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Synthesis and Characterization of Linear Water-soluble γ-Cyclodextrin based Polymers as Drug Carrier Systems

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A series of new linear water-soluble homo and copolymers of γ-cyclodextrin are reported. These water-soluble polymers were synthesized from γ-cyclodextrin (γ-CD) and triazine through a single pot condensation polymerization procedure and the synthetic parameters optimized. Lactose and maltose based γ-cyclodextrin copolymers were also prepared. The physicochemical properties of these synthesized polymers were characterized by FT-IR spectroscopy, XRD analysis, thermogravimetry analysis (TGA) and aqueous solubility determination. The formation of a 1:1 efavirenz (an anti HIV drug)/γ-CD polymer inclusion complex was confirmed from FT-IR and UV–VIS spectroscopy and phase solubility studies. The release performance of efavirenz was investigated through phase solubility and dissolution studies. It was found that these copolymers showed improved drug dissolution abilities.

Keywords: γ-Cyclodextrin polymer; Condensation polymerization; Inclusion complex; Drug release

INTRODUCTION

Cyclodextrins (CDs) comprise a family of commercially available cyclic oligosaccharides, produced from the active enzymes, glycosyltransferases, upon amylase. The three major commercially available cyclodextrins are crystalline and homogeneous substances, which form a torus of 6, 7 or 8 glucopyranose units called α, β or γ-cyclodextrin, respectively [1]. Cyclodextrins (CDs) have assumed great scientific and commercial interest because of their ability to form host–guest inclusion complexes with a number of organic molecules into their hydrophobic cavity. They have been well known for many years for solubilizing a wide range of water-insoluble and nonpolar organic molecules [2–5]. Because of this property, CDs have been used as drug carriers to improve drug stability, solubility, dissolution and formulation [6,7]. It has been found that the low water solubility of poorly water-soluble drugs is enhanced by complexation with the both native and chemically modified cyclodextrins (CDs). For example randomly methylated amorphous β-CD and crystalline heptakis-(2,6-di-O-methyl)-β-CD were the most efficient carriers in this respect [8,9].

A number of derivatives of CDs are known and excellent reviews are available highlighting the wide variety of derivatives available [10,11] though limited work has been done on γ-cyclodextrin derivatives, mainly due to its high price. However this situation is changing, because this derivative has the advantageous property of high water-solubility and an out standing toxicological profile [12].

When the cyclodextrin rings are incorporated into a polymeric main chain they behave differently from their monomeric derivatives. Polymeric derivatives with water-soluble spacers are expected to show better water-solubility as compared to the parent cyclodextrin. Polymeric derivatives of cyclodextrins have been found to be better for drug release applications that the parent cyclodextrin. A number of polymeric derivatives have been reported [13–17] that have been used for drug delivery of antitumoral [18], anti-inflammatory [19] and gene delivery [20]. However all these polymers use the epichlorohydrin route for the synthesis under highly alkaline conditions. Much milder conditions have been used to synthesise polymers from β-CD for the inclusion

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of the fullerene molecule [21]. Thus sugar (lactose and maltose) based γ-cyclodextrin copolymers would show greater solubilizing and binding properties, because of the biologically friendly structure of sugars (lactose and maltose). The synthesized sugar based γ-cyclodextrin copolymers can provide a range of different water-soluble polymers with unique structures. These polymers are potential candidates for various biochemical and biophysical utilization such as chromatographic supports for the separation of proteins, biosensing and drug delivery systems.

Efavirenz ((S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one) is a non-nucleoside reverse transcriptase (RT) inhibitor drug of human immunodeficiency virus type-1. However, efavirenz has an extremely low aqueous solubility (approximately $< 10\,\mu g/ml$ at 25°C). As a result, its bioavailability is limited.

A range of new linear homopolymers and copolymers were synthesized by polycondensation reaction with γ-cyclodextrin and a linker along with lactose and maltose as comonomers. The effect of the synthesized polymers on the inclusion and release performance of efavirenz was investigated through phase solubility and dissolution studies.

EXPERIMENTAL

Materials

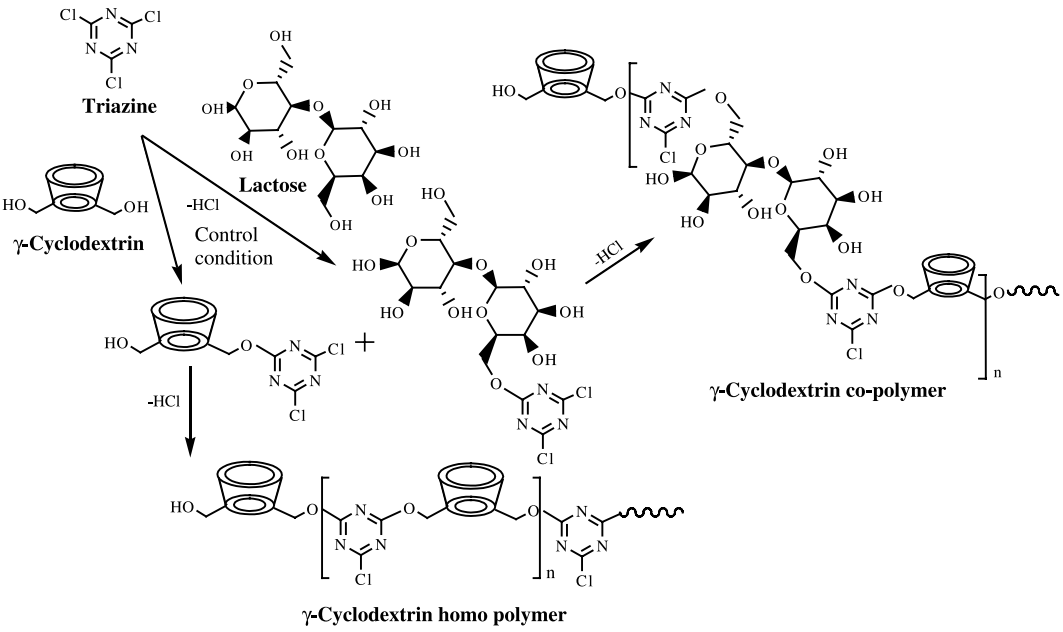
γ-Cyclodextrin (γ-CD) was purchased from Sigma Chemical Company USA and used as received. Cyanuric chloride (CC), lactose and maltose and other chemicals were purchased from local market and used without further purification. Efavirenz was obtained from Ranbaxy Ltd. (Indore, India) as a gift sample and used as received.

TABLE I Polymerization recipe and molecular weight of obtained γ-CD polymers

	γ-CD	CC	Lactose	Maltose	
Sample	(Mole Ratio)				M_n
γ-CD-P-1	1	1	0	0	>3500
γ-CD-P-2	1	1	0	0	>3500
γ-CD-P-3	1	1	0	0	>3500
γ-CD-P-4	1	1	0	0	>3500
γ-CD-P-5	1	1	0	0	>3500
γ-CD-L-P-1	1	2	1	0	>3500
γ-CD-L-P-2	1	2	1	0	>3500
γ-CD-L-P-3	1	2	1	0	>3500
γ-CD-L-P-4	1	2	1	0	>3500
γ-CD-L-P-5	1	2	1	0	>3500
γ-CD-M-P-1	1	2	0	1	>3500
γ-CD-M-P-2	1	2	0	1	>3500
γ-CD-M-P-3	1	2	0	1	>3500
γ-CD-M-P-4	1	2	0	1	>3500
γ-CD-M-P-5	1	2	0	1	>3500

Synthesis of γ-CD Polymers

γ-CD homopolymers as well as sugar based copolymers were synthesized by one-step condensation polymerization. Various ratios were used and are shown in Table I. The reaction is shown in Scheme 1. γ-CD and cyanuric chloride (CC) were taken into two separate flasks and dissolved in required amount of distilled water and different pH's ranging from 8–12 were maintained using 0.1 N NaOH solutions. 10 ml of this γ-CD solution of predetermined pH was taken in a round bottom flask with continuous stirring and the temperature



SCHEME 1 Synthesis of the γ-CD homopolymer, γ-CD-lactose and γ-CD-maltose copolymers.

maintained below 5°C. To this, 10 ml of cyanuric chloride solution was added maintaining the same temperature. After the complete addition the reaction was continued for another 4–5 h and then the contents were allowed to attain ambient temperature (30°C). The polymerization was stopped by addition of 0.1N HCI solution. The solution obtained was dialyzed for 24 h using a dialysis membrane of molecular weight cut-off of 3500. This solution was freeze-dried to get an off-white fluffy product. The synthesized polymers were characterized on a Shimadzu model 8400S FT-IR. Thermal analysis was carried out on a Shimadzu TGA-50 thermal analyzer.

Aqueous Solubilities of CD-polymers

0.6 g of polymer sample 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.

Phase Solubility Studies

Solubility measurement of the efavirenz was carried out by adding 100 mg of efavirenz to 10 ml of 6.8 phosphate buffer solution (PBS) of γ-CD polymer in the 1–7% (w/v) concentration range, in a glass container which was shaken at a constant temperature (25°C) until equilibrium was achieved (72 h). An aliquot was withdrawn and filtered. The efavirenz concentration was determined by measuring the UV absorbance of the saturated solutions at 247 nm wavelength and compared with the calibration curve. The apparent binding constant of the efavirenz/γ-CD polymer complex was calculated from the slope and intercept of the straight line of the phase solubility diagram, using the well known Higuchi–Connors equation [22].

$$K_{1:1} = \frac{\text{slope}}{\text{intercept}\,(1 - \text{slope})}$$

Dissolution Studies

For dissolution studies, efavirenz/γ-CD-polymer complexes were prepared by adopting the procedure described by Arias *et al.* and Mura *et al.* [23,24]. Samples were analyzed spectrophotometrically at 247 nm for efavirenz. The sample powder of drug with polymers (10:90 w/w) ground mixture was 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 μm). Efavirenz concentration in the phosphate buffer solution was obtained by UV–VIS spectrophotometer measurements after calibration at definite intervals of time.

RESULTS AND DISCUSSION

Synthesis

The first step in the synthesis of the γ-cyclodextrin polymers was the preparation of dichlorotriazine sodium salt from the dispersion of cyanuric chloride in water. Cyanuric chloride has three chlorine atoms whose reactivates can be controlled with temperature. The second step is the condensation reaction of two chlorine groups of dichlorotriazine sodium salt with two γ-cyclodextrin (primary —OH) under alkaline conditions and controlled pH and temperature (<30°C). 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 [25]. If the degree of substitution is higher, it would lead to the formation of crosslinked and hence insoluble polymers by reaction with itself. However all the synthesized polymers and copolymers showed higher solubility than the parent cyclodextrin even though the linker is water insoluble. This implies that the polymers obtained were linear with very low degree of branching.

Infra-red Spectroscopy

The FT-IR spectra for the γ-CD polymer, efavirenz and the inclusion complex are shown in Figs. 1a, 1b and 1c, respectively. Apart from the typical absorption peaks for the cyclodextrin moiety, the polymer

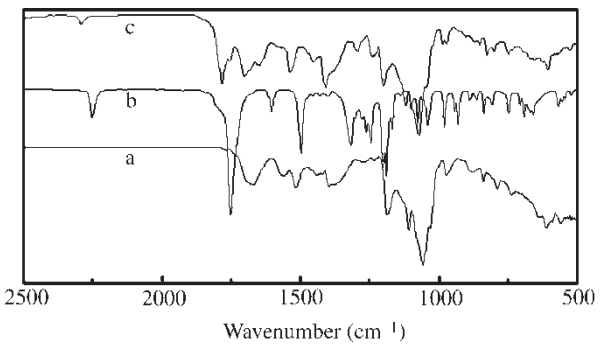


FIGURE 1 FT-IR spectra of (a) γ-CD polymer, (b) Efavirenz and (c) γ-CD polymer/Efavirenz complex.

(1a) also showed a characteristic band at 1766 cm^{-1}. This arises due to the C═N stretching that indicates the presence of the triazine as a part of the polymer chain. Figure 1b shows the spectra for efavirenz with a typical and strong band at 2260 cm^{-1} arising due to the exocyclic triple bond. In the complex of efavirenz/γ-CD polymer (Fig. 1c), the spectra obtained shows that the peaks of efavirenz almost disappear whereas the characteristic peaks of γ-CD-polymers remain strong. The band at about 1000 cm^{-1} is broadened and slightly shifted due to the superposition of the band associated with stretching of the efavirenz. In the complex we observe a shift of the efavirenz characteristic peak from 2260 cm^{-1} to 2239 cm^{-1}, 1751 cm^{-1} to 1741 cm^{-1}, 1242 cm^{-1} to 1238 cm^{-1}. These results indicate the modification of environment of efavirenz due to the formation of drug/γ-CD complex. If it were not so then the spectra would resemble that of a physical mixture of efivarenz and the CD polymer with no shift in the characteristic bands.

Thermal Analysis

The thermograms are shown in Fig. 2. γ-Cyclodextrin shows mass loss in three different temperature regions. The first mass loss at around 107°C is due to the loss of moisture, the second mass loss at 300°C as dehydration of γ-CD, and third mass loss at 367°C as a decomposition of glucose in γ-CD. However in case of the γ-CD homopolymer and copolymers the thermogram is different with the first mass loss at around the same temperature but second mass loss is at a lower temperature of 248°C due to the modification of the cyclodextrin unit with the resulting loss of the crystalline nature of the cyclodextrin molecule. Subsequent loss occurs due to the decomposition of the glucose and the triazine linker.

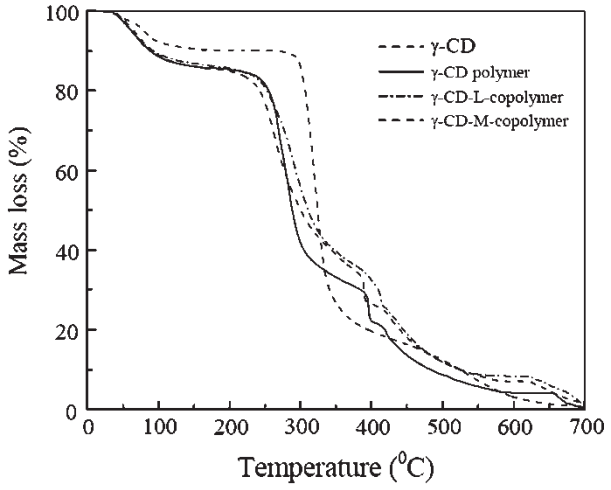


FIGURE 2 TGA curves of the γ-CD polymers.

X-ray Powder Diffractometry (XRD)

The X-ray diffraction pattern of the synthesized γ-CD polymers shown in Fig. 3 were taken. These indicate that the synthesized polymers do not have the typical 2θ values of γ-CD. It can be seen that the synthesized homopolymers and copolymers have an amorphous structure as compared to the crystalline peaks of the parent γ-CD (2θ = 12.5°, 19.6°, 23.0°, 27.0°). These XRD data show that the γ-CD are modified due to the condensation reaction and are converted to amorphous polymer.

Aqueous Solubilities of CD-polymers and Host–guest Interaction

As expected, after polymerization there is significant enhancement of the aqueous solubility of γ-CD as shown in Table II. The low aqueous solubility of parent γ-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 triazine groups via condensation reaction disrupts this intermolecular hydrogen bonding. Since all of the γ-CD polymers are water-soluble it implies that there was no cross linking. There is an almost four fold increase in the solubility of the γ-cyclodextrin on polymerization.

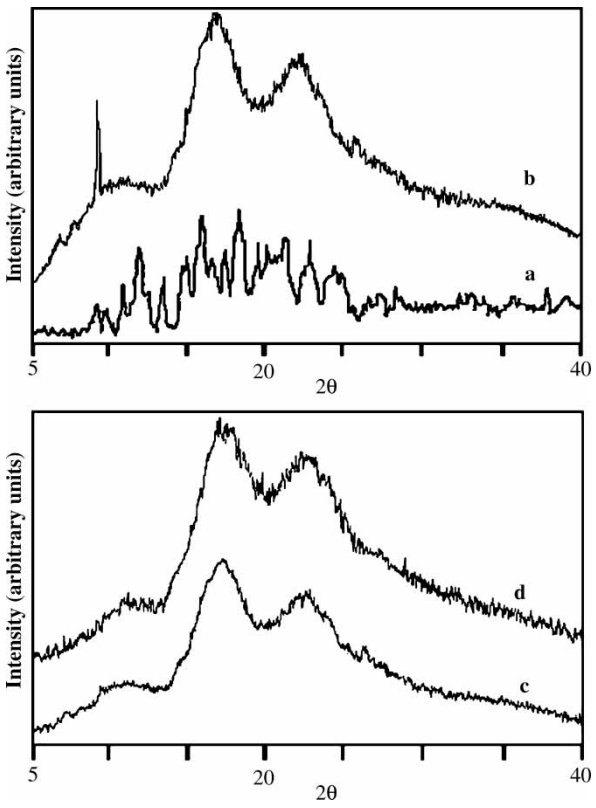


FIGURE 3 X-ray diffractograms of (a) γ-CD (b) γ-CD homopolymer (c) γ-CD-lactose copolymer and (d) γ-CD-maltose copolymer.

TABLE II Aqueous solubility of γ-CD polymers at 25°C

Sample	Aqueous solubility (mg/ml)	Solubility relative to γ-CD
γ-CD	232	1
γ-CD-P-1	812	3.5
γ-CD-P-2	809	3.5
γ-CD-P-3	845	3.6
γ-CD-P-4	872	3.8
γ-CD-P-5	860	3.7
γ-CD-L-P-1	970	4.2
γ-CD-L-P-2	968	4.2
γ-CD-L-P-3	997	4.3
γ-CD-L-P-4	1040	4.5
γ-CD-L-P-5	1060	4.6
γ-CD-M-P-1	958	4.1
γ-CD-M-P-2	870	3.8
γ-CD-M-P-3	901	3.9
γ-CD-M-P-4	925	3.9
γ-CD-M-P-5	938	4.0

The confirmation of the host–guest interaction was obtained from UV absorbance studies. The efavirenz drug shows an absorbance at around 247 nm in methanol and is water-insoluble, whereas γ-cyclodextrin polymer shows no absorbance in this region. The spectra (Fig. 4) of the inclusion complex in water showed the typical absorbance peak corresponding to the efavirenz 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 improved water-solubility of efavirenz is shown in Fig. 5. In 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 ($K_{1:1(\gamma\text{-CD})} = 363\,mol^{-1}$, $K_{1:1(\gamma\text{-CD-P})} = 537\,mol^{-1}$, $K_{1:1(\gamma\text{-CD-L-P})} = 575\,mol^{-1}$, $K_{1:1(\gamma\text{-CD-M-P})} = 585\,mol^{-1}$ calculated according to the molecular weight of γ-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.

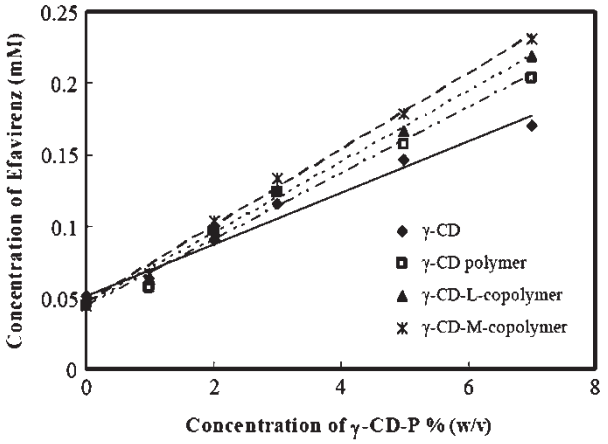


FIGURE 5 Solubility diagrams of efavirenz in γ-CD; γ-CD polymer; γ-CD-L polymer; γ-CD-M polymer.

The values of the stability constant $K_{1:1}$ was with in the range of 100 to 1000 M^{-1} considered the ideal. The γ-CD-lactose and γ-CD-maltose copolymers have better complexing properties or the binding constant compared to the parent γ-CDs, where as γ-CD homopolymers have the better stability compared to the parent γ-CD but lower than that of the copolymers. This can be attributed to the cooperative action in binding between the adjacent γ-CD units and polymer chains. The adjacent units and polymer chain can act like arms of the CD cavities to facilitate the drug inclusion that is helpful for the complexing of large molecules.

Dissolution Studies

The dissolution profiles of the co-ground complexes of the efavirenz with the synthesized γ-CDs polymers are compared with the efavirenz/CDs in Fig. 6. Efavirenz/γ-CD polymers samples show the better dissolution rate and higher cumulative release of the drug dissolution. This is due to the high hydrophilic nature of the synthesized polymers, lowering the interfacial

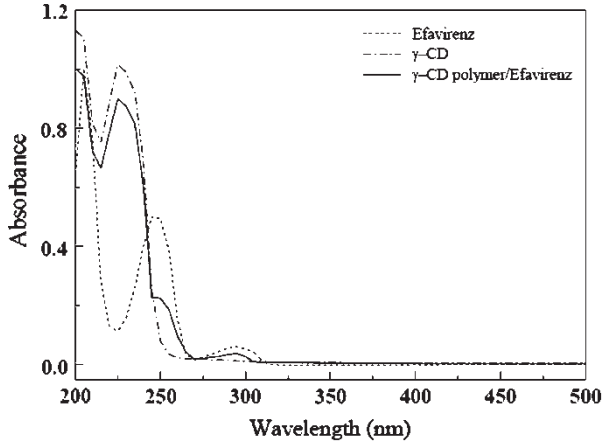


FIGURE 4 UV–VIS spectra of efavirenz in methanol and γ-CD polymer and efavirenz-γ-CD polymer in water.

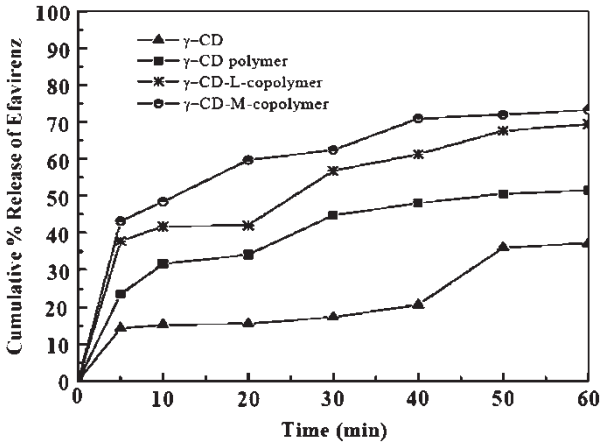


FIGURE 6 Dissolution curves of efavirenz with γ-CD and ground products with water-soluble γ-CD polymers.

tension in between the highly water insoluble drug and water. The high amorphous nature of the synthesized polymers and their water-solubility show a positive impact on the cumulative release of the drug.

CONCLUSIONS

Chain type cyclodextrin-based water-soluble homopolymers and sugar based copolymers, with hydroxyl functional groups have been synthesized by the classical stepwise 'condensation' polymerization. As a linking agent triazine was chosen, because of the temperature dependent reactivity of this unit and the ease of functionalization without the loss of the complexing properties of γ-cyclodextrins. The characteristic of the sugar based copolymers is their potential for the highly water-soluble biocompatible materials. The inclusion complexation of γ-CD polymer with efavirenz significantly enhanced the water solubility of efavirenz. The drug/γ-CD-P complex formation was confirmed from UV–VIS spectra. This polymerization process is simpler and was carried out at ambient temperature and was more controlled than the epichlorohydrin cyclodextrin polymerization process.

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