Synthesis of Cyclodextrin and Sugar-Based Oligomers for the Efavirenz Drug Delivery

Indrajit Shown,[1] *Sudip Banerjee,*[2] *A.V. Ramchandran,*[2] *Kurt E. Geckeler,*[3] *C. N. Murthy**[1]

Summary: In the present work water-soluble lactose based oligomers of β-cyclodextrin were synthesized by a simple and efficient condensation polymerization process. Proposed water-soluble β-cyclodextrin oligomers were prepared by controlled reaction between β-cyclodextrin and a triazine linker and purification by an ultrafiltration process. Similarly, lactose based β-cyclodextrin oligomers were synthesized for enhanced water solubility. The physical and chemical properties of the synthesized polymers were characterized by FT-IR and ^{1}H NMR spectroscopy, XRD analysis, thermogravimetric analysis (TGA) and aqueous solubility determination. Molecular weights of these β-cyclodextrin based oligomers were measured by ESI technique. These β-cyclodextrin based water-soluble oligomers polymers were used as supramolecular carriers for efavirenz (an anti HIV drug), improving the inclusion property and aqueous solubility properties of this drug. These synthesized oligomers were found to improve stability and aqueous solubility of efavirenz on their (1:1) inclusion complex through phase solubility and dissolution studies. Reduced cytotoxicity than the parent β-CD was observed in hemolysis test.

Keywords: condensation polymerization; inclusion complex; phase solubility; supramolecular carriers; water-soluble; β-cyclodextrin

Introduction

Cyclodextrins (macrocyclic oligosaccharides)[1,2] are well known in supramolecular chemistry to solubilize hydrophobic molecules in aqueous media via noncovalent interactions in hydrophobic cavity.[3–6] Cyclodextrins and their derivatives have potential in the pharmaceutical applications due to there extensive use in drug delivery systems (peptide and protein delivery, ophthalmic drug delivery, nasal drug delivery and in many other areas).[7–12] Their ability to accommodate a large variety of non polar organic molecules in their hydrophobic macrocyclic cavities finds numerous drug delivery applications to enhance solubilisation, stabilization and absorption.[13–16]

Among all the macrocyclic oligosaccharides β-CD is cheaper than other CDs, however the low water solubility and cytotoxicity of the β-cyclodextrin limits its further applications in drug formulations.[17] β-cyclodextrin has been shown to alter the cell membrane permeability and so causes hemolysis[18] due to binding and extraction of the cholesterol through its inclusion complex. In addition β-CD damages the renal cell (parenteral administration) by the extraction of cholesterol from kidney membrane and causes nephrotoxicity.[19]

This drawback has stimulated a great deal of research to chemically modify the

[1] Applied Chemistry Department, Faculty of Technology and Engineering, The M. S. University of Baroda, Vadodara-390 002, India
E-mail: chivukula_mn@yahoo.com
[2] Department of Zoology, Faculty of Science, The M. S. University of Baroda, PO Box-51, Kalabhavan, Vadodara-390001, India
[3] Department of Materials Science and Engineering, Gwangju Institute of Science and Technology, Gwangju, South Korea

cyclodextrin molecules by selective conversion of the hydroxyl groups to the other functionalities (hydroxyalkylated CDs, sulfoalkyl ether CDs and ionic CDs)[20–23] to reduce toxicity. Thus, the research interest has been significantly increased in the area of cyclodextrin based polymers synthesis.[24–28] Cyclodextrin based polymers are synthesized by the condensation reaction of the cyclodextrins with a crosslinking agent. Most of the studies found in the literature regarding the cyclodextrin based polymers have been synthesized by using epichlorohydrin as a crosslinking agent for analgesic, anti inflammatory and antibiotic drug delivery systems.[25,29] The physicochemical properties of the synthesized cyclodextrin based polymers were modified according to the different types of cyclodextrins, the spacer, or the ratio between both. These synthesized polymers materials provide modified encapsulation of guest to the host molecules that is different from the parent cyclodextrin.[30–32]

In continuation of our previous work with linear γ-cyclodextrin based polymers as drug carrier systems[33], the main objective of this work was to focus on a cheap polymeric carrier with low toxicity for the efavirenz ((S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one). Efavirenz is one of the anti HIV drugs with poor aqueous solubility (approximately $< 10\,\mu g/ml$ at $25\,^{\circ}C$). It is a non-nucleoside reverse transcriptase (RT) inhibitor drug of human immunodeficiency virus type-1. As a result of its poor solubility, its bioavailability appears to be limited. It was therefore thought that the low aqueous solubility of this drug can be enhanced by complexation with the β-cyclodextrin based polymers, which simultaneously offers the advantages of the amorphous state and CD-type complexation without toxic effects. Keeping this in mind, it was planed to synthesize a series of linear β-cyclodextrin based polymers by the polycondensation reaction with an aromatic linking agent under mild conditions. The physicochemical properties of the modified β-cyclodextrin oligomers were characterized using various techniques including ^{1}H-NMR, FT-IR, TGA, XRD, ESI-mass and aqueous solubility determination. Here we also report the investigations on the β-cyclodextrin based polymers as nonpolar drug carriers and their drug release pattern in vitro with efavirenz. The information obtained from aqueous solubility, phase solubility and hemolysis studies of β-cyclodextrin polymers/drug inclusion complex have been correlated with the β-cyclodextrin molecules. Such systems can be used to improve the aqueous solubility of any other non polar drug, thus enhancing its dissolution rate, leading to a faster on set of action and less cytotoxicity.

Experimental Part

Materials

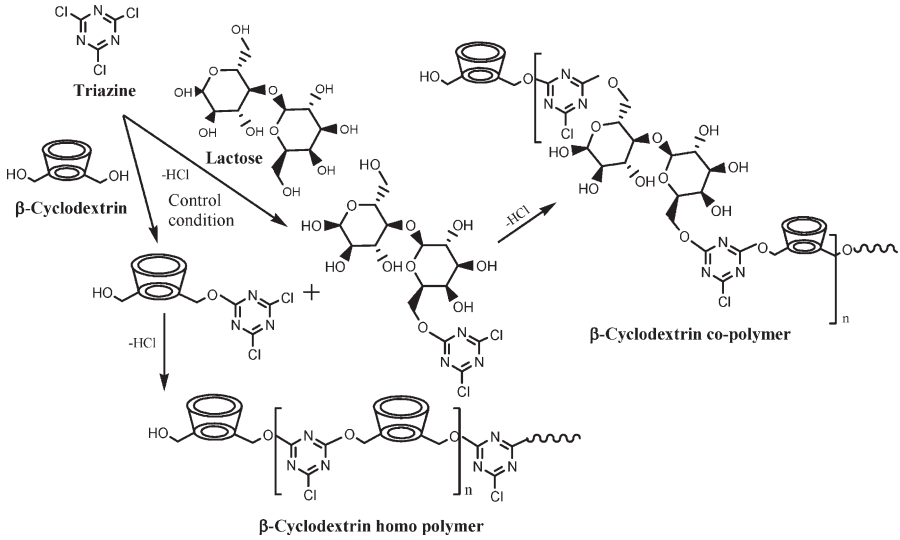
β-Cyclodextrin was provided by Sigmanet Pvt. Ltd, Mumbai as a gift sample. Cyanuric chloride, lactose and other chemicals were purchased from local markets and used without further purification. Efavirenz was obtained from Ranbaxy Ltd. (Indore, India.) as a gift sample and used as received.

Equipment

FT-IR spectra were taken on Shimadzu (8400S) instrument. ^{1}H-NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz at room temperature. The TG analysis of the β-cyclodextrin polymers was done on a Shimadzu, TGA-50 system with a heating rate of $10\,^{\circ}C$/min in the temperature range of 50–$700\,^{\circ}C$. X-ray powder diffraction patterns were taken on a computer-controlled RIGAKU-DMAX-2200. The electrospray ionization (ESI) mass spectra of these polymers were recorded on a platform II quadrapole mass spectrometer (Micromass) fitted with an electrospray ion source. Samples were dissolved in CH_3OH/H_2O (50:50, v/v).

Synthesis of β-cyclodextrin Oligomer

β-cyclodextrin based oligomers were synthesized by one-step condensation



Scheme 1.
Synthetic scheme for the β-cyclodextrin homopolymer and β-cyclodextrin-lactose-co-polymer.

polymerization. The reaction is given in Scheme 1. The typical synthesis procedure fallowed was according to the method described in reference 33. Thus in brief, 10 ml of cyanuric chloride solution was added in a 10 ml of β-CD solution with continuous stirring at controlled temperature and controlled pH. The reaction was continued with stirring, for 4–5 h. Stirring was continued for another 12 h while the contents were allowed to attain ambient temperature (30 °C). The polymerization was stopped by neutralization with 0.1 N HCI solution. The solution obtained was dialyzed for 24 h with a dialysis membrane of molecular weight cut-off 3500. Residual unpolymerised and merely substituted β-CD was separated 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 (Yield 45%).

Using a similar experimental procedure mentioned above the β-CD-lactose-oligomers was synthesized by one step condensation polymerization using various ratios of the reactants. The experimental scheme is given in Scheme 1 and the experimental details given in Table 1.

Aqueous Solubility and Dissolution Experiment of Efavirenz

The aqueous solubility of the synthesized oligomers were measured using a simple technique. 0.1 g of β-cyclodextrin oligomer 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.

The stability measurement of efavirenz was carried out by adding 100 mg of efavirenz to 10 ml of pH-6.8 phosphate buffer solution (PBS) of β-CD oligomer. The % of the β-CD oligomer was varied from 1–7% (w/v). All solutions were prepared in a glass container which was shaken at a constant temperature (25 °C) until equilibrium was achieved (72 h). An aliquot was withdrawn and the efavirenz concentration was determined by measuring the ultraviolet absorbance of the

Table 1.
Polymerization recipe of β-cyclodextrin polymers.

Sample	β-CD: CC: Lactose	pH	Time(hr)	ESI mass (M_n)
β-CD-homopolymer	1: 1: 0	12	17	5680
β-CD-lactose-copolymer	0.5: 1: 0.5	12	17	4876

saturated solutions at 247 nm wavelength and compared with the calibration curve for efavirenz. The apparent binding constant of the efavirenz/β-CD oligomer complexes were calculated from the slope and intercept of the straight line of the phase solubility diagram, using the following equation.[34]

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

The dissolution process of the efavirenz into solution can be describe as fallows: efavirenz/cyclodextrin polymers complexes were prepared by adopting the procedure described by Arias et al. and Mura et al.[35,36] Efavirenz with β-CD oligomer at 10:90 (w/w) ratio was manually ground using mortar and pestle for 10 min. Phosphate buffer solution of pH-6.8 was employed as dissolution medium at $37 \pm 0.5\,^{\circ}C$. The sample powder prepared with polymers 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 the 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 certain periods of time.

Hemolysis Studies

For Hemolysis studies 5% hematocrit solution was prepared by the method described by Ohtani et al.[37] Sodium citrate was added (0.47%) to freshly drawn human blood. The erythrocyte fraction obtained after centrifugation (1000 rpm for 5 min) was washed three times with phosphate buffer (0.154 M sodium chloride and 0.01 M phosphate, pH 7.4) and re-dispersed in a buffer solution to give a hematocrit of 5%. 0.1 ml of cell suspension was added to 2.0 ml of the buffer solution containing β-CDs polymer at various concentrations. Each mixture was incubated for 30 min at $37 \pm 0.5\,^{\circ}C$, and then centrifuged at 1000 rpm for 5 min. The supernatant concentration was measured using a UV-Vis spectrophotometer at 543 nm, which corresponded to the release of hemoglobin from the cells. The degree of hemolysis is presented as percentage of the total efflux of hemoglobin, which was obtained when water was used instead of the buffer solution containing cyclodextrin polymers.

Results and Discussion

β-cyclodextrin based low molecular weight polymers with lactose as a comonomer were synthesized by the one pot condensation reaction with cyanuric chloride as a linker. Cyanuric chloride contains three chlorine atoms with different reactivities, depending on the temperature. In the second and the final step of the condensation reaction two chlorine groups of dichlorotriazine covalently react with two cyclodextrin (primary –OH) under alkaline conditions and low temperature (5–30 °C). With a higher degree of substitution, the solubility of the synthesized β-cyclodextrin oligomers would be reduced to form insoluble polymers by reaction with itself. With the optimized conditions highly water-soluble oligomers were synthesized with low cross linking reactions that was confirmed from the aqueous solubility data shown in Table 2. The low degree of substitution (allow to react 2–3 triazine) per cyclodextrin moiety confirms the linearity of the synthesized polymers. The

Table 2.
Aqueous solubility of β-cyclodextrin polymers at 25 °C.

Sample	Aqueous solubility (mg/ml)	Solubility relative to CD
β-CD	18.5	1.0
β-CD-homopolymer	127.3	6.9
β-CD-lactose-copolymer	135.3	7.3

molecular weights of the synthesized polymers measured by the ESI-mass are shown in Table 1. The β-CD and β-CD-lactose oligomers have molecular weight of 5680 and 4876 respectively.

^{1}H NMR Spectroscopy

The spectra in Figure 1 of β-CD homopolymer and oligomers respectively shows peaks at δ 4.84 (s,7H,C^{1}H), δ 3.82 (t,7H,C^{3}H), δ 3.74 (m,14H, C^{6}H, C^{5}H), δ 3.50 and δ 3.44 (m, 14H, C^{2}H, C^{4}H). The peak near 5.0 ppm is assigned to the anomeric proton attached to the C-1 of the glucose unit, and two broadened peaks between 3.0 and 4.0 ppm correspond to the protons in pyranose rings. The ^{1}H-NMR spectral resonance signal of β-CD-lactose copolymer and β-CD homopolymer look similar due to same structure except a shoulder signal at δ 3.30 ppm of lactose based oligomers. This shoulder in spectra may be due to the presence of lactose, which has a different chemical environment after copolymerization with β-cyclodextrin triazine, when compared to β-cyclodextrin triazine.

Infra-red Spectroscopy

The FT-IR spectra for the β-CD-oligomer, efavirenz and the inclusion complex are shown in Figure 2a, 2b and 2c respectively. Apart from the typical absorption peaks of C-H stretching (2890–2880 cm^{-1}) and bending (1480–1280 cm^{-1}) from normal alkanes for the cyclodextrin moiety, the β-cyclodextrin oligomer(2a) also showed a characteristic C=N stretching at 1747 cm^{-1}. This confirms the formation of polymer with the addition of the triazine as a part of the polymer chain. Figure 2b shows the spectra for efavirenz with a typical exocyclic triple bond stretching at 2260 cm^{-1}. In the complex of efavirenz/β-CD-oligomer (Figure 2c), the spectra obtained shows that the peaks of efavirenz almost disappear whereas the characteristic peaks of β-CD polymers remain strong. The band at about 1000 cm^{-1} is broadened and slightly shifted due to the superposition of the band

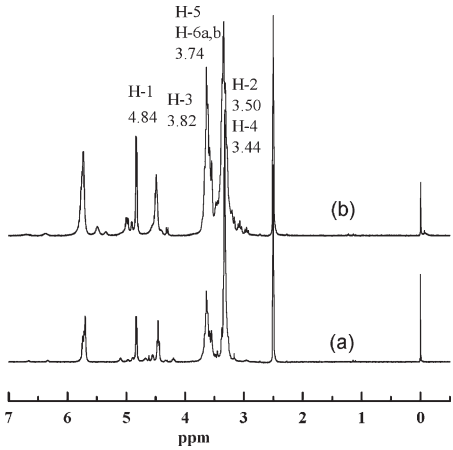


Figure 1.
^{1}H NMR spectra (a) for β-CD-homopolymer and (b) β-CD-lactose co polymer.

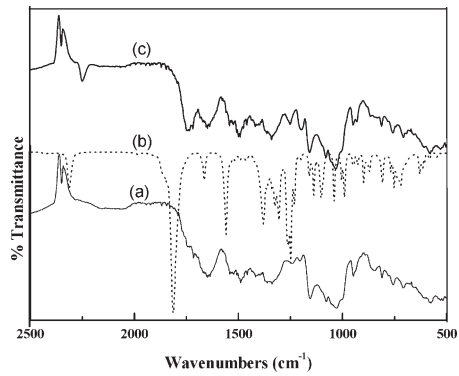


Figure 2.
Stacked FT-IR spectra of (a) β-CD polymer, (b) Efavirenz and (c) β-CD polymer/efavirenz complex.

associated with stretching of the efavirenz. In the complex we observe a shift of the efavirenz characteristic peak from 2260 to 2243 cm^{-1}, 1753 to 1740 cm^{-1}, 1242 to 1235 cm^{-1}. These results indicate the modification of environment of efavirenz due to the formation of drug/β-CD-polymer inclusion complex. If it were not so then the spectra would resemble that of a physical mixture of efavirenz and the β-CD-polymer with no shift in the characteristic bands.

X-ray Diffraction (XRD)

The X-ray diffraction pattern of the synthesized β-cyclodextrin-polymers (Figure 3) shows that the synthesized copolymer does not have typical 2θ values of parent β-CD. It can be seen that the synthesized oligomers have a different structure than that of the parent β-CD ($2\theta = 9$, 12.5, 19.6, 23.0, 27.0, 34.8°) with the total suppression of the crystalline nature of the parent β-CD. These XRD data show that the β-CD is modified due to the condensation reaction and converted to an amorphous polymer. Similar result was observed for the β-cyclodextrin-lactose copolymer.

Thermal Analysis

Thermogravimetric analysis (TGA) curves of synthesized β-cyclodextrin and β-cyclodextrin-lactose oligomers are shown in Figure 4. In the pristine CDs the first mass loss around 103–107 °C is due to the loss of moisture, the second and third mass loss at 300 °C and 360–367 °C respectively, due to decomposition after melting of glucose in cyclodextrin. In case of the β-cyclodextrin-lactose oligomers, first mass loss is around same temperature but second mass loss is at 248 °C and third mass loss at 320 °C. Beyond 390 °C it is due to decomposition of glucose in monomer and the triazine linker.

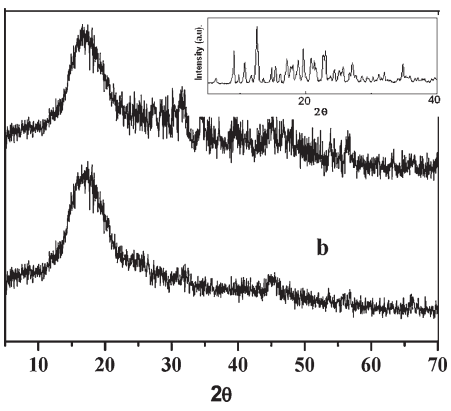


Figure 3.
XRD of (a) β-CD homopolymer, (b) β-CD lactose copolymer. β-CD (Inset).

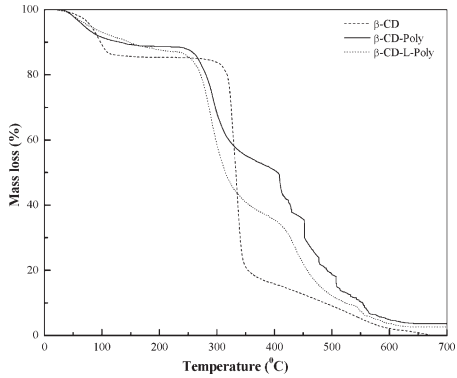


Figure 4.
TGA curves of the β-cyclodextrin polymers.

Aqueous Solubilities of β-cyclodextrin Oligomers

As expected, after polymerization there is significant enhancement of the aqueous solubility of cyclodextrin as shown in Table 2. The solubilities of the synthesized β-cyclodextrin polymers are higher than that of the parent β-cyclodextrins. 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 triazine groups via condensation polymerization disrupt the intermolecular hydrogen bonding, thus increasing the aqueous solubility. In case of sugar based oligomers, lactose provides the unique chemical functionality as water-solubility with the biocompatibility increases the aqueous solubility and the flexibility of

the polymeric chain. On the other hand, high water-solubility of the synthesized β-cyclodextrin-polymers implies that cross linking did not occur. There is an almost 7 fold increase in the solubility of the β-cyclodextrin on polymerization.

Host-Guest Interaction in Aqueous Solution

The confirmation of the host-guest interaction was obtained from UV absorbance studies. The efavirenz shows an absorbance at around 247 nm in methanol and is water-insoluble, whereas β-cyclodextrin- polymer shows no absorbance in this region. The spectra (Figure 5) 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 unique cavity sizes of the β-CD offers a nonpolar environment for the drug and thus has been "solubilized" in water.

The improved water-solubility of efavirenz is shown in Figure 6. In the phase solubility studies by linear relationship between dissolved drug concentration and amount of solubilizing agent, we calculated the binding constants of the drug-polymers inclusion complexes at 25 °C ($K_{1:1(\beta\text{-CD})} = 1125\ mol^{-1}$, $K_{1:1(\beta\text{-CD-P})} = 1551\ mol^{-1}$, $K_{1:1(\beta\text{-CD-L-P})} = 1739\ mol^{-1}$ calculated according to the molecular weight of β-CD repeating unit.). From the phase solubility plot the slope of the diagram is less than one thus the inclusion complex

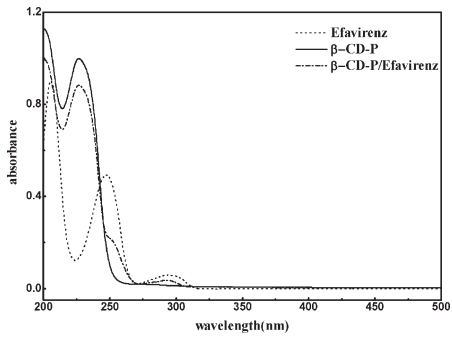


Figure 5.
UV–VIS spectra of efavirenz in methanol and β-CD polymer and efavirenz-β-CD polymer in water.

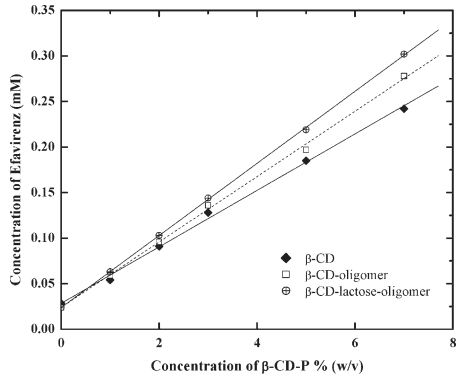


Figure 6.
Phase solubility diagram of efavirenz in β-CDs, β-CD and β-CD-lactose oligomer.

may be of 1:1 stoichiometry. These results show the CD-lactose oligomers have better complexing properties or binding constant compared to the β-CD homopolymer. This can be attributed to the cooperative action in binding between the adjacent CD units and polymer chains. The adjacent lactose units and polymer chain act like arms of the CD cavities to facilitate the drug inclusion that is helpful for the complexing of large molecules.

Dissolution Studies

Figure 7 shows the dissolution profiles of the co-ground complexes of the efavirenz with the synthesized β-CD polymers (homo and

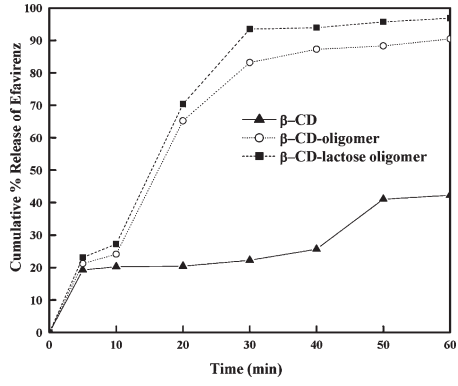


Figure 7.
Dissolution curves of efavirenz with β-CDs and ground products with β-CD homopolymer and β-CD-lactose copolymer.

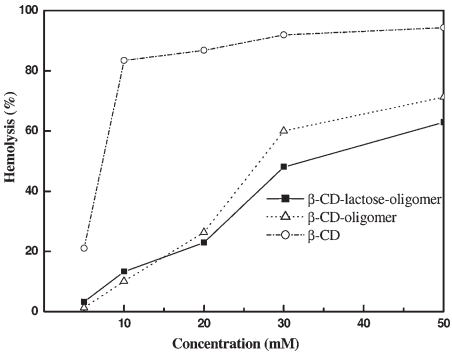


Figure 8.
Hemolytic effect of β-CD, β-CD homopolymer and β-CD-lactose copolymer on human erythrocytes in phosphate buffer (pH 7.4).

copolymer) and compared with the efavirenz/CDs. Efavirenz/β-CD-lactose copolymer show better dissolution rate and higher cumulative release (almost 92–96% release) of the drug dissolution. This is due to the high hydrophilic nature of the synthesized polymers, lowering the interfacial tension between the highly water insoluble drug and water. The highly amorphous nature of the synthesized polymers and the co-ground procedure of the preparation of drug complexes added some positive impact on the cumulative release of the drug.

Cytotoxicity

The result of the cytotoxicity studies is shown in the Figure 8. Very low cytotoxicity or hemolysis was observed in case of synthesized β-CD polymers as compared to the parent β-CD. The lactose based β-CD copolymer shows better hemolytic activity due to the increase in the chain length and thus enhances the structural hindrance. These structural modifications change the microenvironment of the apolar cavities thus inhibiting the formation of inclusion complex with cholesterol that leads to reduced cell damage.

Conclusion

In this work β-cyclodextrins based and lactose based oligomers were synthesized by simpler condensation polymerization process at ambient temperature. The lactose based oligomers have potential for highly water-soluble biocompatible materials. The β-CD-homopolymer and oligomers show enhanced aqueous solubility and better stability for efavirenz. The synthesized β-cyclodextrin oligomers showed low cytotoxycity as compared to the parent β-cyclodextrin.

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