz in the pulsed Fourier transform mode (to an accuracy of ± 0.05ppm). Each spectrum was referenced to DMSO at 0.005ppm as an external reference spectrum was recorded for the preparations. The spectra and chemical shifts values were recorded.

Evaluation of Binary Systems
Phase solubility study-
Phase-solubility studies were performed by the method of Higuchi and Connors. Candesartan, in constant amounts (5 mg) that exceeded its solubility, was transferred to screw capped vials containing 15 ml of aqueous solution of β-CD or at various molar concentrations(0, 3.0, 6.0, 9.0, 12.0, and 15.0 μm). The contents were stirred on rotary shaker for 72 hrs. at 37°C ± 0.1°C and 1200 rpm. The time duration was fixed based on pilot experiment and found to be sufficient to achieve equilibrium of mixture. After reaching equilibrium, samples were filtered through a 0.36 μm membrane filter, suitably diluted and analyzed spectrophotometrically for drug content at 272 nm UV/Visible spectrophotometer,. Solubility studies were performed in triplicate.

Formulation studies-
Tablets containing 50 mg of Candesartan were prepared by direct compression using different excipients like Lactose monohydrate, colloidal silicon dioxide, and magnesium stearate. Tablets containing complexes (equivalent to 50 mg Candesartan) prepared by kneading and co evaporation method were also prepared similarly using less quantity of lactose. The blend was compressed on a six-station single rotary machine using round-shaped, flat punches to obtain tablets having thickness 3–4 mm and hardness 3–5 kg/cm2. The tablets were studied in 6 replicates for release profile of Candesartan using the same method described in dissolution studies.

Dissolution Studies-
Dissolution studies of Candesartan in powder form, and Complexes with β-CD were performed to evaluate drug release profile. Dissolution studies were performed on USP dissolution apparatus type II with 900 ml dissolution medium 0.1 N HCl (P11.2) at 37°C ± 0.5°C at 75 rpm for 8 hr. At fixed time intervals 5 ml aliquots were withdrawn, filtered, suitably diluted and assayed for Candesartan content by measuring the absorbance at 272 nm. (Pilot experimental data Candesartan indicated no change in the l max of Candesartan due to the presence of CD’s in the dissolution medium.) Equal volumes of fresh medium (pre-warmed to 37°C) were replaced into the dissolution medium to maintain constant volume throughout the test period. Dissolution studies were performed in 6 replicates, and calculated mean values of cumulative drug release were used while plotting the release curves.
Table 1. Solubility of Pure Candesartan Stability constant (Kc), and Correlation Coefficient (R^2) as obtained from the Candesartan-β-Cyclodextrin Phase Solubility Diagrams

<table>
<thead>
<tr>
<th>Medium</th>
<th>G</th>
<th>Kc (M⁻¹)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.5</td>
<td>0.271</td>
<td>218</td>
<td>0.9319</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>0.281</td>
<td>291</td>
<td>0.8931</td>
</tr>
<tr>
<td>pH 6.9</td>
<td>0.751</td>
<td>322</td>
<td>0.9013</td>
</tr>
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</table>

Table 2. Chemical shifts for β-cyclodextrin in 1:1 and 1:2 molar ratios with Candesartan

<table>
<thead>
<tr>
<th>Sample</th>
<th>Molar Ratio</th>
<th>H(1)</th>
<th>H(3)</th>
<th>H(5)</th>
<th>H(2)</th>
<th>H(4)</th>
<th>H(6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan/βcd</td>
<td>1:1</td>
<td>2.43</td>
<td>3.21</td>
<td>3.71</td>
<td>3.52</td>
<td>3.91</td>
<td>6.76</td>
</tr>
<tr>
<td>Candesartan/βcd</td>
<td>1:2</td>
<td>2.67</td>
<td>3.38</td>
<td>4.43</td>
<td>5.42</td>
<td>5.98</td>
<td>6.11</td>
</tr>
</tbody>
</table>

Figure 2. Phase solubility diagram of Candesartan-β-cyclodextrin at different pH

Results and Discussion

Phase Solubility Studies-
The phase solubility diagram β-cyclodextrin – Candesartan system in water can be characterized as A_l type phase solubility curve, which suggests that the molar ratio of the complex is 1:3 the stability constant was found to be 78.65. Results of the phase solubility studies are shown in Figure 2 that presents the solubility profiles of Candesartan as a function of increasing concentrations of β-CD in aqueous solution at different pH values (4.5, 5.0, and 6.9). As can be seen, the solubility of the drug increased linearly with the increase of β-CD concentration (≤3 μM), giving rise to A-type solubility diagrams. This linear candesartan-β-CD correlation, with a slope of less than 1, suggests the formation of a 1:1 (mol/mol) candesartan-β-CD inclusion complex, at the different studied pH values. The calculated stability constant values were 139, 314, and 219 M⁻¹, respectively, at pH (a) 4.5, (b) 5.0, and (c) 6.9 (Table 1). Figure 2 Candesartan indicating that Candesartan-β-cyclodextrin complexes (1:1 molar ratio) are sufficiently stable. In fact, values of obtained stability constants are always within the range of 100 to 1000 M⁻¹, which is believed to Candesartan an ideal value. The solubility differences of candesartan as a function of the method used for the preparation of the solid binary products, that is, physical mixture and freeze-dried products was also studied. It has been found that when the freeze-dried product candesartan-β-CD (1:2 ratio) was used, the drug solubility increased by a factor of 3 compared with that of the physical mixture. Moreover, it was 6-fold higher than that of the pure candesartan.

Ultra Violet Spectroscopy studies
The UV spectrum for Candesartan consists of two peaks, one at 272nm and other at 311 nm (not very prominent) and a point of inflexion at 258 nm. The molar absorptivities at these wavelengths were determined as 0.651, 0.872 respectively. The point of inflexion at 242nm (ε = 0.652) has been assigned to butane group by reference to the UV
spectrum for butane 1 group bonded to a non-conjugated molecule shows Candesartan ring system structure obtained with UV. In the spectrum for Candesartan, the absence of a peak at 280 nm and the

**FT-IR studies**

The IR spectra of β-cyclodextrin, Candesartan and β-cyclodextrin – Candesartan complex (1:1 molar ratio) is shown Figure 3. Two biphenyl peaks at 1571 cm\(^{-1}\) and 1621 cm\(^{-1}\) characterize the spectrum of Candesartan. A broad band at 1680cm\(^{-1}\) characterizes the spectrum for β cyclodextrin, which is due to the glycoside linkages. The spectrum for the physical mix and kneaded preparation are more or less the summation of those for the hydroxyl group peaks of Candesartan at 1823 cm\(^{-1}\) & 1781cm\(^{-1}\). In contrast in the spectrum for the Candesartan / β-cyclodextrin freeze dried preparation the Candesartan hydroxyl peaks are Candesartan individually observed and instead appear as a single broad band around 1431 cm\(^{-1}\). The cyclodextrin glycoside peak is unchanged in the presence of Candesartan both as freeze preparation and as a physical mixture.

**\(^{13}\)C- NMR Studies**

In each of the spectra, whether in the presence or absence of Candesartan, the signals due to H(2) are clearly visible around 5.0 ppm, whereas in the range 4.0-3.5 ppm, the spectral patterns from the cyclodextrin signals in the presence of Candesartan are quite different from those observed in its absence. Inspection of the spectra shows that the differences are not due to new signal multiplicities, but instead, arise from changes to the chemical shifts of the signals, in particular, changes to the signals from H(3) and H(5). The signals have moved downfield and these movements are consistent irrespective of the Candesartan/β-cyclodextrin molar ratio. However, the δ values in the 1:3 and 1:5 mixtures show that a change in molar ratio does not similarly affect each signal. In the absence of Candesartan the H (3) signals from β-cyclodextrin give rise to a singlet at 3.63 ppm. In the presence of Candesartan the β-cyclodextrin H (3) signals are shifted up field, such that one signal of the triplet is obscured by the signal due to H (6) and other two signals appears at 3.9 ppm, on the up field side of the H. In the absence of Candesartan the H signals are almost completely obscured by H signal. In the presence of Candesartan β-cyclodextrin H signals are shifted up field so that they are completely visible around 2.54 ppm. (Figure 4)

![Figure 3. FT-IR spectrum of β-cyclodextrin, Candesartan and Candesartan-β-cyclodextrin inclusion complex](image-url)
In Vitro Dissolution Studies
The dissolution studies was carried out with Candesartan and its complexes and physical mixture using dissolution medium 0.1 N HCl. 30 min (dissolved within 60 min), time to dissolve 50% drug (t50%) and mean dissolution time are reported in Table 3. The data revealed the onset of dissolution of pure Candesartan was very low (29.22 % within 30 min). It is evident that the dissolution rate of pure Candesartan is very low (49.46 % in 4 hr.) Inclusion complexes CHH, CNB, IOH and COOH significantly enhanced dissolution rate of Candesartan (85–91%) 8 hrs) [8]. Moreover, candesartan diffusion rate was higher from the control-saturated solution. These results confirm that β-CDs do not interact significantly with the silicone membrane. Moreover, although drug diffusion was very similar for all the freeze-dried products, candesartan diffusion rate slightly decreased
as the CD amount increased. This behaviour is the consequence of different drug thermodynamic activity in the studied systems. In fact, for saturated solutions, the thermodynamic activity is constant despite the amount of dissolved drug. In addition, only free molecules can diffuse through the rate-limiting membrane. Therefore, the drug activity is only related to its free molecules in solution. In the presence of β-CD, candesartan solubility increases because of the formation of the inclusion complexes, but the actual thermodynamic activity is lower than that of the drug alone. Similar results were recently reported by different authors. The diffusion profile obtained from the saturated solution is very irregular (R² = 0.9583) with high standard deviations. This is a consequence of the higher instability of this system when compared with the candesartan-β-CD binary products, which gave a very regular candesartan diffusion profile (always R² > 0.873) with standard deviation less than 2%. Therefore, data obtained from the diffusion study suggest that β-CD is able to both stabilize the system and regularize the diffusion profile.

**Conclusion**

Results obtained during this study showed that β-CD is able to improve candesartan dissolution properties. The best results were obtained from freeze-dried product, in which a true inclusion of candesartan with β-CD was confirmed by studies both in the solid and liquid phase. Despite their different solubility, drug diffusion through a model silicone membrane was higher for the saturated drug solution than the freeze-dried inclusion complexes that were able to stabilize the system leading to a more regular diffusion profile.

Table 3. Dissolution Efficiency and Dissolution Percentage Values at 25and 90 Minutes and Time to dissolve 50% Drug for Candesartan and Candesartan-β-Cyclodextrin Systems*

<table>
<thead>
<tr>
<th></th>
<th>DE25</th>
<th>DP35</th>
<th>DE50</th>
<th>DP90</th>
<th>T50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan</td>
<td>2.0</td>
<td>3.53</td>
<td>18.79</td>
<td>48.91</td>
<td>&gt;70</td>
</tr>
<tr>
<td>PM</td>
<td>11.56</td>
<td>18.82</td>
<td>38.71</td>
<td>59.84</td>
<td>55</td>
</tr>
<tr>
<td>FD</td>
<td>35.73</td>
<td>69.64</td>
<td>74.13</td>
<td>86.60</td>
<td>&lt; 35</td>
</tr>
</tbody>
</table>

*DE Candesartanicates dissolution efficiency; DP, dissolution percentage; t 50%, time to dissolve 50% of drug; CANDESARTAN, Candesartan; PM, physical mixture; and FD, freeze-dried. DE was calculated from the area under the dissolution curve at 15 and 60 minutes and is expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time.

Figure 5. Dissolution Profiles of Candesartan and its complexes with β-Cyclodextrin in simulated gastric fluid

Figure 6. Dissolution Profiles of Candesartan and its complexes with β-Cyclodextrins.
References


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