CHAPTER 4

Applications of Cyclodextrin based Polymers and Bis (β-Cyclodextrin)

4.1 Aqueous Solubilization of Cefpodoxime proxetil and Carbamazepine by Cyclodextrin based Polymers

4.1.1 Brief Review

About 90% of medicines, in pharmaceutics the active ingredients are in the form of solid materials. The number of newly developed drugs having a poor solubility and thus exhibiting bioavailability problems after oral administration is steadily increasing. Estimates by the pharmaceutical companies are that about 40% of the drugs in the pipeline are poorly soluble, and as high as 60% of compounds come directly from the synthesis route[1]. The solubility of drug plays an important role in disposition, since the maximum rate of drug transport across a biological membrane is the result of permeability and solubility. According to the biopharmaceutical classification system (BCS), aqueous solubility and permeability are the most important parameters affecting drug bioavailability.

Cefpodoxime proxetil (CPDX) is an orally administered extended spectrum third generation antibiotic of the cephalosporin class prescribed for the treatment of various respiratory tract and urinary tract infections[2]. The structure of CP is shown in Figure 4.1.1. The major drawback to the use of this cephalosporin class antibiotic drug is its poor aqueous solubility, resulting in low bioavailability i.e. ~ 50% when administered orally[3]. The relative instability of cefpodoxime proxetil has been a major cause of its limited use. It is a highly hydrophobic drug. Therefore, it was proposed to develop a delivery system of cefpodoxime proxetil with enhanced aqueous solubility and improved dissolution rate with the synthesized polymer. As a result its bioavailability is limited.

Carbamazepine (CBZ) is one among the poorly water-soluble drug. The structure of carbamazepine shown in Figure 4.1.2. Carbamazepine chemically 5H-dibenzo[b,f]azepine-5-carboxamide was discovered by chemist Walter Schindler at J. R. Geigy AG (now part of Novartis) in Basel, Switzerland, in 1953 is an antiepileptic drug widely used in the treatment of epilepsy and bipolar disorder[4]. Carbamazepine (CBZ) is used for anticonvulsant and anti-neuralgic effects.

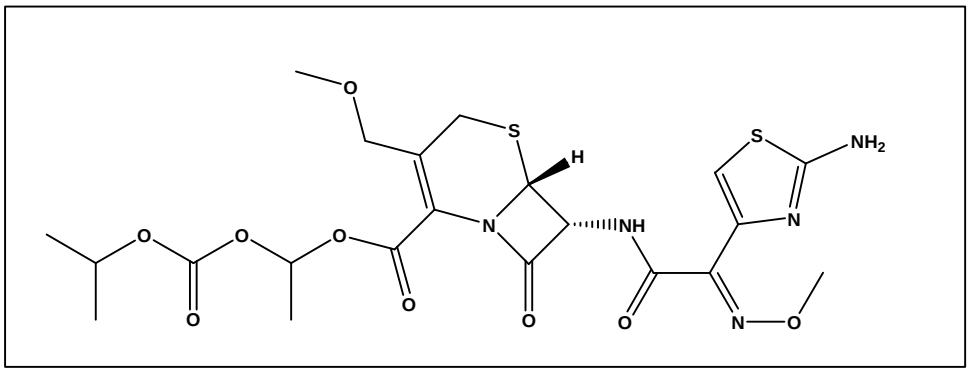


Figure 4.1.1 Chemical structure of Cefpodoxime proxetil

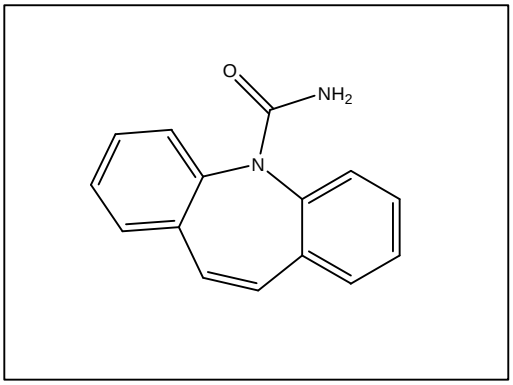


Figure 4.1.2 Chemical structure of Carbamezapine

The popularity of this drug is related to several beneficial properties, including proven efficacy in controlling different types of seizures[5,6]. CBZ is poorly soluble in water with erratic oral absorption and bioavailability less than 70%[7,8]. CBZ comes under Biopharmaceutical Classification System (BCS) Class II drugs which exhibits very low aqueous solubility and high permeability characteristics[9]. The controlled delivery system for CBZ would also be beneficial for patients with epileptic seizures, because CBZ will be released in a controlled manner[10-14]. The aqueous solubility of CBZ at 25^{o}C is 0.1 mg/mL nearly practically insoluble in water[15]. Thus, the absorption dissolution rate of CBZ is very poor. CBZ is known to form an inclusion complex with cylodextrins, which increase the aqueous solubility, dissolution and bioavailability in aqueous media[16,17]. Cyclodextrins can both enhance and hamper drug delivery through artificial membranes[18]. Thus, the cyclodextrin based polymers synthesized in previously were used as a carrier for the poorly water-soluble antibiotic drug cefodoxime proxetil and antiepileptic drug carbamazepine, with the intention that these synthesized cyclodextrin based polymers can change the solubility of CPDX and CBZ and alter the stability of the drug/cyclodextrin polymers inclusion complex with better release performance.

4.1.2 Experimental

4.1.2.1 Materials

β-Cyclodextrin based polymers synthesized previously were used for solubilization of drugs Cefpodoxime proxetil (CPDX) and carbamazepine (CBZ) and were purchased from Sigma-Aldrich.

4.1.2.2 Measurements

The FTTR spectra were recorded on a Shimadzu (8400S) infrared spectrophotometer at 30 scans by using a KBr pellet method. UV-Visible spectra were recorded on a Shimadzu UV-2450 spectrophotometer.

4.1.2.3 Synthesis of β-CD-P-CPDX Inclusion Complex

The synthesis of the β-CD-P-CPDX inclusion complex (Scheme 4.1.1) was achieved in a mixed aqueous solvent system containing ethanol and water (typically 50:50, v: v),

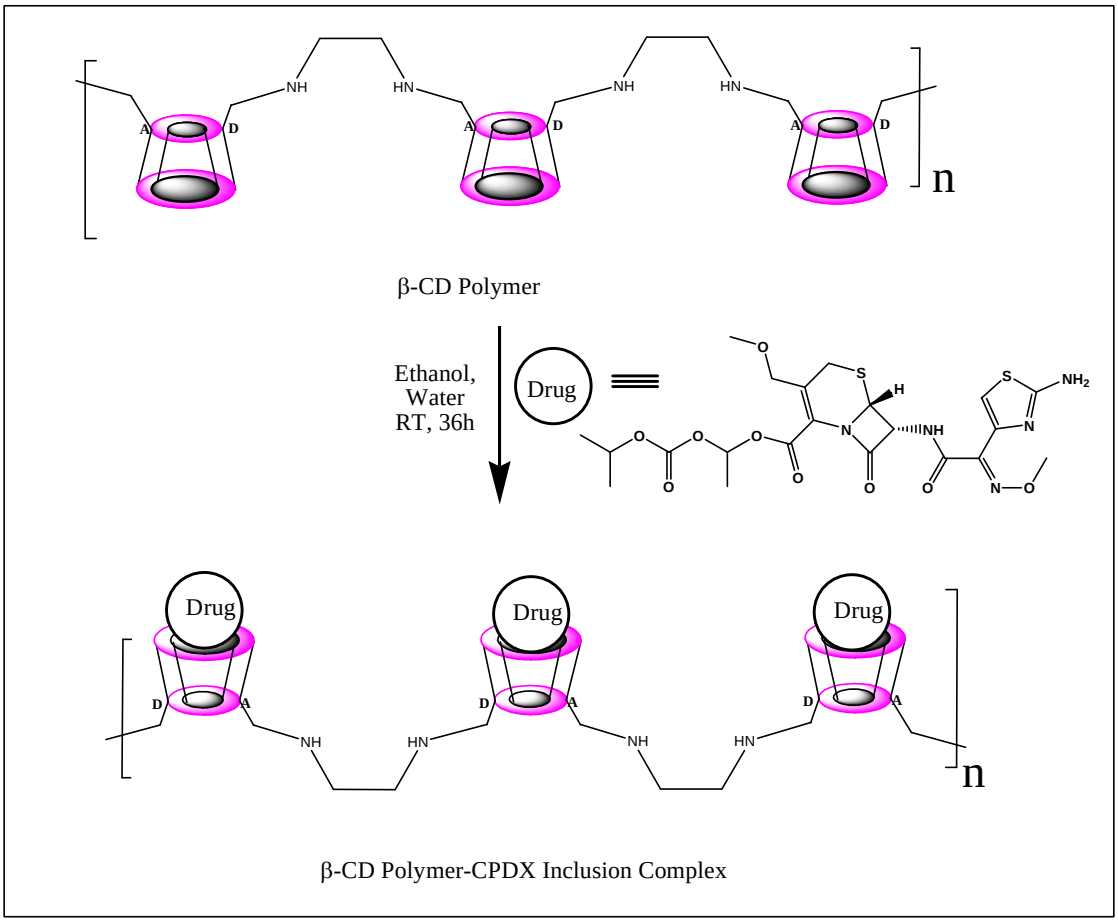
where both β-CD-P and CPDX formed a homogeneous reaction medium. The inclusion of the CPDX into the β-CD-P was monitored over a period of 4 hrs by UV-Vis spectroscopy. The β-CD-P-CPDX inclusion complex was isolated by removing the ethanol by rotavapor and subsequent addition of distilled water. The complex was purified in aqueous solution by membrane filtration using a polymer membrane with a molecular mass cut-off of 650 Da and finally freeze-drying of the aqueous suspension yield the white solid powder of β-CD-P-CPDX inclusion complex.

4.1.2.4 Synthesis of β-CD-P-CBZ Inclusion Complex

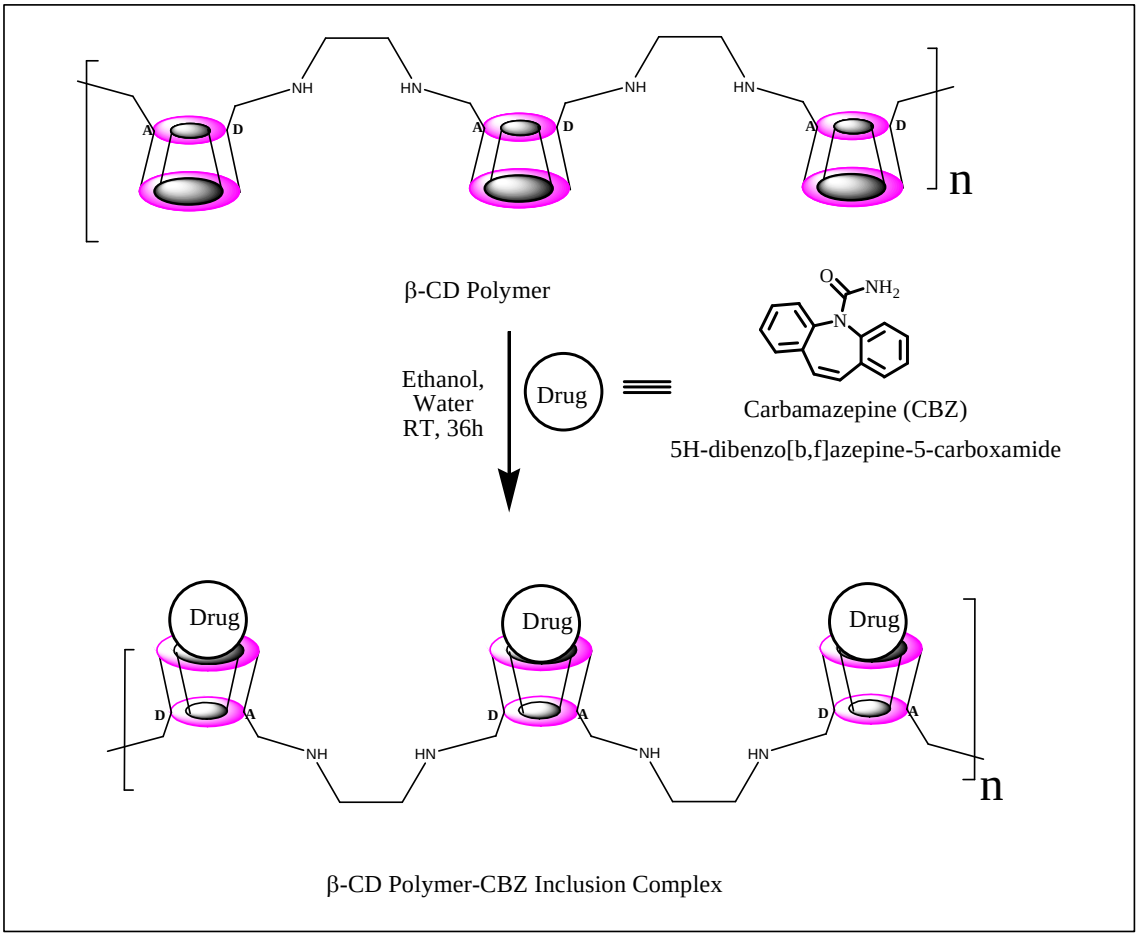
The synthesis of the β-CD-P-CBZ inclusion complex (Scheme 4.1.2) was achieved in a mixed aqueous solvent system containing ethanol and water (typically 50:50, v:v), where both β-CD-P and CBZ formed a homogeneous reaction medium. The inclusion of the CBZ into the β-CD-P was monitored over a period of 4 hrs by UV-Vis spectroscopy. The β-CD-P-CBZ inclusion complex was isolated by removing the ethanol by rotta vapor and subsequent addition of distilled water. The complex was purified in aqueous solution by membrane filtration using a polymer membrane with a molecular mass cut-off of 650 Da and finally freeze-drying of the aqueous suspension yield the white solid powder of β-CD-P-CBZ inclusion complex.

4.1.2.5 Phase Solubility

The phase solubility studies give an idea of the ease with which CPDX and CBZ forms an inclusion complex. The phase solubility of the CPDX and CBZ with pristine β-CD and synthesized β-CD polymers was measured using a simple one pot technique. The phase solubility studies were carried out in phosphate buffer solution according to the method described by Higuchi and Connors. Accurately weighed sample of CPDX and CBZ in quantities exceeding its aqueous solubility (100 mg) was added into vials containing 10 mL of 7.2 phosphate buffer solution (PBS) of various concentration of β-CD or β-CD polymer (10mg, 20mg, 30mg, 40mg, 50mg). All solutions were prepared in a glass vials which shaken at constant temperature (25^{o}C) until equilibrium was achieved (48h). This amount of time was considered sufficient to reach equilibrium and then filtered through a 0.45μm membrane filter to obtain a clear solution and insoluble residue was omitted. The CPDX and CBZ concentration was



Scheme 4.1.1 Scheme shows the formation of inclusion complex of drug CPDX with β-CD-EDA-P in mixed solvents system.



Scheme 4.1.2 Scheme shows the formation of inclusion complex of drug CBZ with β-CD-EDA-P in mixed solvents system.

determined by measuring the UV absorbance of the saturated solutions at 285 nm wavelength and compared with the calibration curve. The apparent binding constant of the CPDX/β-CD and CBZ/β-CD polymer inclusion complex was calculated from the slope and intercept of the straight line of the phase solubility diagram, using the well-known Higuchi-Connors equation.

$$K_{1:1} = \frac{Slope}{Intercept\,(1 - Slope)}$$

4.1.2.6 Dissolution Studies

The release performance of the CPDX and CBZ drugs with the synthesized polymers were measured by dissolution studies. The dissolution of β-CD-P-CPDX and β-CD-P-CBZ inclusion complex were determined by adopting the procedure described by M. J. Arias *et al.*[27] and P. Mura *et al.*[28]. Samples were analyzed spectrophotometrically at 285 nm for both CPDX and CBZ drugs. The freeze dried inclusion complexes of the drugs with polymers (100 mg) were added to 75 mL of water in a 150 mL beaker and stirred at 100 rpm with a glass three-blade propeller centrally immersed in a beaker 20 mm from the bottom. At appropriate time intervals, suitable aliquots were withdrawn with a filter-syringe (pore size 0.45 mm) and absorbance was measured. Both CPDX and CBZ concentrations in the phosphate buffer solution were obtained by UV-Vis spectrophotometer measurements after calibration at definite intervals of time. Fresh dissolution medium was added to maintain a constant volume after each sampling.

4.1.3 Results and Discussions

4.1.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra for the β-CD polymer, CPDX and the inclusion complex are shown in Figure 4.1.3. Inspection of IR spectrum of β-CD Polymer shows prominent absorption bands at 3377 cm^{-1} (for O-H stretching vibrations) 2927 cm^{-1} (for C-H stretching vibrations) and 1157 cm^{-1} and 1026 cm-1 (for C-H, C-O stretching vibration). Figure 4.1.3 b shows the spectra for CP shows absorption band at 3312cm^{-1} for amide linkage. Figure 4.1.3 c shows the spectra for inclusion complex of CPDX/ β-CD-P shows the peak of pure drug CPDX at 3312cm^{-1} due to amide bond has shifted to lower

frequency 3214cm^{-1} which clearly shows the interaction of amide linkage of CPDX with the hydroxyl groups of the β-CD molecule. The FTIR spectrum of CBZ (Fig. 4.1.4 b) exhibited the absorption band at 1678 cm^{-1} arising due to the C=O stretching of primary amide along with typical aromatic stretching bands. In the FTIR spectrum of CBZ encapsulated β-CD-Urea (Fig. 4.1.4 c), a distinct band notice for the CBZ at 1678 cm^{-1}, this typical absorption band was shifted to a higher frequency at 1684 cm^{-1} due to the formation of the inclusion complex. It was also observed that spectra of CBZ encapsulated β-CD polymer, displayed that the peaks of CBZ almost disappear whereas the characteristic peaks of β-CD polymers remains strong. Thus, the positions and relative intensities of few bands were affected due to host-guest interaction, which confirmed the formation of an inclusion complex between CBZ and β-CD polymer. If it were not so, then the spectra of CBZ encapsulated would resemble that of a physical mixture of CBZ with β-CD polymers with no shift in the characteristic bands.

4.1.3.2 Aqueous Solubility of β-CD-polymer and Host–guest Interactions

As expected, after polymerization there is significant enhancement of the aqueous solubility of β-CD. The low aqueous solubility of parent β-CD is attributed to the intermolecular hydrogen bonding between the secondary hydroxyl groups, which are unfavorable to the interaction between cyclodextrin and surrounding water molecules. The introduction of ethylenediamine groups via condensation reaction disrupts this intermolecular hydrogen bonding. Since β-CD polymer is water-soluble it implies that there was no cross linking. There is an almost seven fold increase in the solubility of the β-cyclodextrin on polymerization.

The confirmation of the host–guest interaction was obtained from UV absorbance studies. The CPDX drug shows an absorbance at around 259 nm in methanol and is water-insoluble, whereas β-cyclodextrin polymer shows no absorbance in this region. The spectra in Figure 4.1.5 shows the inclusion complex in water showed the typical absorbance peak corresponding to the CPDX drug implying that the drug has been encapsulated in the cyclodextrin cavity. The CD cavity offers a nonpolar environment for the drug and thus has been “solubilized” in water. The UV-Vis absorbance spectra of CBZ in ethanol, β-CD polymer in water and CBZ encapsulated β-CD polymer in water was shown in Figure 4.1.6.

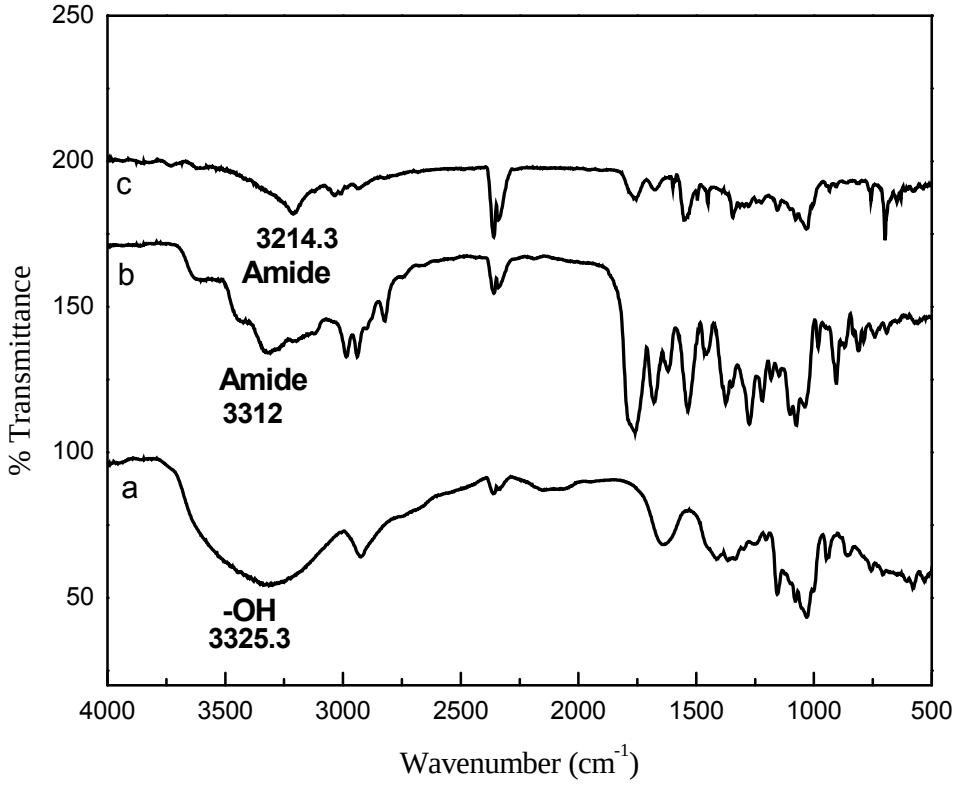


Figure 4.1.3. FTIR absorption spectra of (a) CPDX (b) β-CD polymer and (c) β-CD polymer/CPDX complex

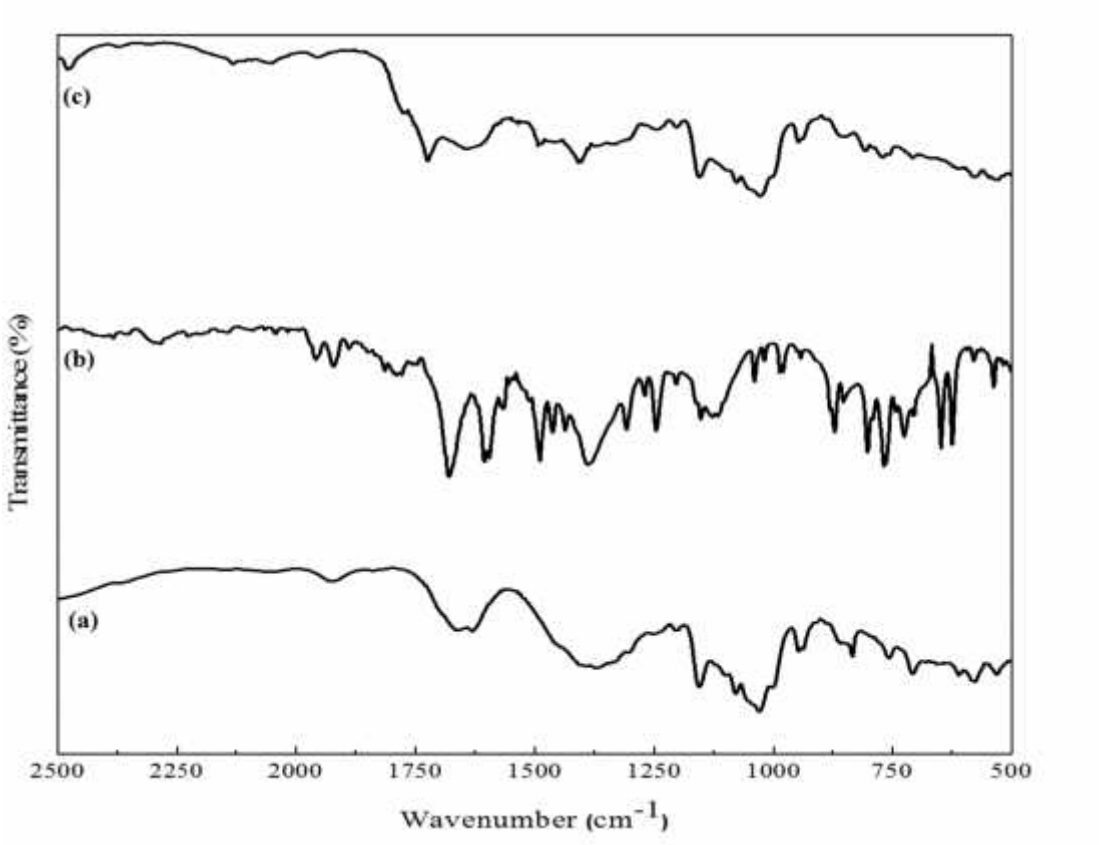


Figure 4.1.4 FTIR spectra of (a) β-CD-Urea-P, (b) CBZ, (c) CBZ encapsulated β-CD-Urea.

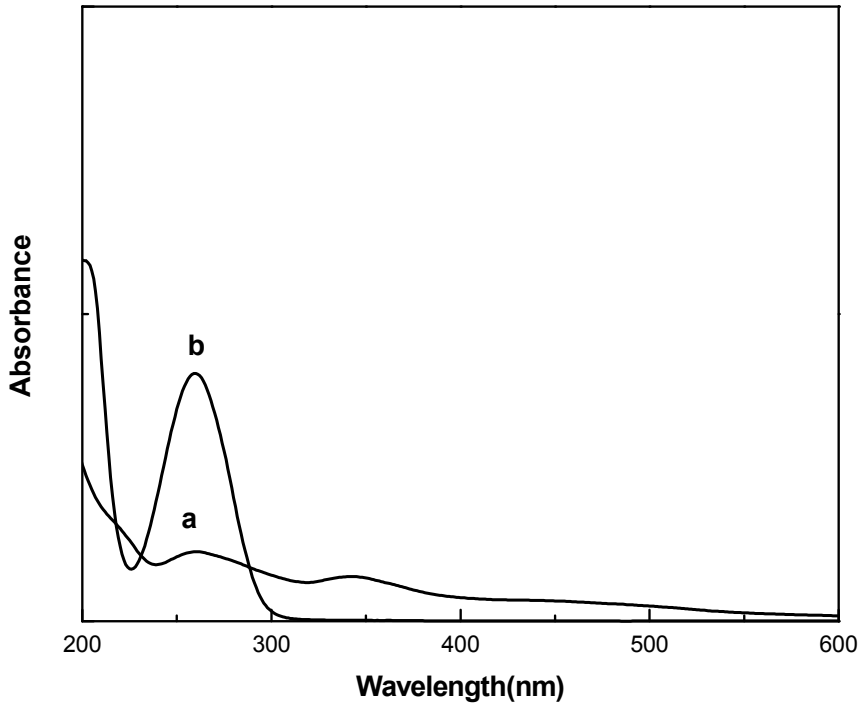


Figure 4.1.5. UV-VIS absorbance of (a) β-CD Polymer in water (b) CPDX in methanol

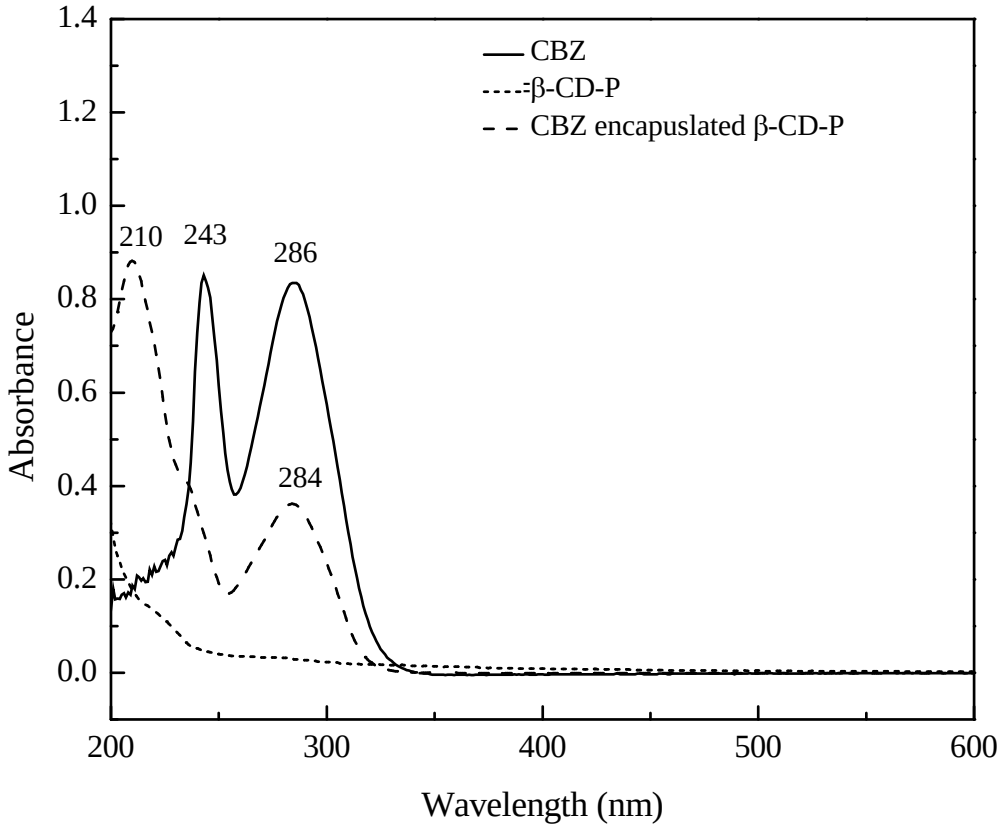


Figure 4.1.6 UV-Vis spectra of CBZ in ethanol, β-CD polymer in water and CBZ encapsulated β-CD polymer in water.

The CBZ drug shows two absorbance maximum at around 243 nm and 286 nm in ethanol and it was water-insoluble, whereas the β-CD polymer shows no absorbance in this region. However, the spectrum of the CBZ-β-CD-P inclusion complex in water showed the typical absorbance peak corresponding to the CBZ drug with considerable blue shift in the absorbance maximum implying that the drug CBZ has been encapsulated into the non-polar cavity of β-CD-P.

4.1.3.3 Phase Solubility Diagrams of the β-CD-P/CPDX and β-CD-P/CBZ inclusion complexes

The improved water-solubility of CPDX and CBZ are shown in Figure 4.1.7 and Figure 4.1.8 respectively. From the phase solubility studies by linear relationship between dissolved drug concentration and amount of solubilizing agent, we calculated the binding constants of the complexes at 25°C ($K1:1_{(\beta\text{-CD})}$= 1035 mol^{-1} and $K1:1_{(\beta\text{-CD-P})}$=1529 mol^{-1} for CPDX and $K1:1_{(\beta\text{-CD})}$= 1035 mol^{-1} and $K1:1_{(\beta\text{-CD-P})}$= 1136 mol^{-1} for CBZ calculated according to the molecular weight of β-CD repeat unit.). From the phase solubility plot the slope of the diagram is less than one thus the inclusion complex may be of 1:1 stoichiometry. These results show the β-CD-Polymer have better complexing properties or binding constant compared to the parent β-CD. This can be attributed to the cooperative action in binding between the adjacent CD units and polymer chains. The ethylenediamine and urea units on polymer chains act like arms of the CD cavities to facilitate the drug inclusion that is helpful for the complexing of large molecules.

4.1.3.4 Dissolution Studies

The dissolution profiles of the CPDX and CBZ with the synthesized β-CD polymers were compared with the CPDX/β-CD and CBZ/β-CD in Figure 4.1.9 and Figure 4.2.0 respectively. CPDX/β-CD and CBZ/β-CD polymer samples showed better dissolution rate (almost 90-96%) and higher cumulative release of the drug dissolution. This is due to the high hydrophilic nature of the synthesized polymers, lowering the interfacial tension in between the highly water insoluble drug and water. The high amorphous nature of the synthesized polymers and the water-solubility show a positive impact on the cumulative release of the drug.

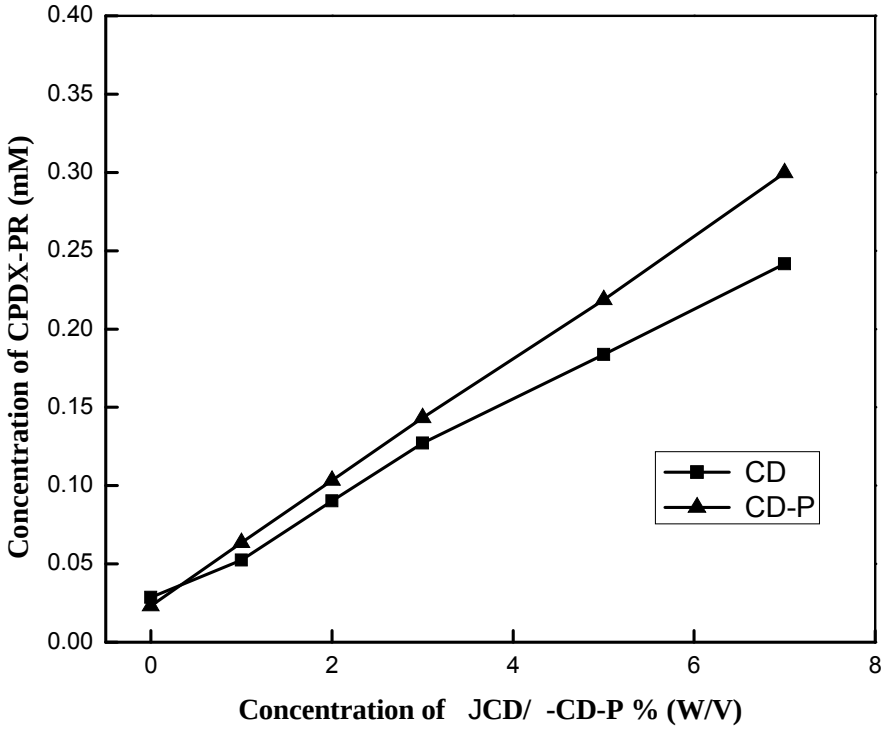


Figure 4.1.7 Phase solubility diagram of CPDX in β-CD and β-CD-Polymer

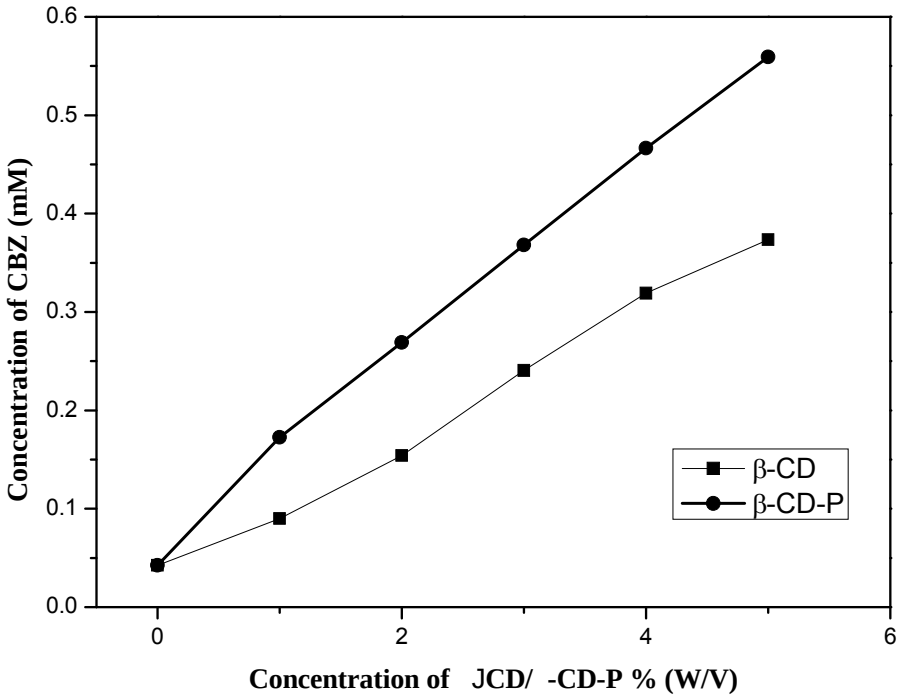


Figure 4.1.8 Phase solubility diagram of CBZ in β-CD and β-CD-Polymer

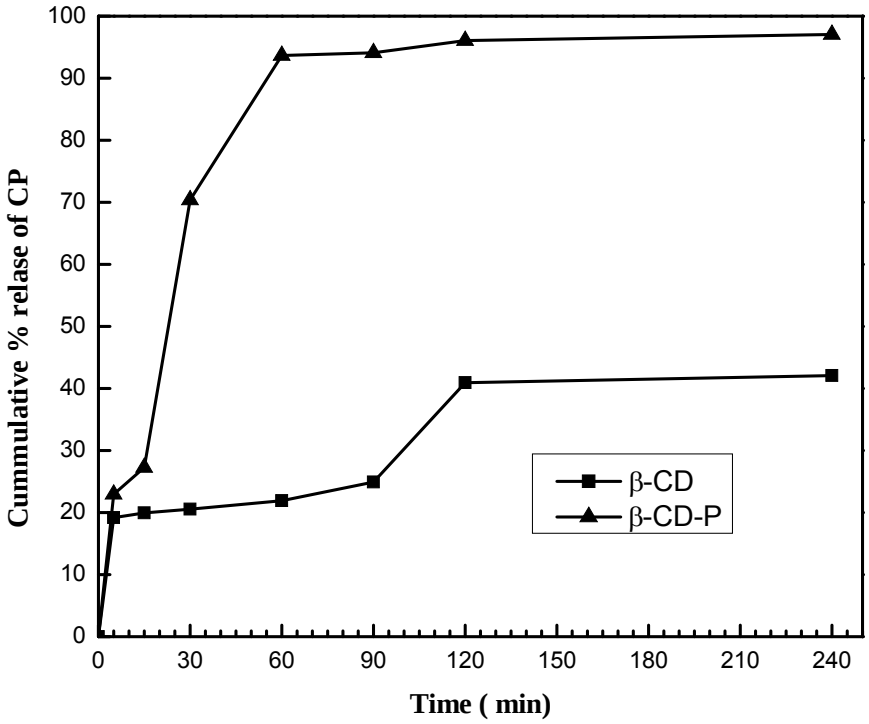


Figure 4.1.9 Dissolution curves of CPDX with β-CD and β-CD Polymer

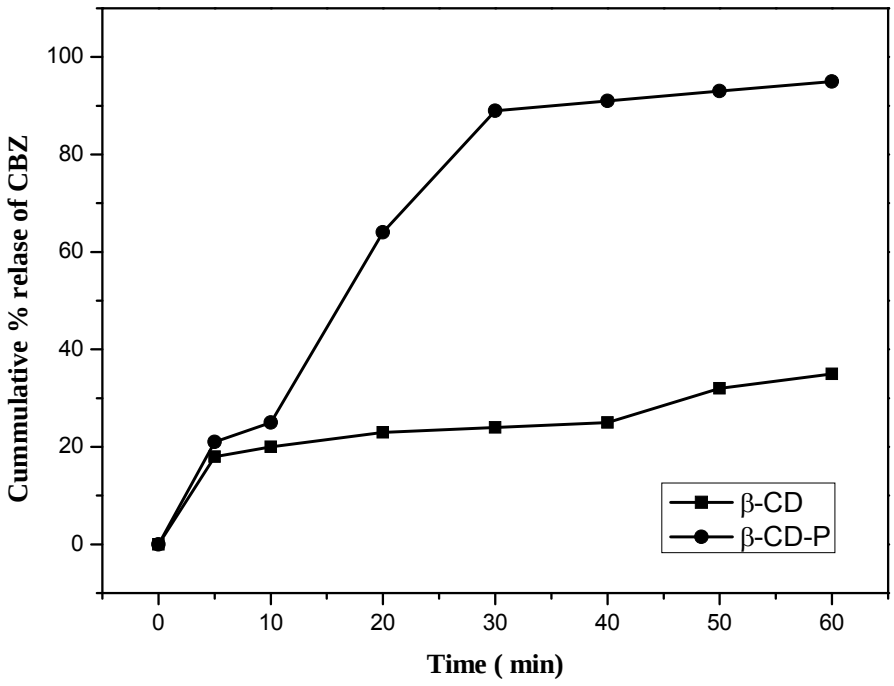


Figure 4.2.0 Dissolution curves of CBZ with β-CD and β-CD Polymer

References

1. E. Merisko-Liversidge, *Nanocrystals*: In: Marty JJ. Ed. Particles. Orlando: Marcel Dekker, **2002**.
2. M. T. Borin, L. Khwashioki, B. Idris, K. Mulaya, *Antimicrob. Agent Chemother.,* **1990**, *34*, 1094-1109.
3. H.C Neu, C. Nai-Xun, P. Labthavikul, *Antimicrob. Agent Chemother.,* **1984**, *26(2)*, 174-180.
4. W. Schindler, F. Hafliger, *Helvetica Chimica Acta*, **1954**, 37(2), 472-483.
5. M. Sitges, L.M. Chiu, V. Nekrassov, *Neurochem. Int*., **2006**, 49, 55-61.
6. K.A. Voudris, A. Attilakos, E. Katsarou, A. Drakatos, S. Dimou, S. Mastroyianni, A. Skardoutsou, A. Prassouli, A. Garoufi, *Epilepsy Res.*, **2006**, 70, 211-217.
7. Eds P. O. Anderson, *Handbook of Clinical Drug Data, 9th Ed*., Appelton & Lange, **1999**.
8. W. Liu, L. Dang, S. Black and H. Wei, *J. Chem. Eng. Data*, **2008**, 53(9), 2204-2206.
9. H. van de Waterbeemd, *Eur. J. Pharm. Sci*., **1998**, 7, 1-3.
10. H. El-Zein, L. Riad, A. A. El-Bary, *Int. J. Pharm.,* **1998**, 168, 209-220.
11. E. Chan, H.S. Lee, S. S. Hue, *Br. J. Clin. Pharmacol.*, **2001**, 51, 567-576.
12. J. H. Hu, K.P. Johnston, R. O. Williams, *Eur. J. Pharm. Sci.*, **2003**, 20, 295-303.
13. D. Douroumis, A. Fahr, *Eur. J. Pharm. Biopharm.*, **2006**, 63, 173-175.
14. H. Friedrich, B. Fussnegger, K. Kolter, R. Bodmeier, *Eur. J. Pharm. Biopharm*., **2006**, 62, 171-177.
15. J. S. Smith, R. J. MacRae and M. J. Snowden, *Eur. J. Pharm. Biopharm*., **2005**, 60, 73-80.
16. L. S. Koester, C. R. Xavier, P. Mayorga and V. L. Bassani, *Eur. J. Pharm. Biopharm*., **2003**, 55(1), 85-91.
17. A. S. Jain, A. A. Date, R. R. S. Pissurlenkar, E. C. Coutinho and M. S. Nagarsenker, *AAPS PharmScitech*., **2011**, 12(4), 1163-1175.
18. T. Loftsson, S. B. Vogensen, M. E. Brewster, F. Konraosdottir, *J. Pharm. Sci*., **2007**, 96(10), 2532-2546.