# ANALYSIS OF ANTIHYPERTENSIVE COMPOUND B CYCLODEXTRIN INCLUSION COMPLEX BY FOURIER TRANSFORM INFRA RED, ULTRAVIOLET-VISIBLE ABSORPTION SPECTROPHOTOMETER AND $^{13}$C NMR SPECTROSCOPY

MUKESH CHANDRA SHARMA, SMITA SHARMA[a]
*School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshshila Campus, Khandwa Road, Indore- 452 001, Madhya Pradesh, India*
*[a]Department of Chemistry Yadhunath Mahavidyalya Bhind- 477001 Madhya Pradesh, India*

An attempt has been made to enhance the solubility and dissolution of Candesartan by complexation using β-cyclodextrin. Complexes were prepared by physical mixture, kneading, and spray drying methods. UV Spectroscopy, Fourier Transform Infrared Spectroscopy and $^{13}$H Nuclear Magnetic Resonance evaluated the prepared complexes. Release profile of the drug from the complexes were studied in pH 6.5 and pH 3.5 and it was found that preparation showed lesser release characteristic as compared to the complex prepared by kneading and freeze drying method.Cyclodextrins are cyclic oligosaccharides, which have the ability to form host/guest inclusion complexes both in solution and in solid state. They have been used extensively to increase the solubility of poorly soluble drugs[1, 2]. Amongst the existing cyclodextrins, β-cyclodextrin has been used extensively to modify the physico-chemical properties[3-5].Candesartan is a antihypertensive agent, which is used in the treatment of hypertension. This drug is practically insoluble in water and has a longer onset of action. Therefore an attempt has been made to improve the aqueous solubility of Candesartan by complexing it with β-cyclodextrin, thus enhancing its dissolution rate, thereby showing a faster onset of action.

(Received October 24, 2009; accepted November 16, 2009)

*Keywords*: Inclusion complex, Angiotensin II, β-cyclodextrin, $^{13}$CNMR

## 1. Introduction

Various techniques for the improvement of the solubility of poorly water-soluble drugs include micronization[1] formation of inclusion complexes with cyclodextrin[2] formation of amorphous drug[3] and formation of solid dispersions with hydrophilic carriers [4-8] have been utilized. In the present work β-cyclodextrin (β-CD) has been used to increase the solubility and dissolution rate of Candesartan. Candesartan (Figure[1]) 2-ethoxy-1-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-1, 3-benzodiazole-6-carboxylic acid. it is an orally active specific angiotensin II, $AT_1$ receptor antagonist, and clinically effective drug in the treatment of hypertension. it is slightly soluble in alcohol, practically insoluble in water. Due to its hydrophilic nature (octanol/water partition coefficient 5.8 at pH of 7.6). Cyclodextrins (CD's) with their Candesartaner-shaped cavities capable to form inclusion complexes with a wide range of commonly used drugs by taking the whole molecule or part of it into the cavity and known to improve the aqueous solubility of drugs. Many drugs such as hydrocortisone, itraconazole, omeprazole, mitomycin, nitroglycerin, voriconazole etc. have been complexed with CD's and formulated for enhancing solubility and therapeutic activity. [9-10] Even IRB *β*-cyclodextrin (*β*-CD) complexes has been reported to enhance its solubility. [11] In previous year's cyclodextrins (CDs) have been recognized as an important constituents of pharmaceutical excipients. They are cyclic oligosaccharides consisting of (α-1, 4)-linked α-D-glucopyranose units, with a relatively hydrophobic central cavity and a hydrophilic outer surface.

## 2. Material and methods

### *2.1 Formulation of Candesartan- β-cyclodextrin inclusion complexes*

Materials *β*-CD (Mole. Wt. 1135) were generous gift from Gangwal Chemicals Pvt. Ltd. Mumbai and Candesartan were obtained from SUN Pharma Baroda India. All other reagents and chemicals were of analytical reagent grade. The solubility studies were performed by adding an excess amount of Candesartan to the aqueous solution of β-cyclodextrin at various concentrations (5-25 µg/ml). The contents were stirred for 48 hrs at 38±1º. After equilibrium, the samples were filtered and absorbance read at 311 nm.The inclusion complex was prepared in the molar ratio of 1:3 and 1:5. Physical mixtures were prepared by triturating together the powders for 30 min in a clean, dry pestle and mortar. The kneaded dispersion was prepared by wetting the powders with cyclohexane. It was kneaded to get a paste like consistency and stirring continued till it starts peeling off from the walls of the mortar. It was then dried in a hot air oven at 80º for 20 min. The solutions were then frozen in freezer at (200 k) and then dried to constant weight using freeze dryer for around 55 hrs. Cyclohexane was taken, as solvent and both the drug and β-cyclodextrin were completely soluble in it. The FT-IR spectra of the samples were recorded on FT-IR Magma instrument using NaCl disc technique. Very small quantities of samples of β-cyclodextrin were added to NaCl, mixed well in pestle/mortar to form a uniform powder. This mixture is then placed in a dye set subjected to dye press under vacuum to form NaCl and sample disc (translucent). These discs are then placed in the FT-IR instrument, and observations are noted in full scan and then in the range of 3500nm-520nm. Fourier transform infrared spectroscopy (FTIR) spectra of pure Candesartan and β-CD, as well as their binary products, were obtained using a FTIR Magma IR750 by II instrument using NaCl disc method. Analysis was performed at room temperature.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. 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 (10 ml). The cuvette was then stoppered and shaken. Further aliquots were then cumulatively added to the cuvette to give a final Candesartan concentration of $1.0 \times 10^{-7}$ µg/ml$^{-1}$ 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. The spectrum was recorded between 200-370nm and the absorbance measured at 267,298 and 356 nm. The reference solutions were similarly prepared except than an equivalent aliquot of 95% ethanol was substituted for the Candesartan 95% $^{v}/_{v}$ ethanol aliquot. NMR spectra were recorded with 500 MHz $^{13}$C NMR machine Solutions of Candesartan, β-cyclodextrin and Candesartan/ β-cyclodextrin (1:3 and 1:5 molar ratios) were prepared by filtering a saturated solution of the respective material in DMSO through a cotton wool plug in a 2 mm capillary tube. NMR spectrometer operating at 500MHz in the pulsed Fourier transform mode (to an accuracy of ± 0.05 ppm). Each spectrum was referenced to DMSO at -0.01ppm as an external reference. A spectrum was recorded for the preparations. The spectra and chemical shifts values were recorded.

Dissolution studies of Candesartan in powder form, and Complexes with ß-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 ($P^H$1.2) at 37 $^0$C ± 0.5 $^0$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 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 $^0$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. The dissolution medium used was 900ml of phosphate buffer (pH 6.1). The drug and the inclusion complex were filled in so as to contain Candesartan tablet. At various time intervals, 10 ml sample was withdrawn and absorbance was measured of the filtered

sample, suitably diluted, at the maximum at 320nm and replaced with fresh dissolution medium. Experiment was performed in triplicate. 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 (fig.1).

### *2.2 Analysis of Candesartan- β-cyclodextrin inclusion complexes*

Quantitative IR analyses of each sample were performed using sodium chloride (NaCl) discs referenced to a potassium bromide background on a FT-IR instrument. The IR spectra for all the 1:3 and 1:5 (molar ratio) Candesartan / β-cyclodextrin freeze dried preparations are essentially the same. In each case significant spectral change for the freeze-dried preparations only observed in the range of 3500-520$cm^{-1}$. Figure 2 and 3 shows the IR spectra of β-cyclodextrin-Candesartan in 1:3 and1: 5 (ratios). The spectrum for Candesartan is characterized by tetrazole peaks at 2305 $cm^{-1}$ and 1883$cm^{-1}$. A broad band at 1652$cm^{-1}$ characterizes the spectrum for β cyclodextrin, which is due to the glycosidic linkages. The spectrum for the physical mix and kneaded preparation are more or less the summation of those for the biphenyl rings of Candesartan at 1649$cm^{-1}$ and 1675 $cm^{-1}$. The cyclodextrin glycosidic peak is unchanged in the presence of Candesartan both as freeze preparation and as a physical mixture.

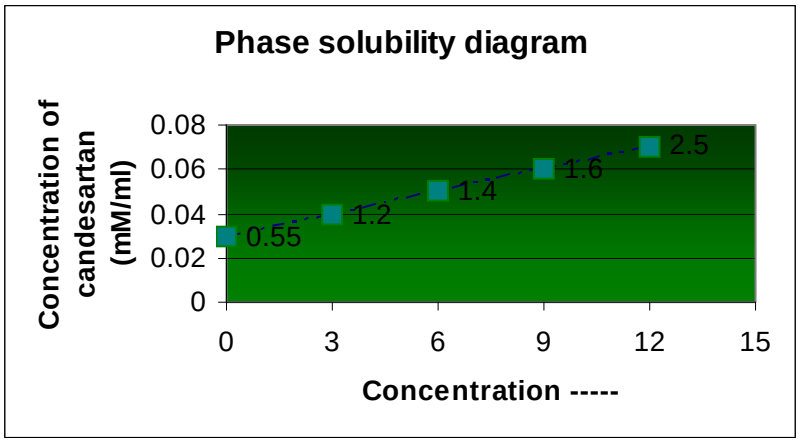


*Fig.1. Phase solubility diagram.*

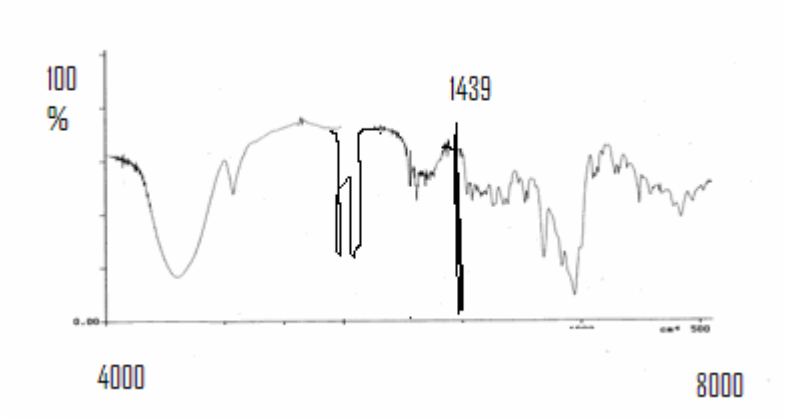


*Fig.2. FT-IR spectrum of freeze-dried Candesartan- β cyclodextrin preparation in 1:3 ratio*

The UV spectrum for Candesartan (figure 3) consists of two peaks, one at 298nm and other at 336nm and a point of inflexion at 242nm.

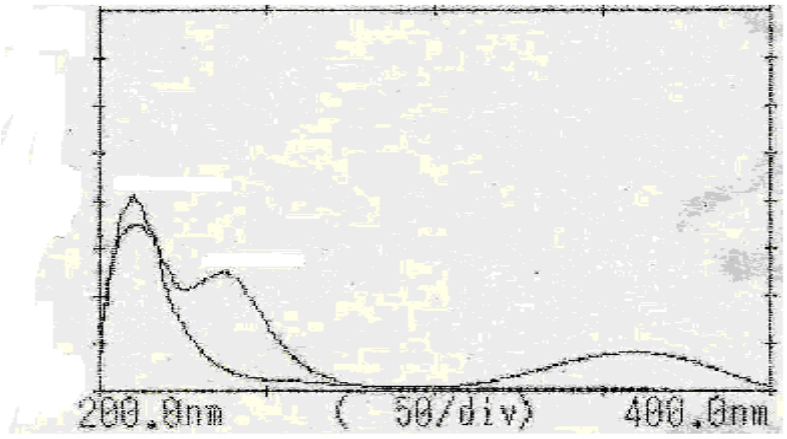


*Fig 3: UV spectrum of Candesartan/ β-cyclodextrin in the region of 240-360nm*

Assignment of the peaks at 265nm and 318nm have been made with reference to spectra for teterazole and hydroxyl group, both of which show major peak at 260nm and 335 nm; the former is taken to correspond to the major peak of Candesartan. In the spectrum for Candesartan, the absence of a peak at 280nm and the presence of a slightly less prominent one at 321 nm may be due to conjugation between the butyl group and the tetrazole ring. $^{13}$CNMR technique was also used to assess the degree of complexation.

| chemical shift(Δδ) ppm |
|---|

*Table 1. Shows the changes in chemical shifts for β-cyclodextrin in 1:3and 1:5 ratios with Candesartan.*

| Sample | Ratio | H(4) | H(7) | H(9) | H(11) |
|---|---|---|---|---|---|
| can/βcd | 1:3 | 5.28 | 4.43 | 6.61 | 7.03 |
| can/βcd | 1:5 | 2.31 | 2.39 | 5.43 | 6.89 |

The signals have move downfield and these movements are consistent irrespective of the Candesartan/ βcyclodextrin 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(4) signals from β-cyclodextrin give rise to a triplet at 7.20ppm (downfield to H(9)). In the presence of Candesartan the β-cyclodextrin H(6) signals are shifted upfield, such that one signal of the triplet is obscured by the signal due to H(6) and other two signals appears at 6.9ppm, on the upfield side of the H(6). In the absence of Candesartan the H(5) signals are almost completely obscured by H(6) signal. In the presence of Candesartan β-cyclodextrin H(5) signals are shifted upfield so that they are completely visible around 2.35ppm.Changes to the proton signals of Candesartan in the presence of β-cyclodextrin suggest that the butyl and hydroxy signals are not shown as they are obscured by the cyclodextrin proton signals in the range of 6.00ppm-4.0ppm. Changes in chemical shifts are marked in presence of β-cyclodextrin, in particular. Dissolution profiles of Candesartan and its complexes with b-cyclodextrin in phosphate buffer (pH 6.9). The *in vitro* dissolution rate profiles of pure Candesartan drug powder, Candesartan marketed prep and

complexes with b-cyclodextrin prepared by physical mixture, kneading method and freeze drying method.

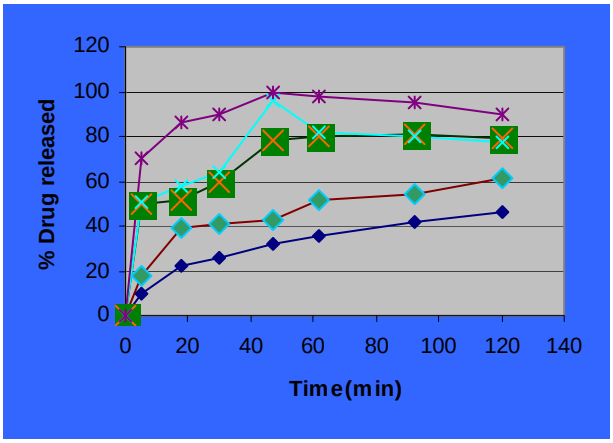


*Fig. 4*

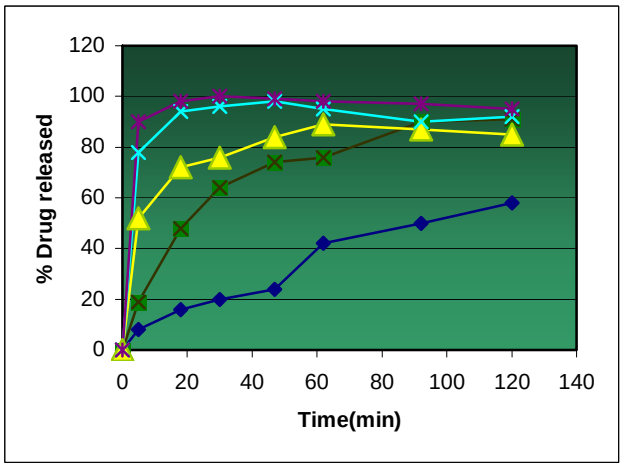


*Fig. 5*

The dissolution profile[11] of the inclusion complexes prepared by different methods is shown in fig 4: Dissolution profiles of Candesartan and its complexes with b-Cyclodextrinin simulated gastric fluid without pepsin (pH 3.9) and Fig 5: Dissolution Profiles of Candesartan and its complexes with b-Cyclodextrinin in phosphate buffer (pH 6.5).It can be seen that after 15 min only 15.1% of pure drug and is dissolved, and even after 90 min only 62.3% of the drug goes into the solution whereas in case of Candesartan – β-cyclodextrin inclusion complex prepared by kneading method and FD method, 65.2% drug was released within 21 min and almost complete release (90.1% and 95.8%, respectively) was seen after 50 min in pH4.2. The release of the drug from the marketed formulation was 62.1% after 15 min and 90% after 90 min. In phosphate buffer (pH 6.9) 9.2% of pure Candesartan was released after 15 min and at the end of 1 hours 68.9% drug went into the solution whereas in case of Candesartan- β-cyclodextrin inclusion complex prepared by Kneeded and FreezeDried method 82.6% and 88.3% of the drug was released after 15 min and almost complete release was observed at 60 min. In case of the marketed formulation, the percentage release was 65 % after 25 min and 96.1% after 90 min. It can be concluded that an inclusion complex of Candesartan with β-cyclodextrin could be prepared successfully by Kneading and Freeze-drying method in a molar ratio of 1:3 and 1:5 and this was confirmed by

solubility studies, FT-IR, UV and NMR. Dissolution studies of the Kneaded and Freeze Dried complex exhibited almost complete in vitro dissolution profile.

### 3. Summary and conclusions

In the first instance, the spectroscopic studies indicate that water-soluble complexes are formed between Candesartan and β-cyclodextrin.With regard to the nature of the included substrate, the general consensus made from the spectroscopic analysis is that both the hydroxyl, butyl, biphenyl and tetrazole ring systems of Candesartan are included by β-cyclodextrin. $^{13}C$ NMR analysis indicates that both the hydroxyl, butyl, biphenyl groups are included. In case of the complex formed with β-cyclodextrin, NMR results indicate that there are two substrates on the Candesartan molecule, the hydroxyl, butyl, biphenyl ring systems. Since it is physically impossible for an entire Candesartan molecule to be included by β-cyclodextrin then at least two different 1:5 complexes. On the basis of spectroscopic analysis three things can be concluded, firstly β-cyclodextrin cavities are occupied by hydroxyl,butyl,biphenyl groups and since more of substrate is included by β-cyclodextrin macrocycles and hence more extensive interactions are established in the complexes; secondly, β-cyclodextrin can form 1:3 complexes with indole group, and finally,1:5 complexes are possible with β-cyclodextrin, which, in addition to Van der Waal's interactions are also stabilised by intramolecular hydrogen bonds between macromolecules.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.In addition to describing gross structure of each complex, interpretation of spectroscopic data has allowed some of the finer structural details of the complexes to be deduced. In some cases there is no precedent from the literature for the interpretation given and these are supported by models, such as the changes in the chemical shift to the cyclodextrin protons oriented at the entrance of the cavities, which are considered due to an interaction between a protruding uncomplexed region of the Candesartan molecule and the groups of rimming entrance to the cavity. Also the changes to the IR spectrum of Candesartan in the region of the carbonyl bands, which are considered to be indicative of the substrate being included

### References

[1] Gupta M.K, Vanwert A, Bogner RH. J Pharm Sci. **92**, 551. (2003)
[2] Cavallari. C, Abertini B, Gonzalez-R. ML, Rodriguez L. Eur J Phar Biopharm. **65**, 73(2002).
[3] Corrigan O.I. Thermochim Acta. **248**, 245-258. (1995)
[4] Chiou W.L, Riegalman S. J Pharm Sci, **60**, 1281-1302. (1971)
[5] Ambike A.A, Mahadik K.R, Paradkar .A.Int J Pharm.**282**, 151-162. (2004)
[6] Paradkar A, Ambike AA, Jadhav BK, Mahadik K.R. Int J Pharm, **271**, 281-286(2004)
[7] Van den Mooter G, Wuyts M, Blaton N,Eur J Pharm Sci., **12**, 261-269(2001)
[8] Jung J, Yoo S, Lee S, Kim K, Yoon D, Lee K.. Int J Pharm, **187**, 209-218(1999).
[9] Szejtli J., Med. Res. Rev.**14**, 353–386, (1994)
[10] Szejtli J., IUPAC, Pure Appl. Chem.**76**, 1825-1845 (2004)
[11] Hirlekar R., Kadam V.AAPS Pharma Sci Tech. **10**, 276-281(2009)
[12] Nalluri B.N,Chowdary K.R,Murthy K.R., Hayman A.R., Becket G. AAPS Pharm SciTech, **1**, 2 (2003).