# Chapter 3

## Bis(β-Cyclodextrin) and β-Cyclodextrin polymers

### 3.1 Brief Review

Covalently linked CD dimers (linked CD dimers) have attracted attention as long guest possessing hydrophobic groups at either end tend to be complexed with one hydrophobic group in each CD of the linked CD dimer[1-5]. When two CDs are joined through a linker, x, in a dimer, $(CD)_2X$, the stability of the host-guest complex $(CD)_2X.G$, in which the guest G has two hydrophobic binding sites, is sometimes increased by 10-100 times by comparison with that of CD.G[6]. There are three main types of linked CD dimers (Figure 3.1). The CDs are generally linked at the primary faces $C^{6A}$ (head), although there are examples of dimers linked at the $C^{2A}$and $C^{3A}$ positions[7] (tail). Statistically, there are six types of linked CD dimers which involve $C^{6A}$-$C^{6A}$, $C^{6A}$-$C^{3A}$, $C^{6A}$-$C^{2A}$, $C^{3A}$-$C^{3A}$, $C^{3A}$-$C^{2A}$ and $C^{2A}$-$C^{2A}$ substitutions.

Cyclodextrin dimers tethered by the spacer (or linker) of different sizes and shapes may afford distinctly different binding abilities and molecular selectivities. Hence, diverse functional groups such as alkanedioates[8, 9], disulfides[10, 11], dipyridines[12, 13] and imidazole[14, 15] have been used as the linker between two cyclodextrin units. Unexpectedly, cyclodextrin dimers tethered with oligo (ethylenediamine) units have rarely been synthesized and therefore their molecular recognition behavior has not been extensively investigated, except for the study of Tabushi et al.[16] and a short report by Liu et al.[17]. There is an inherent advantage for the oligo(ethylenediamine) tether incorporated in bis(cyclodextrin), since the tether group can ligate to transition metal ions, thus enabling us to modify, and potentially switch the original binding ability through the metal ligation. However, cyclodextrin dimers tethered with ethylenediamine units have been synthesized but their molecular recognition behavior with $C_{60}$ is not known. The synthesis and characterization of bis(cyclodextrin) was according to procedure described in literature[18] and discussed in this chapter. The application of bis(cyclodextrin) in the aqueous solubilzation of $C_{60}$ will be discussed in the next chapter. The synthesized bis(cyclodextrin) was characterized by FTIR, $^1$H NMR and $^{13}$C NMR.

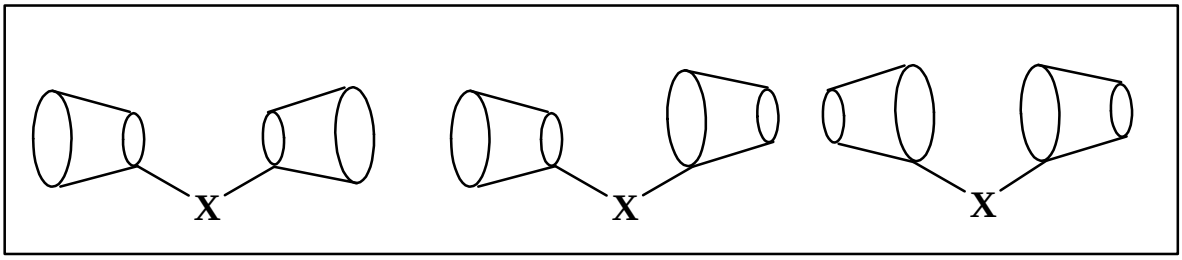



**Figure 3.1** Three main types of Linked CD dimers

In recent years, the excellent enhanced properties of cyclodextrin polymers have inspired for the developments of novel synthesis of cyclodextrin polymers and biomaterials[19, 20]. These systems have to be extensively evaluated in different field of chemistry concerning their safety and efficacy, making future novel applications of these materials in the biomedical and pharmaceutical field feasible. A cyclodextrin polymer is defined as a unit of more than three cyclodextrin molecules covalently coupled or cross-linked together to enable cooperative complexing with several guest molecules. This characteristic makes them invaluable in the field of pharmaceutical and drug delivery. The most common pharmaceutical application of CDs and their chemical derivatives are to enhance the aqueous solubility of the complex species, photo stability and bioavailability of the complex drugs and eventually to reduce side effects[21-26]. The weaker aqueous solubility of the cheapest β-cyclodextrin can limit it practical application towards drug delivery systems[27]. One of the effective approaches to improve the aqueous solubility of β-cyclodextrin is to prepare β-cyclodextrin based polymers consisting β-cyclodextrin as a part of the main-chain[28, 29]. The reported methods of synthesis of β-cyclodextrin polymers only deal with crosslinking of β-cyclodextrin with different reactants or attachment of β-cyclodextrin on the synthesized polymers[30-35]. Linear cyclodextrins based polymers have several advantages, such as higher water-solubility, inclusion ability and moderate cavity size[36, 37]. Controlling the degree of substitution and reactivity is an important phenomenon in balancing the water-solubility of the β-cyclodextrin based polymers. For this purpose, it is necessary to design the synthesis methods which have controlled the reactivity with no more crosslinking[38]. However, there still exists a need in the art for linear cyclodextrin polymers in which the cyclodextrin moiety is part of the main chain and not a pendant moiety off the main chain and a method for their preparation[39]. Cyclodextrin polymers are of interest of many researchers owing to their unique complex forming property and higher hydrophilicity. Solubilization of hydrophobic drug with biocompatibility and controlled release across a membrane and increased stability constant are some of the major applications of water-soluble cyclodextrin based polymers[40]. The first cyclodextrin polymers reported were prepared by cross-linking with epichlorohydrine[41]. A crosslinked structure is produced when cyclodextrins are reacted with bi- or polyfunctional reagents. The most widely used cross linking agents are diepoxides and diisocyanate. The major problem in polycondensation of cyclodextrin with cross-linking agent is no control over the degree of cross-linking and resulted

product has less water-solubility. There are some reports[42] in which they synthesized ethylenediamine linked β-cyclodextrin dimer by reacting 6-monotosyl β-cyclodextrin with ethylenediamine. The synthesis and charecterization of ethylenediamine linked β-cyclodextrin based polymer without cross linking was discussed in this chapter and its application in solubilization of non-polar drug i.e. cefpodoxime proxetil (CP) was discussed in the next chapter. The synthesized cyclodextrin polymers were characterized by FTIR, $^{1}$H NMR, $^{13}$C NMR, XRD, TGA and ESI-MS.

## 3.2 Experimental

### 3.2.1 Materials

β-Cyclodextrin was obtained from Signet Chemical Corporation, Mumbai as a gift sample and dried overnight under a vacuum oven at 60$^{o}$C, then used without further purification. Cyanuric chloride, urea, thiourea, ethylenediaine and DMF were purchased from Merck India. Double distilled water was used throughout the experiments. Monotosyl β-cyclodextrin and capped β-cyclodextrin were used as monomers for the syntheses of bis(β-cyclodextrin) and β-cyclodextrin polymers.

### 3.2.2 Measurements

Fourier transform infrared spectra (FT-IR) were recorded on a Shimadzu (8400s) spectrophotometer at 30 scans by KBr pellet method at $10^{-4}$ resolution. Thermogravimetric analysis (TGA) was made on a Shimadzu, TGA-50 system with a heating rate of 10$^{o}$C/min in the temperature range of 25-700$^{o}$C. X-ray powder diffraction patterns were taken on a computer-controlled RIGAKUDMAX-2200. NMR spectra were recorded on a Bruker Avance spectrometer operating at 400 MHz at room temperature. Samples were prepared in $D_2O$ containing TMS as an internal standard.

### 3.2.3 Synthesis of ethylenediamine linked β-CD dimer

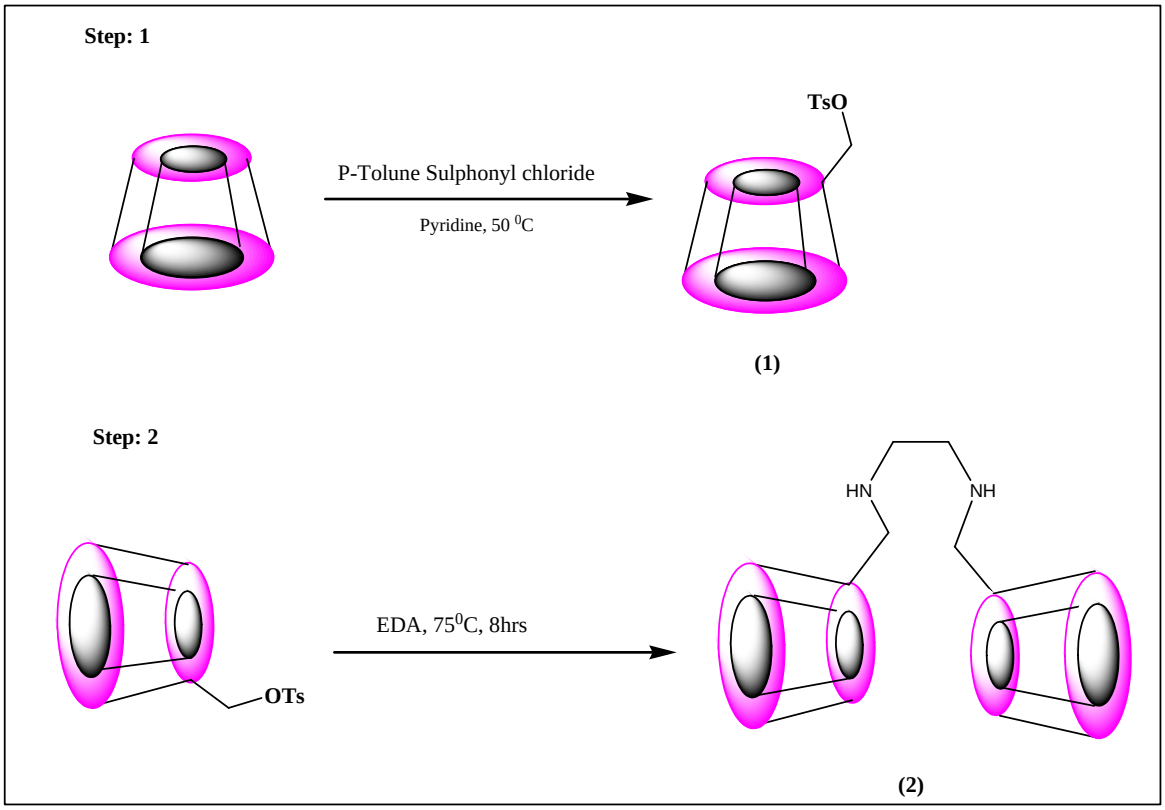
As shown in Scheme 3.1, 6-OTs-β-CD (1) was prepared by the reaction of p-tosyl chloride with β-CD in dry pyridine according to Refs. 43 and 44. Then, compound (1) was converted to ethylenediamine linked β-CD dimer (2) in 67.1% yield. Compound (1) (5.0 g) was dissolved in 50 mL dry dimethylformamide (DMF), and 0.2 mL ethylenediamine was added into the mixture. The colour of solution was yellowy. The mixture was reacted for 8h at 75$^{0}$C under nitrogen. The reaction mixture was

concentrated under reduced pressure and then poured into acetone (400 mL). The precipitates formed were collected on a glass filter and dried under reduced pressure. Compound (2) (0.97 g) was obtained after repeated recrystallization from water.

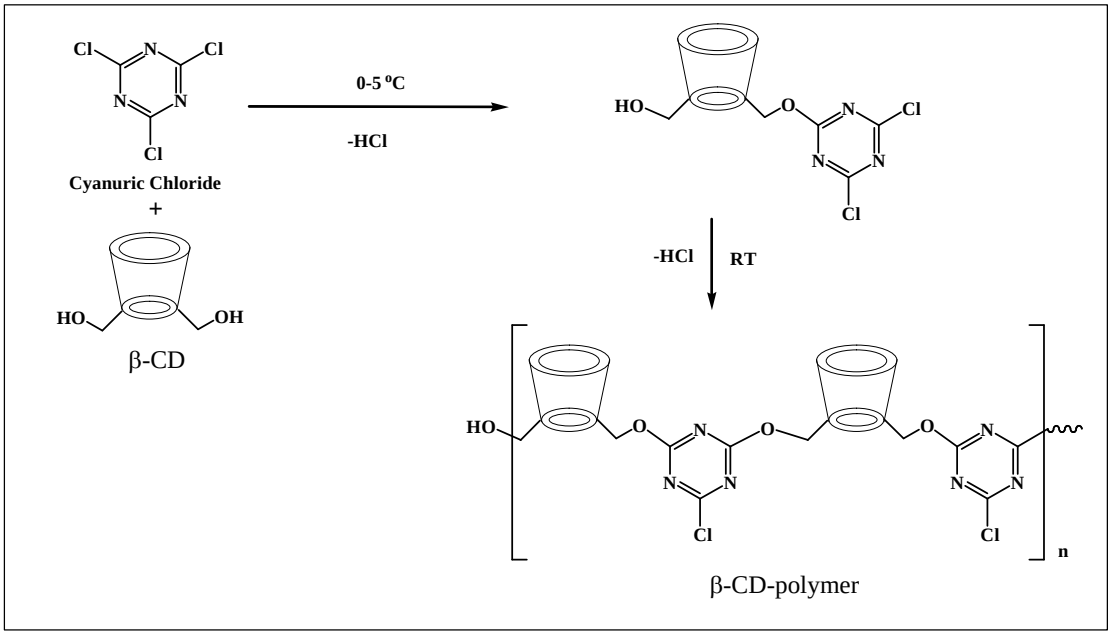
### 3.2.4 Synthesis of β-Cyclodextrin based Polymers

#### (1) Cyanuric chloride linker

β-CD and Cyanuric chloride as a linker based polymer was synthesized by one-step condensation polymerization using short linker cyanuric chloride. The reactions were shown in Scheme 3.2. Typically, a measured quantity of β-CD (1 mole) and cyanuric chloride (1 mole) were taken in different flasks and dissolved separately in the required amount of distilled water and pH was maintained using 1N NaOH solution. 10 mL of this β-CD solution of predetermined pH (>12) was taken in a round bottom flask and kept in ice bath with continuous stirring, the temperature being maintained between 0-5$^{o}$C. To this, 10 mL of the cyanuric chloride solution was added drop wise with the help of pressure equalizing funnel. During the addition, temperature was maintained between 0-5$^{o}$C. After the addition the reaction was continued with stirring, temperature was maintained between 0-5$^{o}$C for 4-5h. Stirring was continued for 12h while the contents were allowed to attain ambient temperature (30$^{o}$C). The polymerization was stopped by addition of 0.1N HCI solution. The polymers were purified in aqueous solution by membrane filtration using a polymer membrane with a molecular mass cut-off of 1000 Da. Residual unpolymerized and substituted β-CD monomers were removed in order to investigate the properties of the high molecular weight polymer fractions only. The solution obtained was directly freeze-dried to get an off-white fluffy product.




**Scheme 3.1** Synthesis of Bis(β-Cyclodextrin) or β-Cyclodextrin Dimer



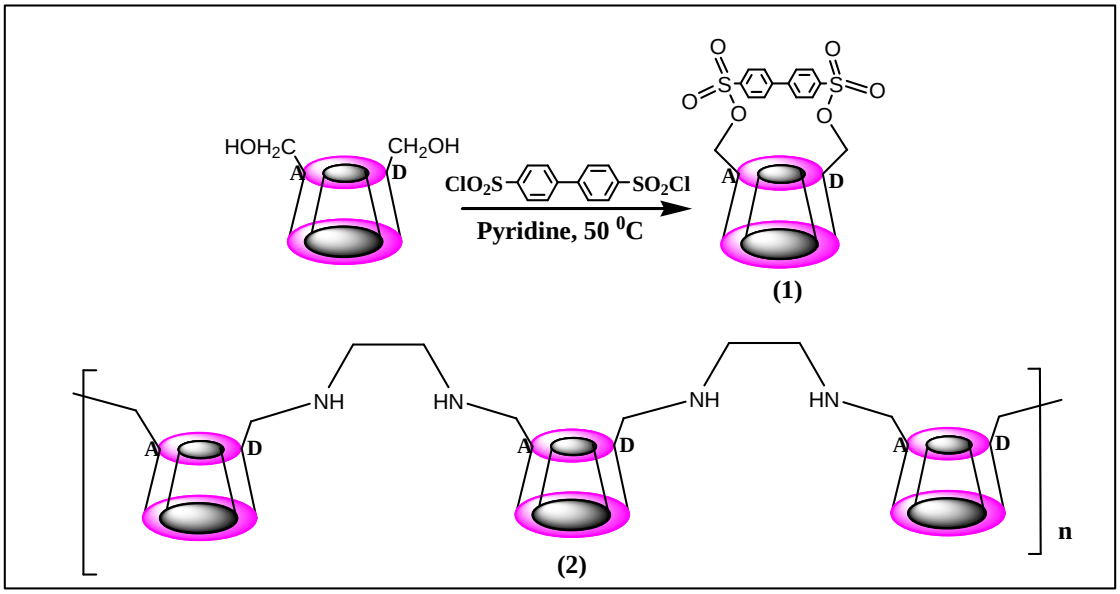

**Scheme 3.2** Synthesis of β-cyclodextrin-cyanuric chloride based polymers.

**(2) Ethylenediamine Linker**

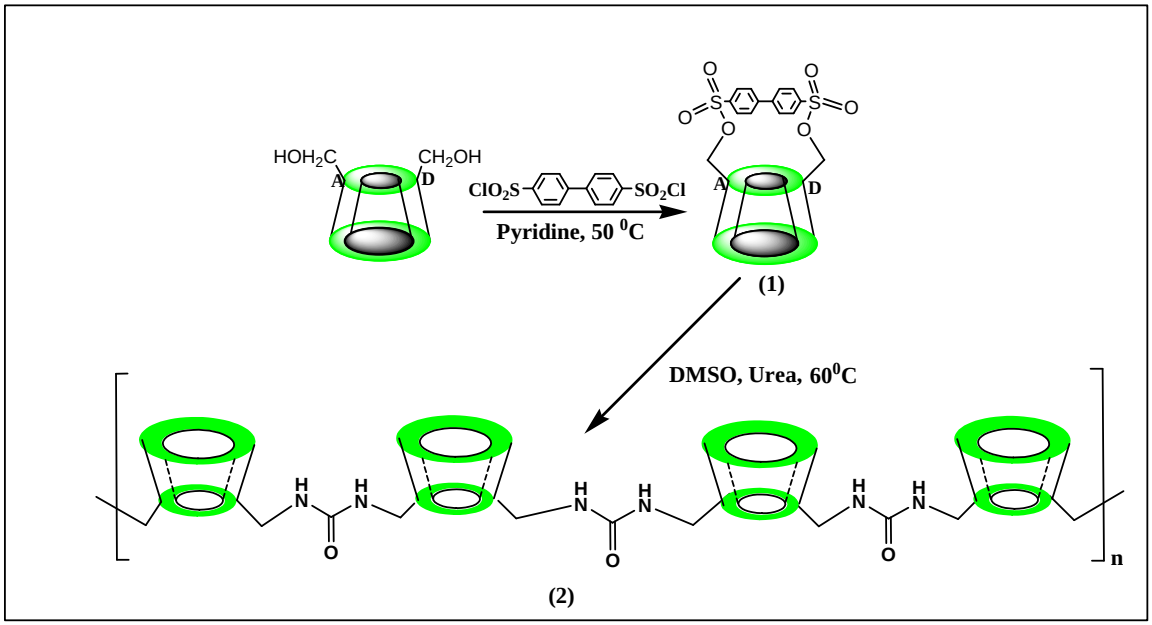
Biphenyl-4, 4'disulfonyl-A, D-capped β-cyclodextrin (2 g) (which was discussed in chapter 2B) was dissolved in 30ml dry N, N'-dimethylformamide (DMF), and 0.5mL ethylenediamine was added into the mixture. The color of the solution was yellowy. The mixture was reacted for 8 h at 80 $^0$C under nitrogen. The reaction mixture was concentrated under reduced pressure and then poured into acetone (400ml). The precipitates formed were collected on a glass filter and dried under reduced pressure. Compound (2) (0.97g) was then subjected to ultra filtration for one day over a polymer membrane with a molar mass cut-off 2Kg moL$^{-1}$ for the removal of any unreacted ethylenediamine , the byproduct of 4,4' biphenyl sulphonic acid and any capped cyclodextrin. The resulting solution was lyophilized to dryness to get slight yellow powder which is highly soluble in water. The Synthesis is shown in scheme 3.3.

**(3) Urea or Thiourea Linker**

β-Cyclodextrin based polymers were synthesized by the condensation polymerization of difunctional β-CD monomer with difunctional short linker (urea or thiourea) to give linear main-chain β-CD polymers as shown in scheme 3.4. Capped β-CD (1.355 gm, 1 mmole) was dissolved in 50 ml DMSO stirred and degassed with nitrogen atmosphere after obtained clear solution, short linker urea (0.06 gm, 1 mmole) or thiourea (0.076 gm, 1 mmole) was added. The solution was stirred under reflux for 12 hrs at 60$^0$C. There after pour the solution into 400mL acetone .The resulted concentrated solution was lyophilized to obtained solid white polymers. The polymers were dried under vacuum overnight at room temperature.




**Scheme 3.3.** Synthesis of Ethylenediamine linked β-Cyclodextrin polymer (2)




**Scheme 3.4** Reaction scheme for the synthesis of β-cyclodextrin polymer with short linker Urea.

### 3.2.5 Determination of Aqueous Solubility of Polymers

The aqueous solubility of the synthesized β-CD polymers were measured using a simple technique. 0.1 g of β-cyclodextrin polymer was added to 0.5 ml of water to ensure the solution reaching saturation. The solution was mechanically shaken for 4 h and then incubated overnight at room temperature. The solution was then filtered through a microfilter-syringe. The filtrate was dried in an oven for sufficient period until a constant weight was reached. The solubility was estimated in terms of the weight of sample in the saturated solution and solution volume. This was repeated to get constant values with in an error of ±0.05 g.

## 3.3 Results and Discussion

The β-CD-cyanuric chloride polymer was successfully synthesized under controlled temperature condition by using cyanuric chloride (2,4,6-trichloro-1,3,5-trazine) as the short linking agent between two β-cyclodextrin monomers. Cyanuric chloride can act as an excellent central linker because of all three chlorine atoms react at different temperatures region. This allows a possible synthetic methodology for forming linear highly water-soluble polymers. The first step in the synthesis of the β-cyclodextrin polymers was the preparation of dichlorotriazine sodium salt from the dispersion of cyanuric chloride in water. The second step is the condensation reaction of two chlorine groups of dichlorotriazine sodium salt with β-cyclodextrin under alkaline conditions, controlled pH (<10) and controlled temperature (<30$^{o}$C). The preparation of the dichlorotriazine sodium salt reduces the degree of substitution of the reactive groups from DS = 1.0 to 0.4 due to the precomplexation effect. This implies that there are only 2–3 reactive triazine groups per cyclodextrin molecule whose reactivity has been shown to be between 2.3–2.7. If the degree of substitution is higher, it would lead to the formation of crosslinked and hence insoluble polymers by reaction with itself. However, the synthesized polymer showed higher solubility than the pristine β-cyclodextrin even though the linker cyanuric chloride is insoluble in water. This implies that the polymers obtained were linear with very low degree of branching.

The low molecular weight ethylenediamine linked β-CD polymer was synthesized by a one pot condensation reaction. The first step was the preparation of biphenyl-4, 4’disulfonyl-A, D-capped β-cyclodextrin from the parent β-CD in pyridine. In the second and the final step of the condensation reaction is nucleophillic addition of two

amine groups of ethylenediamine on electrophillic carbons of biphenyl-4, 4'disulfonyl-A, D-capped β-cyclodextrin. By choosing ethylenediamine as a linker there is no possibility of cross-linking which was confirmed from aqueous solubility data shown in Table 3.1. The molecular weight of the polymer measured by ESI- mass is shown in Figure 3.10. The ethylenediamine linked β-CD polymer has number average molecular weight ($M_n$) is 7847. This low weight indicates that there are approximately 6-7 CD units per chain.

Similarly, β-cyclodextrin-urea or thiourea based polymers were synthesized by condensation reaction. The urea and thiourea has been effectively used as linking agent between capped β-cyclodextrin.

### 3.3.1 Aqueous Solubility of β-CD Polymers

As expected, after polymerization there is a significant enhancement of the aqueous solubility of β-CD. The solubility values for β-CD polymers at 25$^o$C are shown in Table 3.1. The water-solubility of the synthesized β-CD polymers is almost seven times higher than that of the pristine β-CD. The low water-solubility of pristine β-CD is attributed to the intramolecular hydrogen bonding between the secondary hydroxyl groups, which are unfavorable to the interaction between β-CD and surrounding water molecules. The β-CD molecule is rigid as compared to the α-CD and γ-CD the reason for this difference in water-solubility is the presence of one glucopyranose unit in distorted in the α-CD and the non-polar flexible structure of the γ-CD. The introduction of cyanucric chloride or thiourea or ethylenediamine as linker via condensation reaction disrupts this intermolecular hydrogen bonding, thus increasing the aqueous solubility even though the triazine linker insoluble in water. Since all of the β-CD polymers are highly water-soluble it implies that there was no cross linking occurring during the polycondensation reaction. The solubility data reported here are average of two reading and have an accuracy of ±0.1. However, a general observation is that the β-CD-CC polymer has a lower water-solubility as compared to the other β-CD polymers.

**Table 3.1** Aqueous Solubility and Molecular Weight of β-CD Polymers at 25°C

| **Samples** | **Aqueous solubility mg/mL** | **Solubility Relative to β-CD** |
|---|---|---|
| β-CD | 18 | 1.00 |
| β-CD-CC-P-1 | 124 | 6.88 |
| β-CD-EDA-P-2 | 136 | 7.4 |
| β-CD-Urea-P-3 | 125 | 6.97 |
| β-CD-Thiourea-P-4 | 127 | 7.10 |

### 3.4 Characterization of Bis(β-Cyclodextrin)

#### 3.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the β-CD-OTs and Bis(β-CD) are shown in Figure 3.2. The FTIR spectrum (Figure 3.2 a) of β-CD-OTs showed the typical characteristic absorption bands at 3331 $cm^{-1}$ (-OH stretching hydrogen bonded), 2930 $cm^{-1}$ (C-H stretching), 1641 $cm^{-1}$ (OH bending), 1365 (OH deformation), 1168 $cm^{-1}$ (C-O-C stretching and OH bending), 1080 $cm^{-1}$ 1032 $cm^{-1}$ for (C-O-C stretching) and 1176$cm^{-1}$ for ($SO_2$ stretching). The FTIR spectra of the Bis(β-CD) (Figure 3.2 b ) showed all the typical characteristic absorption bands of β-CD and also disappearance of 1176 $cm^{-1}$absorption band arising due to $SO_2$ which suggests that elimination of tosyl group from tosylated CD and formation of new bond with ethylenediamine between two β-CD moieties.

#### 3.4.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

In this reaction, the tosyl group is displaced through nucleophilic substitution by the ethylenediamine group. The complete disappearance of the tosyl group in the aromatic region of both the $^{1}H$ (7.76 ppm and 7.44 ppm Figure 3.3.a) and $^{13}C$ NMR (128 to 145 ppm) spectra provided evidence for the attachment of ethylenediamine on CD moiety. Other important peaks observed from the $^{1}H$ NMR spectrum of Bis(β-CD) was shown in Figure 3.3b and chemical shift values as follows: clusters of proton signals can be observed between 3 to 5.5 ppm of β-cyclodextrin's glucose units protons {4.95 ppm (br S, 7H anomeric proton), 3.3-4.7 ppm (54 H, H-3, H-5, H-6, H-2, and H-4)} and a new singlet at 2.9 ppm assigned to the methylene protons of the ethylenediamine group. The N-H signals are thought to be embedded in the multiplet featuring in the region 3.72-3.45 ppm.

The $^{13}$CNMR data in Figure 3.4 reveals that there is one additional peak at 37.04 ppm which was introduced during the synthesis of compound Bis(β-CD). The signal was assigned to the NH-C of the ethylenediamine group. The resonances of C-1 to C-5 were not greatly affected and same as parent β-CD. However, the resonance of the substituted carbon (C-6') at 31.51 ppm was greatly shifted. This fact constituted evidence that the substitution occurred on the primary hydroxyls of the CD macrocycle.

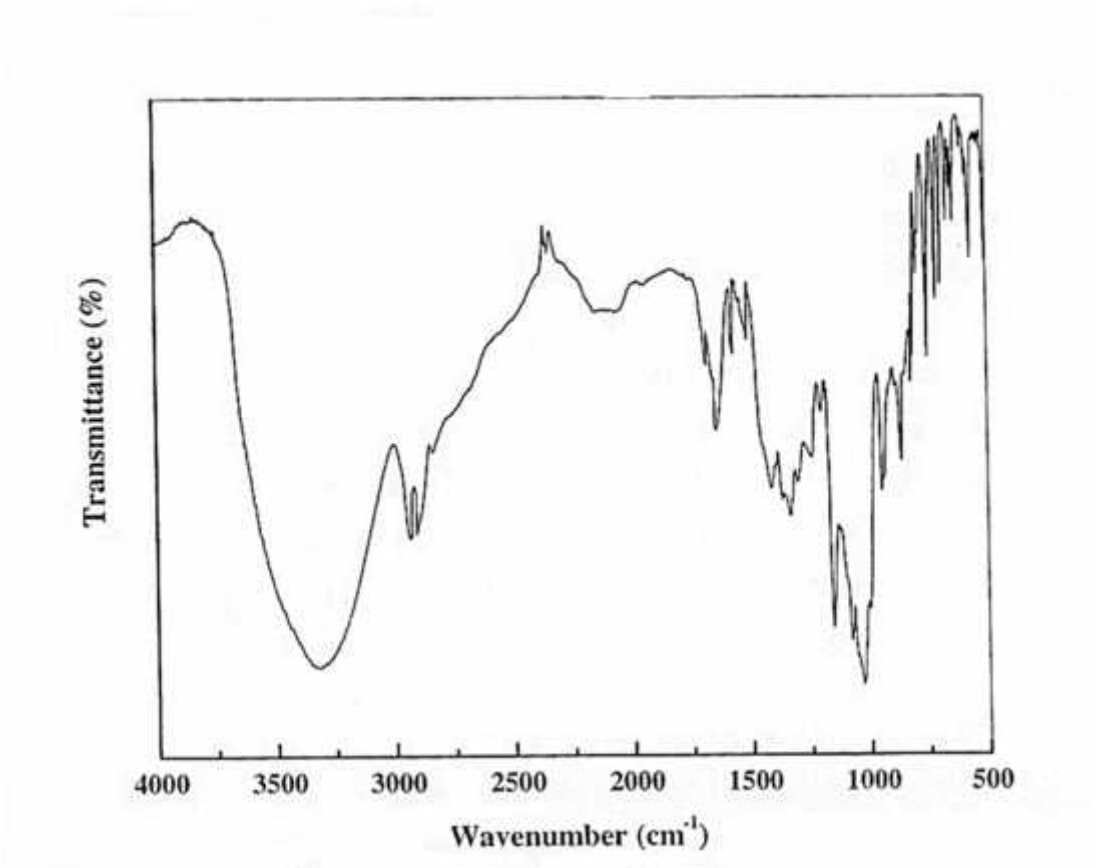



**Figure 3.2** a) FTIR spectra of the β-CD-OTs

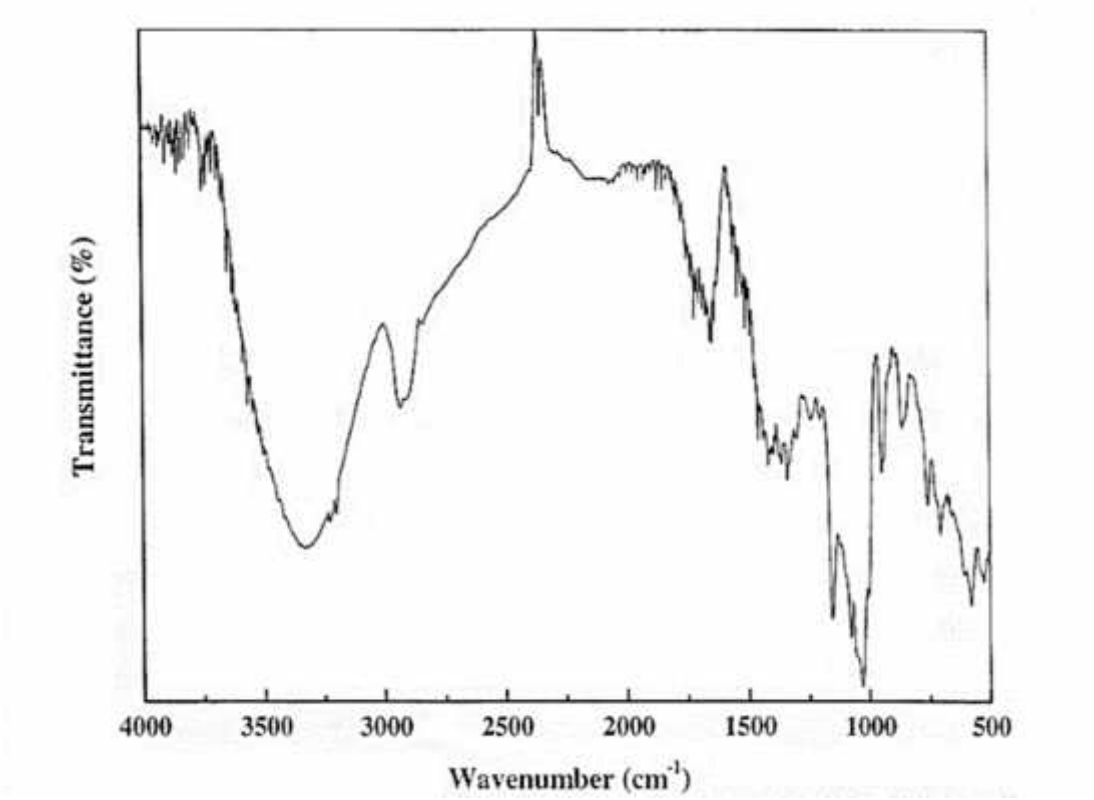



**Figure 3.2** (b) FTIR spectra of the β-CD-Dimer

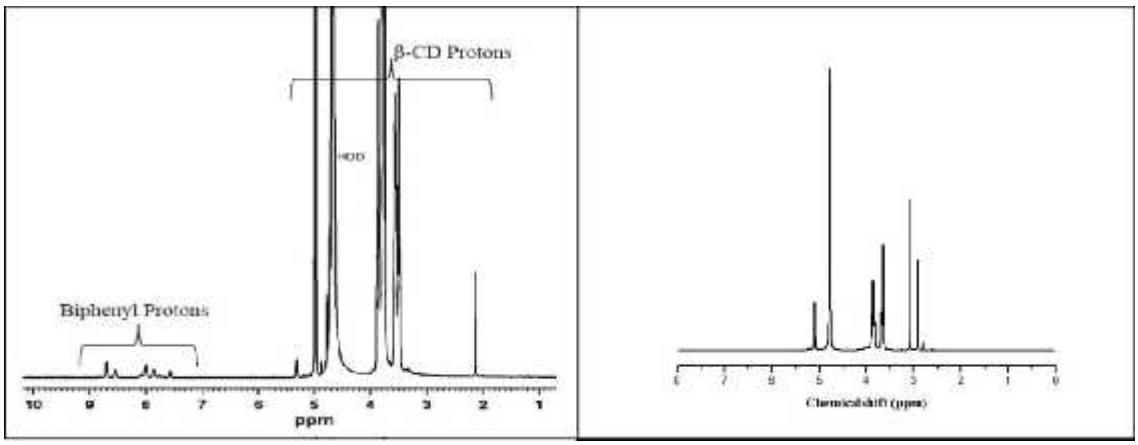



**Figure 3.3** $^{1}H$ NMR spectra of (a) Capped β-CD, (b) Bis (β-CD) in $D_2O$

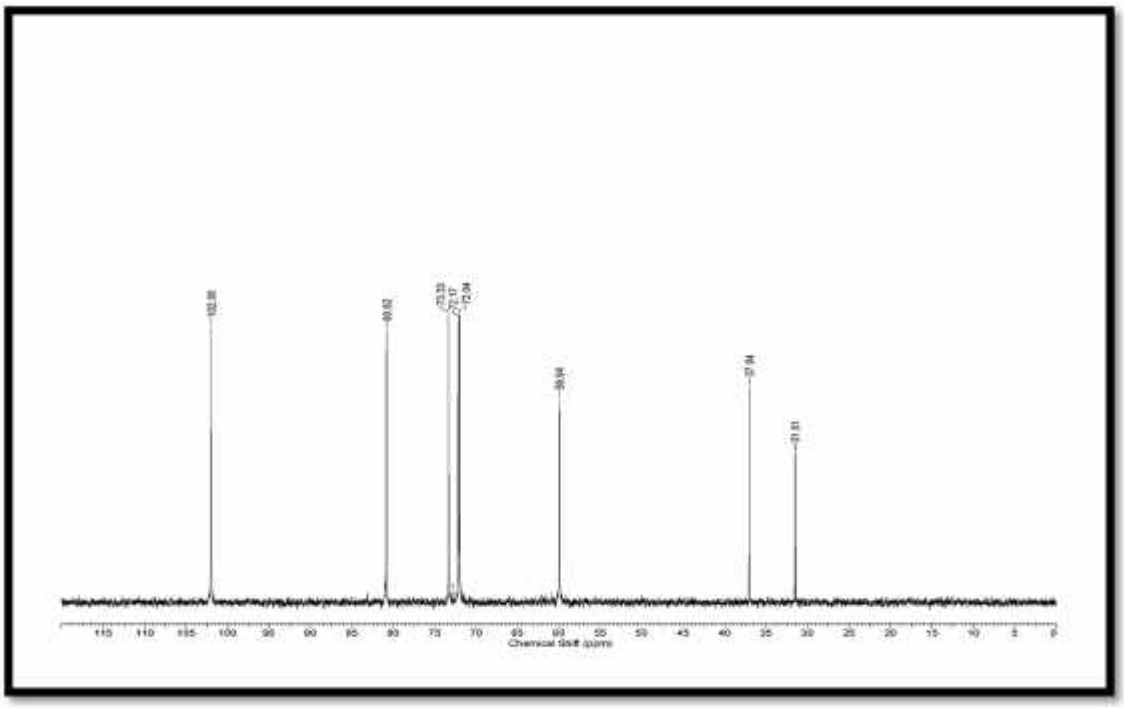



**Figure 3.4** $^{13}C$ NMR spectra of Bis(β-CD) in $D_2O$

## 3.5 Characterization of β-CD-CC and β-CD-EDA Polymers

### 3.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the β-CD, β-CD-CC and β-CD-EDA polymers are shown in Figure 3.5. The FTIR spectrum (Figure 3.5 a) of pristine β-CD showed the typical characteristic absorption bands at 3331 $cm^{-1}$ (-OH stretching hydrogen bonded), 2930 $cm^{-1}$ (C-H stretching), 1641 $cm^{-1}$ (OH bending), 1365 (OH deformation), 1168 $cm^{-1}$ (C-O-C stretching and OH bending), 1080 $cm^{-1}$ and 1032 $cm^{-1}$ for (C-O-C stretching). The FTIR spectra of the β-CD-CC polymer (Figure 3.5 b ) showed all the typical characteristic absorption bands of β-CD and also absorption bands arising due to the linker cyanuric chloride at 1724 $cm^{-1}$ assigned to the -C=N stretching vibration of the cyanuric chloride which is absence in the spectrum of the pristine β-CD. This suggests that triazine is now part of the polymer chain. The FTIR spectra of the β-CD-EDA polymer (Figure 3.5c) showed all the typical characteristic absorption bands of β-CD and the bands arising due to the linker ethylenediamine were merged with absorption bands of –OH groups.

### 3.5.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

The chemical composition of the β-CD polymers has been determined by $^{1}H$ NMR spectroscopy. The $^{1}H$ NMR spectra of the β-CD, β-CD-CC polymer and β-CD-EDA polymer are shown in Figure 3.6. The $^{1}H$ NMR spectral resonance signals of β-CD and β-CD polymer look similar due to similar structure. The pristine β-CD (Figure 3.6 a) showed the proton signals at 4.92 δppm (s, 7H, $C^{1}H$), 3.81 δppm (t, 7H, $C^{3}H$), 3.72 δppm (m, 14H, $C^{6}H$, $C^{5}H$), 3.51 δppm (d, 7H, $C^{2}H$) and 3.43 δppm (m, 7H, $C^{4}H$). The $^{1}H$ NMR spectra of β-CD-CC polymer (Figure 3.6 b ) showed all the typical proton signals of parent β-CD, except some up filed shift of proton signals and a shoulder signal at 3.30 δppm. This shoulder peak was observed in the spectra of polymer may be due to the presence of linker between β-CDs which afford the changes in the chemical environment of β-CD after polymerization. The contribution of β-CD and cyanuric chloride is calculated from integrating the anomeric proton of β-CD (4.92 δppm) and the combine signals of the remaining protons of β-CD.

In case of EDA based polymer, one extra zproton signal arising at 3.60 δppm was depicted to the $–CH_2$-$CH_2$- linkage of the EDA, which is absence in the spectra of β-CD and β-CD-CC polymer.

The $^{13}C$ NMR spectra of the β-CD, β-CD-CC polymer and β-CD-EDA polymer are shown in Figure 3.7. The $^{13}C$ NMR spectrum of pristine β-CD (Figure 3.7 a) showed the typical glucose unit's carbon chemical shifts at 101.2 δppm (C-1), 81.1 δppm (C-4), 73.1 δppm (C-3), 72.0 δppm (C-5), 71.8 δppm (C-2) and 60.3 δppm (C-6). The $^{13}C$ NMR spectra of β-CD-CC polymer and β-CD-EDA polymer (Figure 3.7b and 3.7c) showed all these typical carbon chemical shifts of pristine β-CD and also some extra carbon chemical shifts at 154 δppm (C=N), 71.6 δppm (C-Cl) and 60.1 δppm (C-$CH_2$-) arising from linker cyanuric chloride and EDA. This result confirmed the incorporation of linker in to the main-chain of the polymers.

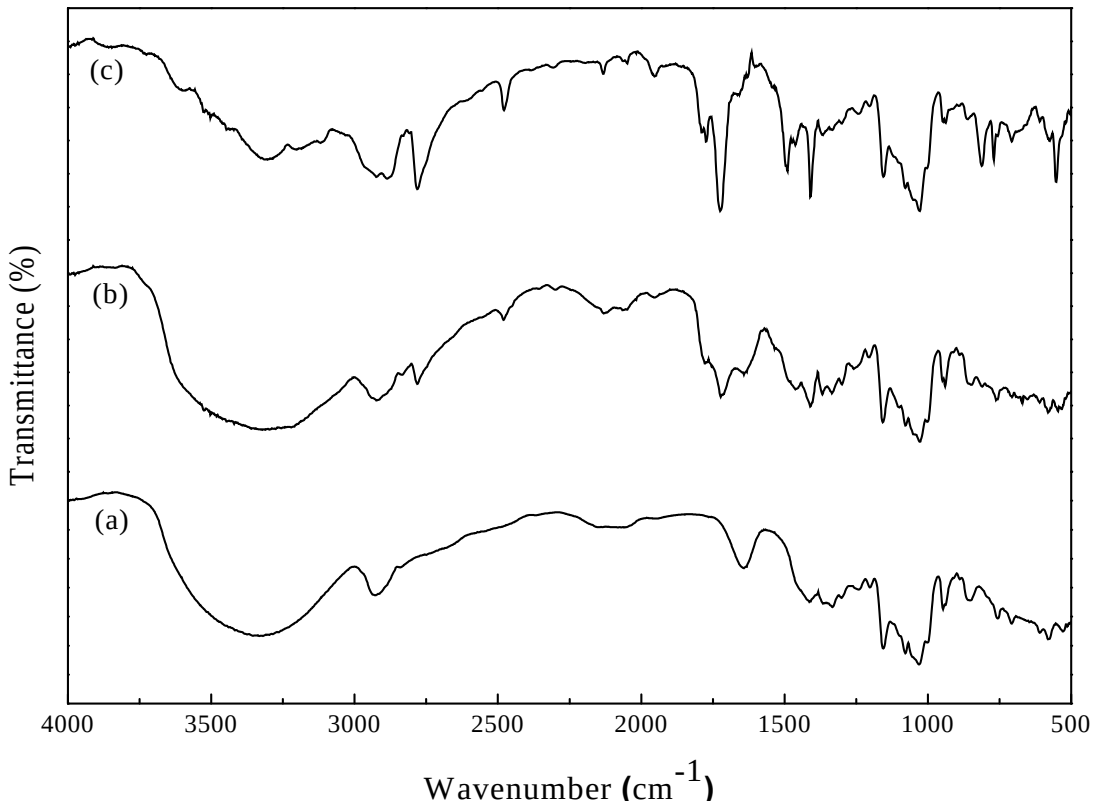



**Figure 3.5** FTIR spectra of (a) β-CD, (b) β-CD-CC polymer and (c) β-CD-EDA polymer.

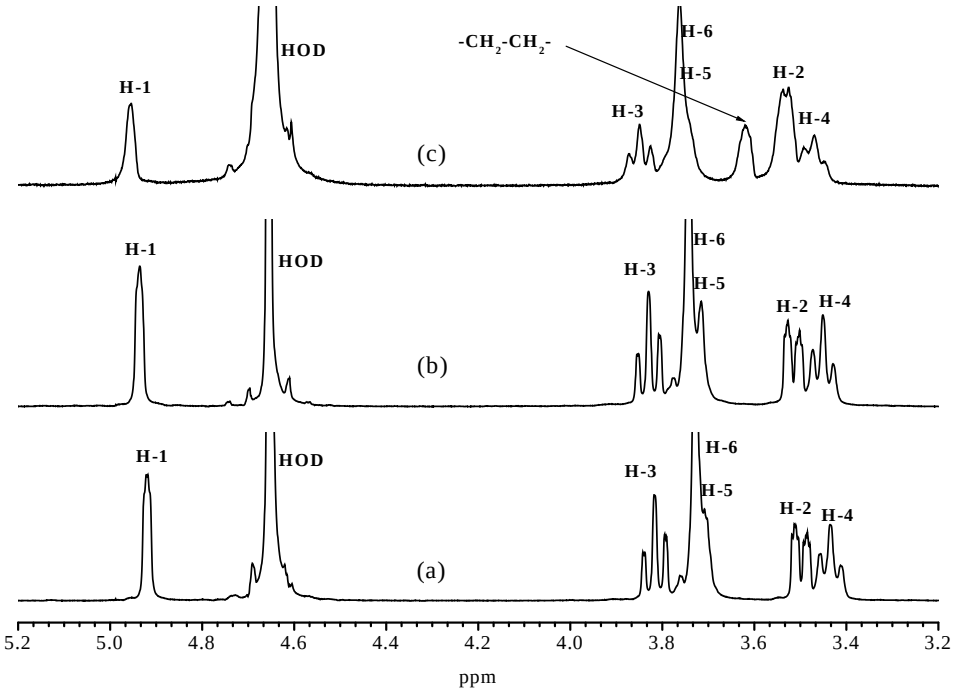



**Figure 3.6** $^{1}H$ NMR spectra of (a) β-CD, (b) β-CD-CC polymer and (c) β-CD-EDA polymer in $D_2O$.

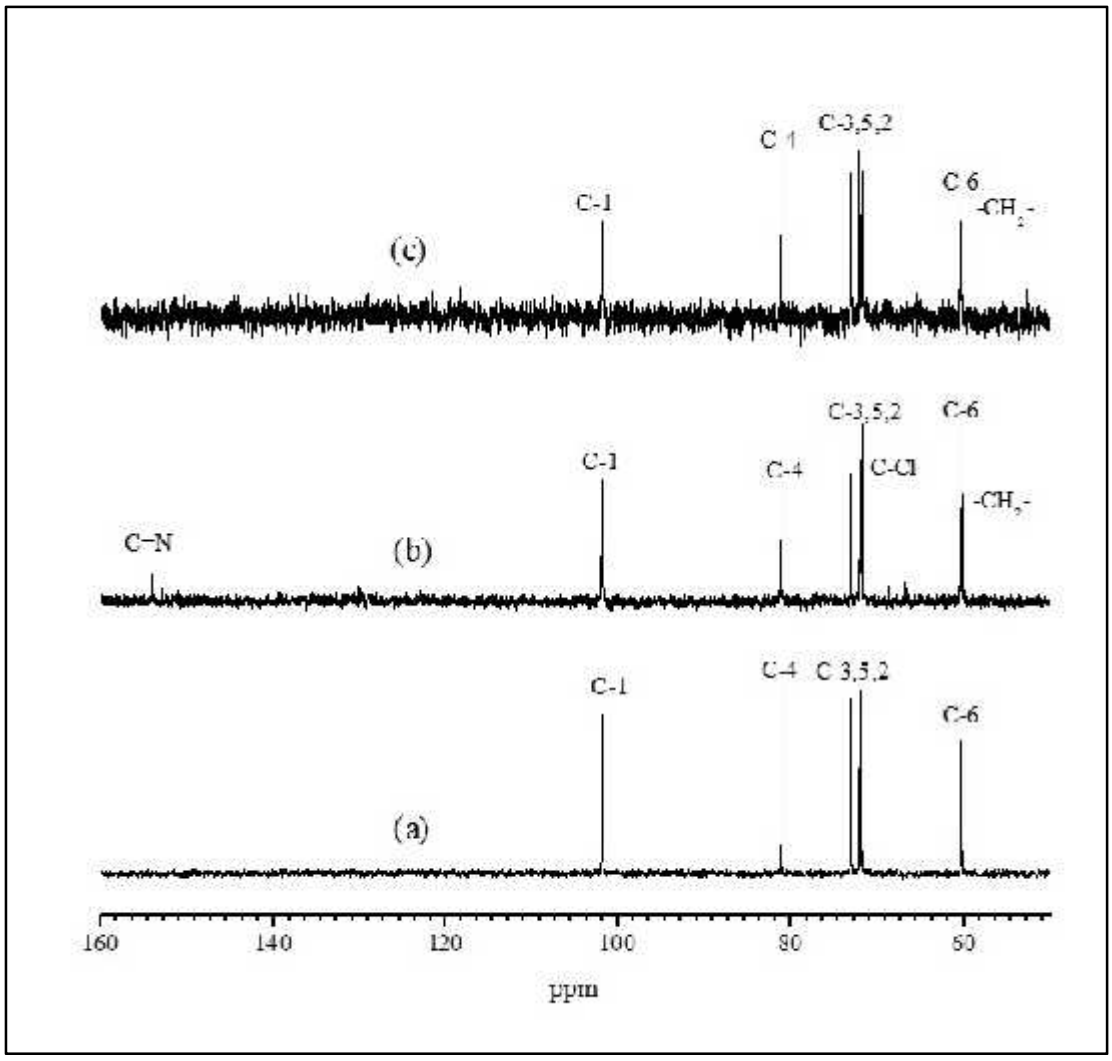



**Figure 3.7** $^{13}C$ NMR spectra of (a) β-CD, (b) β-CD-CC polymer and (c) β-CD-EDA polymer in $D_2O$.

### 3.5.3 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis gives the thermal stability of the polymers and also gives an idea about the presence of different structural units through their degradation pattern. The thermal analysis of the β-CD, β-CD-CC polymer and β-CD-EDA polymer are shown in Figure 3.8. The degradation pattern showed weight loss at three different temperature regions. The first weight loss at 106$^{o}$C was due to the loss of moisture, the second weight loss at 300$^{o}$C as dehydration of β-CD, and third weight loss at 367$^{o}$C as a decomposition of glucose in β-CD. However, in case of the β-CD-CC polymer and β-CD-EDA polymer, the thermogram was different; the first weight loss at around the same temperature due to the loss of moisture but second weight loss was at a lower temperature due to the formation of polymers of the β-CD unit with the resulting loss of the crystalline nature of the β-cyclodextrin molecule. Subsequent loss occurs due to the decomposition of the glucose units present in β-cyclodextrin, triazine linker and ethylenediamine linker.

### 3.5.4 X-ray Diffraction (XRD)

The X-ray diffraction pattern of the β-CD, β-CD-CC polymer and β-CD-EDA polymer are shown in Figure (3.9). The X-ray diffraction pattern of the synthesized β-CD-CC polymer and β-CD-EDA polymer (Figure 3.9b and 3.9c) showed that the polymers do not have typical 2θ values of the pristine β-CD. It can be seen that the synthesized polymers have a different structure than that of the pristine β-CD (2θ = 9, 12.5, 19.6, 23.0, 27.0, and 34.88) with the total suppression of the crystalline nature of the pristine β-CD. These XRD data showed that the β-CD was modified due to the condensation reaction with linker's cyanuric chloride and ethylenediamine and converted to amorphous polymers.

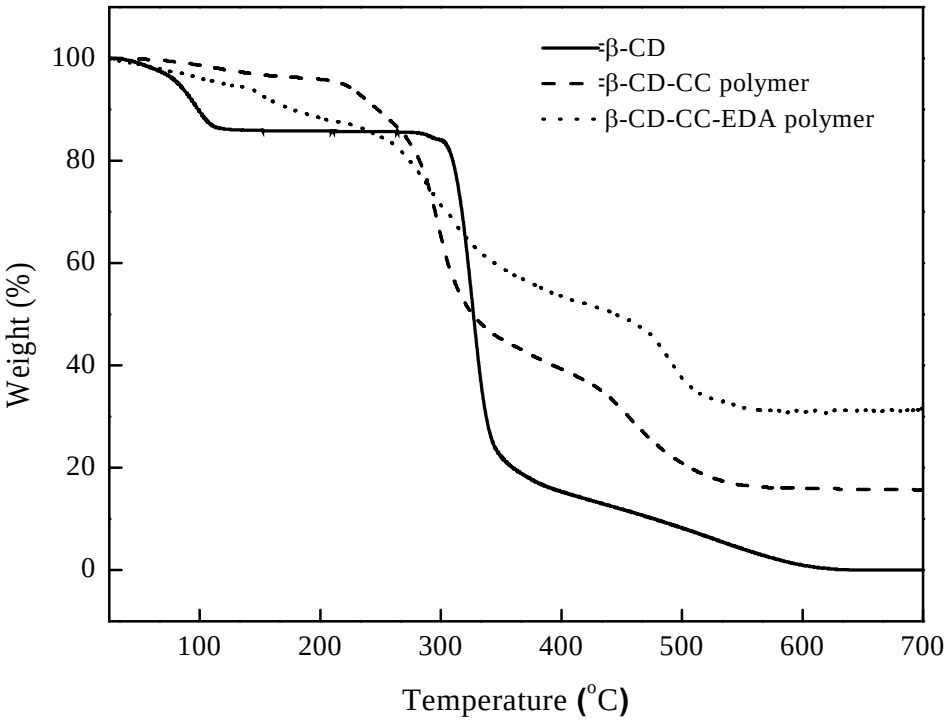



**Figure 3.8** TGA curves of β-CD, β-CD-CC polymer and β-CD-EDA polymer.

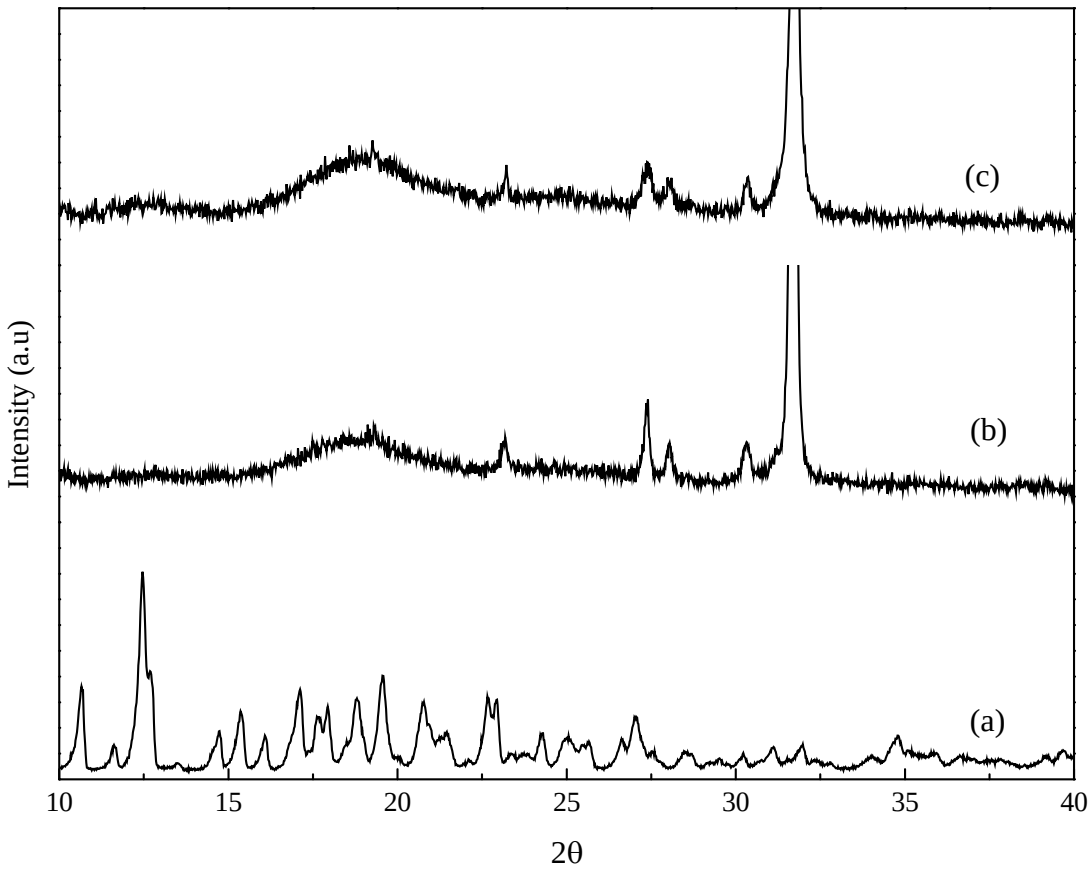



**Figure 3.9** XRD patterns of (a) β-CD (b) β-CD-CC polymer and (c) β-CD-EDA polymer.

### 3.5.5 Electron Ionization (ESI) mass spectroscopy

Electrospray ionization ESI permits measurement of much larger molecular weights by virtue of the fact that it produces charged ions. Since mass spectrometer normally measure mass to charge ratio, increasing the number of charges on the ion effectively multiplies the mass range of the instrument. Mass measurement accuracy of the order 0.01% is possible with this technique. In the analysis, the unknown sample is introduced into an ion source in a liquid stream containing an organic modifier and an acid delivered from a pump driven microliter syringe. Different types of acids are used in ESI. Typically the solvent used is 50% methanol in water containing 5% acetic acid. The electrospray ionization gives multiple peaks for each natural and synthetic bio-polymers. Generally this is a preferred method for the determining the mass of proteins, oligonucleotides and bio-polymers.

The ESI-mass spectra of the synthesized cyclodextrin polymers (β-CD-CC polymer and β-CD-EDA polymer) are shown in Figure 3.10a and 3.10b. The molecular weight results of the synthesized polymers indicates that almost 4 to 5 parent cyclodextrin molecules are attached by the condensation polymerization process.

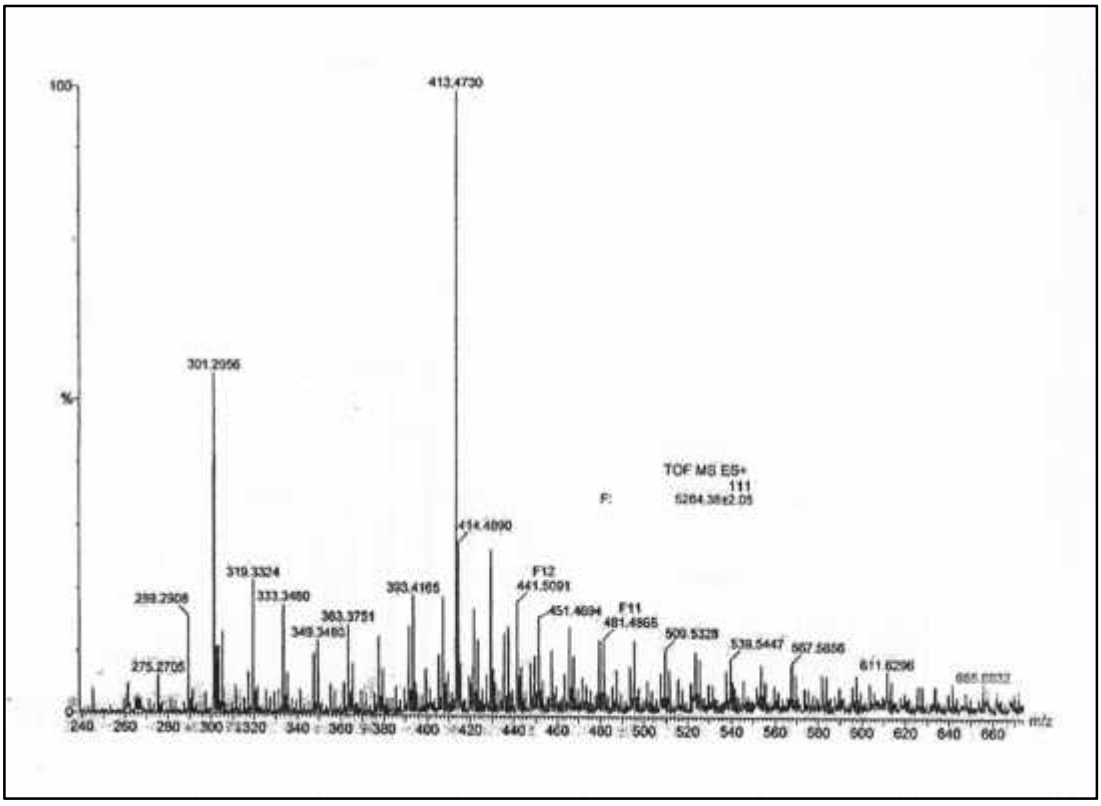



**Figure 3.10 (a)** ESI-mass of β-CD-CC polymer

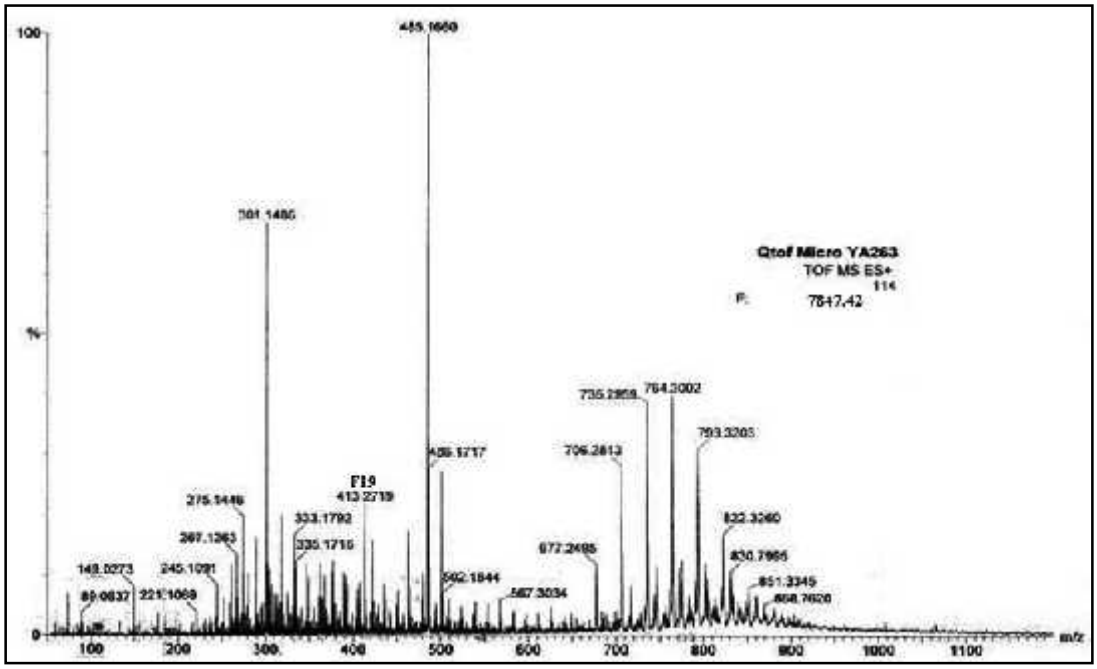



**Figure 3.10 (b)** ESI-mass of β-CD-EDA polymer

## 3.6 Characterization of β-CD-Urea polymers

### 3.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of Capped β-CD, Urea and β-CD-Urea polymer are presented in Figure 3.11. The FTIR spectral data (Figure 3.11c) clearly showed an evidence of polycondensation between Capped β-CD and Urea with the appearance of a new absorption band ascribed to the secondary amide (-NH-C=O) stretching vibration (1632 $cm^{-1}$) arising near to the primary amide (-$NH_2$-C=O) stretching vibration (1668 $cm^{-1}$) which confirms condensation of capped β-CD and Urea.

### 3.6.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

Further corroboration of condensation reaction between selectively difuctionalized β-CD and short linker urea comes from the NMR analysis. The $^{1}H$ NMR spectra of capped β-CD and β-CD-Urea polymer are shown in Figure 3.12a and Figure 3.12b, respectively. The $^{1}H$ NMR spectra of capped β-CD and β-CD-Urea polymer was totally different due to the different structural features. One new proton signal was observed at 5.94 δppm for the β-CD-Urea polymer correspond to the proton of urea linker. The disappearance of the signals of the aromatic protons confirms the attachment of urea linker on to the polymer chain. Similarly $^{13}C$ NMR spectra of the capped β-CD and β-CD-Urea polymer in Figure 3.13(a) and 3.13(b) shows all the carbon chemical shifts of the modified β-CD along with one extra carbon chemical shift at 162.6 δppm carbonyl (-C=O) carbon due to the incorporation of urea linker in to the main-chain of the polymer with β-CD and disappearance of aromatic carbon chemical shifts of Capped β-CD.

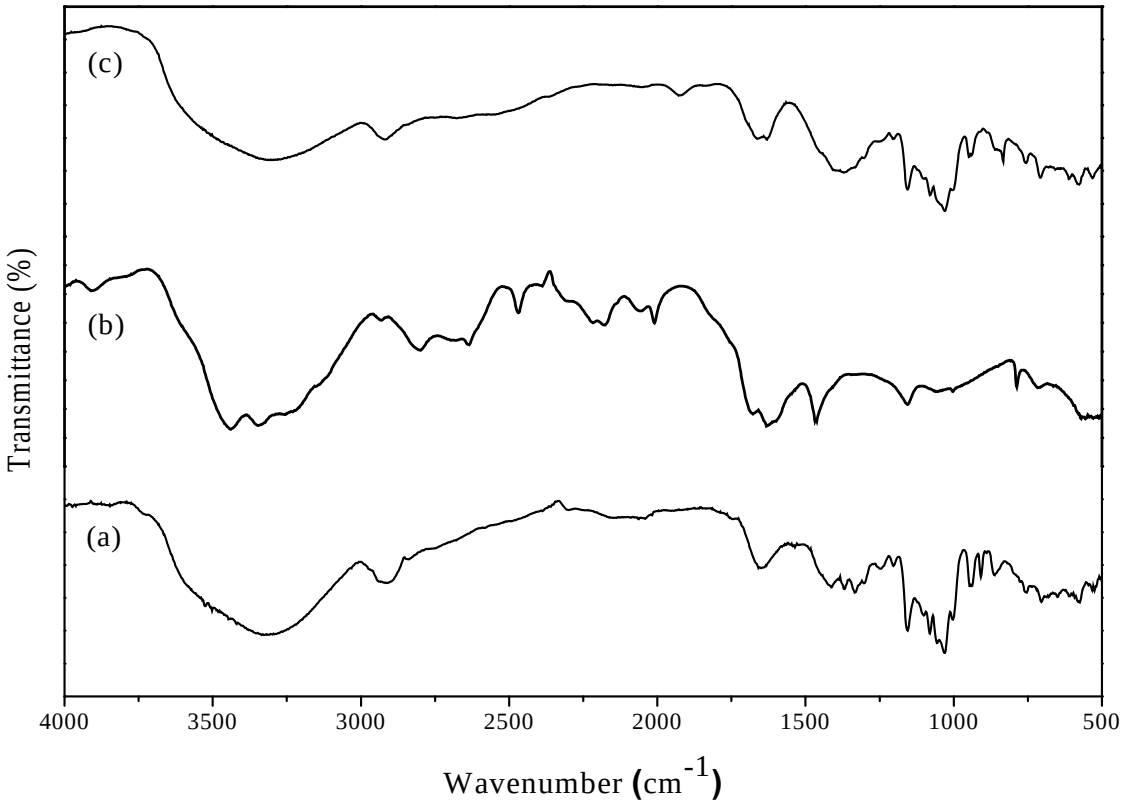



**Figure 3.11** FTIR spectra of (a) Capped β-CD, (b) Urea and (c) β-CD-Urea polymer.

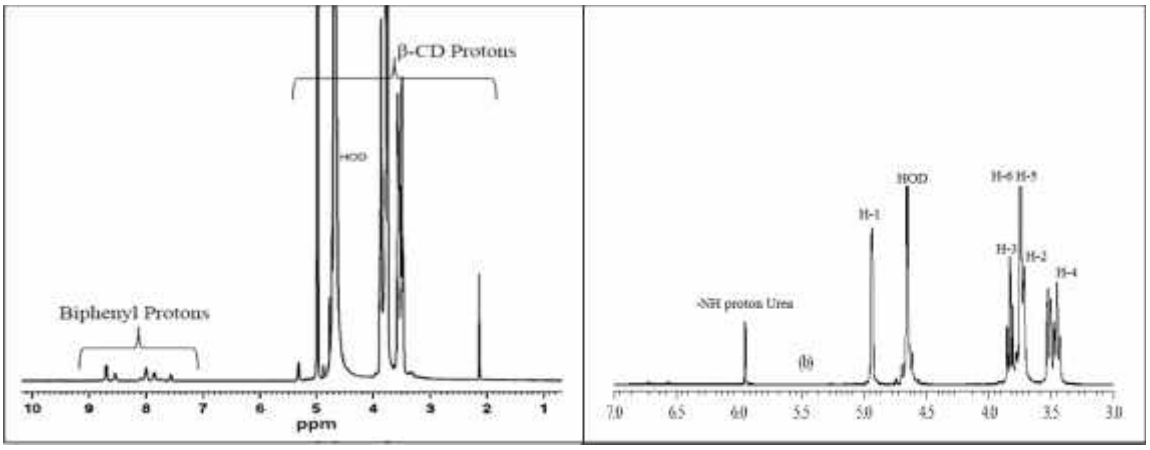



**Figure 3.12** $^{1}$H NMR spectra of (a) Capped β-CD and (b) β-CD-Urea polymer in $D_2O$.

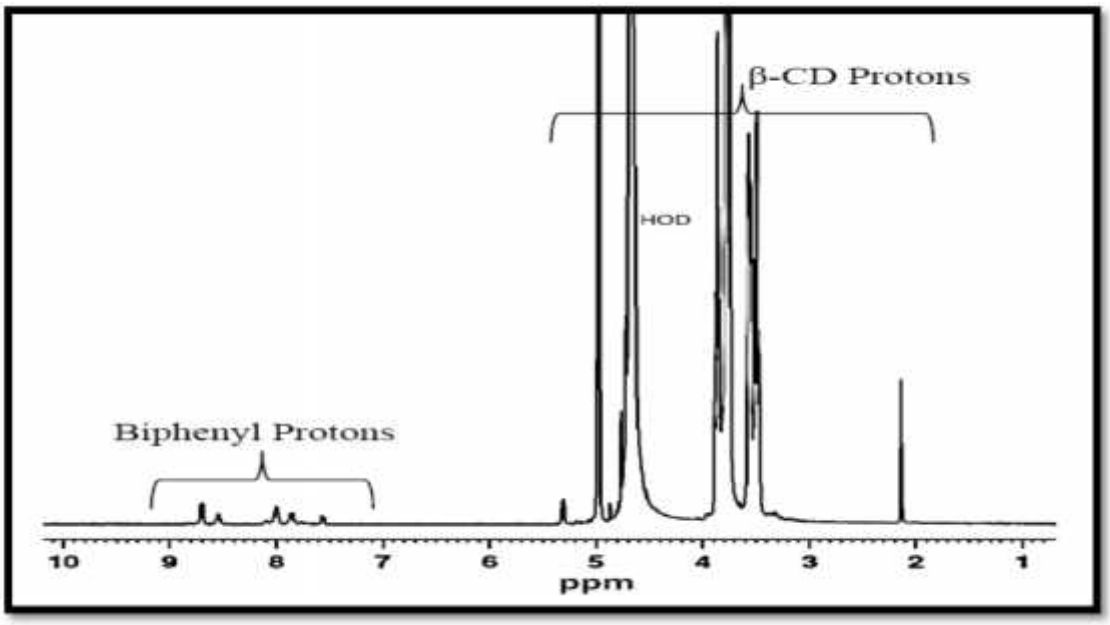



**Figure 3.13 (a)** $^{13}C$ NMR spectra of Capped β-CD

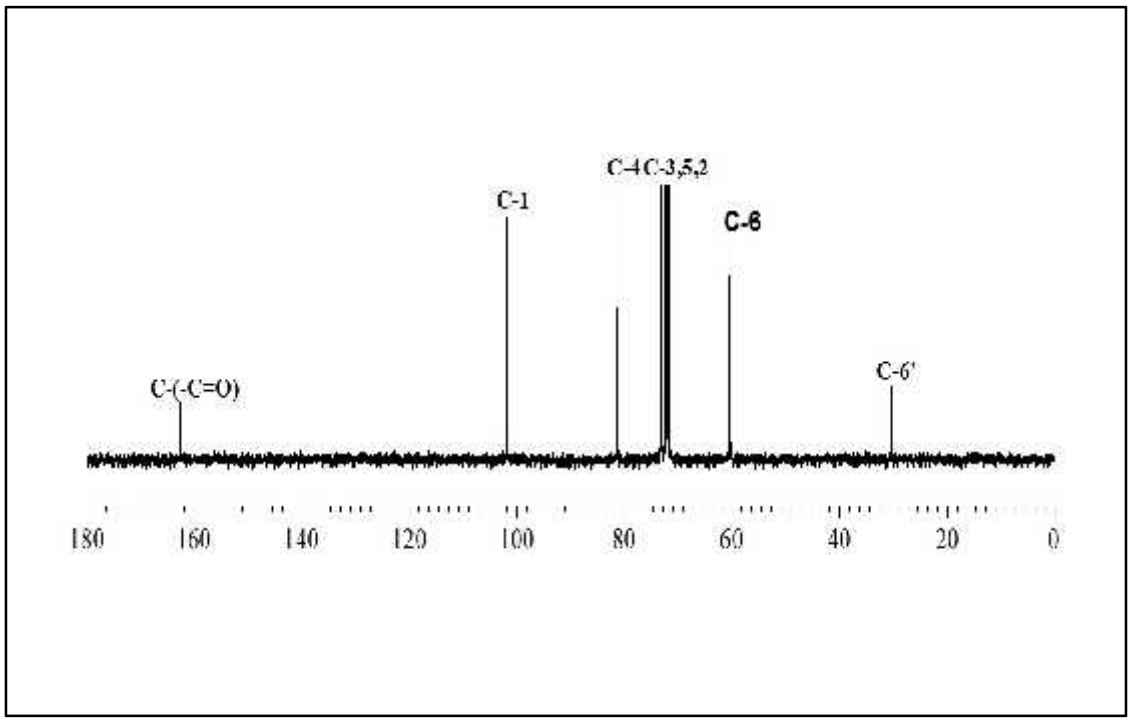



**Figure 3.13 (b)** $^{13}C$ NMR spectra of β-CD-Urea polymer in $D_2O$.

### 3.6.3 Thermogravimetric Analysis (TGA)

The thermal analysis of β-CD and β-CD urea polymer are shown in Figure 3.14. The degradation pattern showed weight loss at three different temperature regions. The first weight loss at 106$^{o}$C was observed due to the loss of moisture and structural water molecules, the second mass loss at 285$^{o}$C as dehydration of β-CD, and third mass loss at 335$^{o}$C as a decomposition of glucose in β-CD. However, in case of β-CD polymer the thermogram was different; the first weight loss at around the same temperature due to the loss of moisture and structural water molecules but second weight loss was at a lower temperature due to the formation of polymer of the β-CD unit with the resulting loss of the crystalline nature of the cyclodextrin molecule. Subsequent weight loss occurs due to the decomposition of the glucose and urea linker after that β-CD polymer is quite stable above 500$^{o}$C.

### 3.6.4 X-ray Diffraction (XRD)

The X-ray diffraction patterns of Capped β-CD and β-CD-Urea polymer are shown in Figure 3.15 (a) and (b). The synthesized β-cyclodextrin-polymer (Figure 3.15 b) does not have typical 2θ values of the Capped β-CD. It can be seen that the synthesized polymer have a different structure than that of the Capped β-CD (2θ = 9, 12.5, 19.6, 23.0, 27.0, and 34.88) (Figure 3.15 a) with the total suppression of the crystalline nature of the β-CD. The XRD pattern of the β-CD-Urea polymer showed that broadening of peak is due to the condensation reaction and converted to an amorphous polymer.

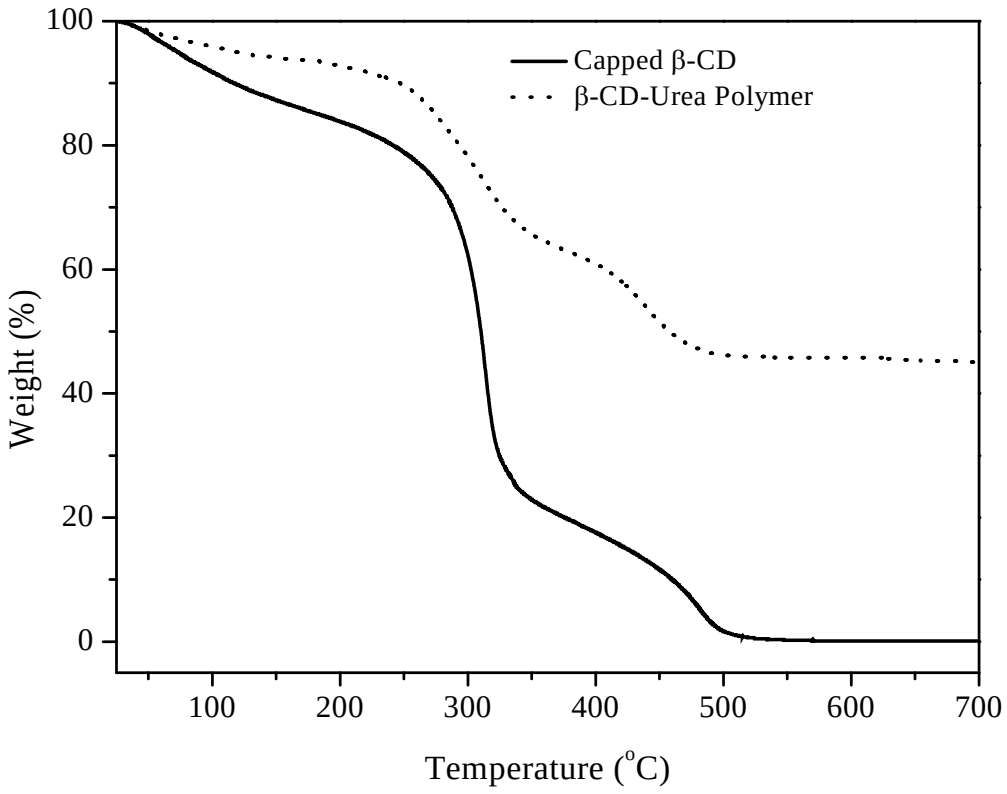



**Figure 3.14** TGA curves of Capped β-CD and β-CD-Urea polymer.

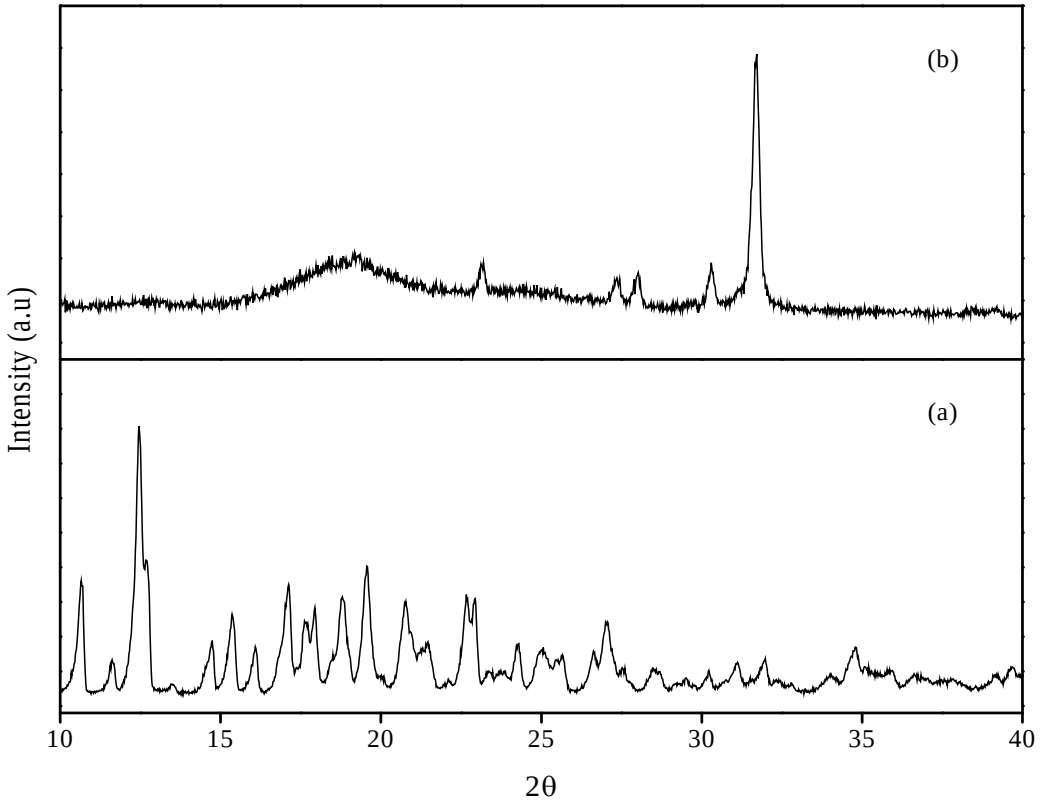



**Figure 3.15** XRD curves of (a) Capped β-CD and (b) β-CD-Urea polymer.

## 3.7 Conclusions

Linear highly water-soluble main-chain β-CD-CC and β-CD-EDA based polymers, with hydroxyl functional groups have been synthesized by using short linkers cyanuric chloride (CC) and ethylenediamine (EDA) between two β-CDs by condensation polymerization procedure respectively. Cyanuric chloride used as a short linker between two β-CDs, owing to its temperature dependent reactivity of all the three chlorine atoms, which controlled the cross-linking and formation of insoluble polymers and in the case of ethylenediamine the main advantage is elimination of cross-linking by taking advantage of ethylenediamine as a linker. Similarly, using condensation polymerization procedure linear polymers of β-CD-urea or thiourea were also synthesized by the nucleophillic addition on electrophilic carbons of capped β-CD with urea or thiourea as a linker. The CD polymers are highly water-soluble and biocompatible materials.

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