J Incl Phenom Macrocycl Chem (2011) 69:383–392
DOI 10.1007/s10847-010-9764-x

ORIGINAL ARTICLE

Synthesis and characterization of amphiphilic star copolymer of beta-cyclodextrin and polypropylene oxide and their application as nanocarriers

N. S. Saindane · D. M. Bramhane · P. R. Vavia

Received: 30 October 2009 / Accepted: 1 March 2010 / Published online: 16 March 2010

Abstract The present study was aimed at synthesizing and characterizing star copolymers of β-cyclodextrin and exploring their application as nanocarriers. The copolymers of β-cyclodextrin and polypropylene oxide were synthesized by using ring opening polymerization, catalyzed by base, under high temperature and pressure. The polymers of different molecular weight were synthesized by increasing chain length of polypropylene oxide at optimized temperature, pressure and concentration of catalyst. The structure of synthesized polymer was confirmed by IR and NMR. Molecular weight and molecular weight distribution was evaluated by hydroxyl number and gel permeation chromatography respectively. Amphiphilic nature of the polymers was evaluated by determining the solubility in water and different organic solvents. For the evaluation of polymer as a nanocarrier, Ibuprofen was selected as model drug. Loading efficiency and release of Ibuprofen from the complex were also investigated. It was observed that, with increase in the molecular weight of the polymers, loading capacity was increased.

Keywords Star polymer · β-Cyclodextrin · Nanocarrier · Ibuprofen

Introduction

β-Cyclodextrin (β-CD) is torus-shaped cyclic oligosaccharides containing seven glucose units. The individual glucose units are held in a C-1 chair conformation and they are joined together by α-1,4 glycosidic linkages to form a cyclic structure [1]. The interior cavity of β-CD is relatively hydrophobic and the most characteristic feature of β-CD is the ability to form inclusion complexes through host–guest interactions [1]. β-CD is the most largely produced cyclodextrin and has been widely used in many fields including pharmaceuticals, foods, cosmetics, chemical products and technologies [2].

In pharmaceutical industry, it has been mainly explored for increasing water solubility of poorly soluble drugs, ultimately increasing their bioavailability, increasing the stability of drugs, prevention of the drug–drug or drug–additive interactions, reducing or eliminating unpleasant smells or tastes of the drugs [2]. However, β-CD has some serious drawbacks like low aqueous solubility (18.5 mg/mL) of β-CD leading to precipitation of complexes of many lipophilic drugs, low complexing ability, when diluted with bloodstreams or gastric fluid leads to rapid decomplexation and precipitation of drugs, hemolytic effect and no controlled drug release [3].

To overcome these serious drawbacks, various CDs derivatives have been developed. Among them, methylated CDs, hydroxyalkylated CDs and ionic CDs are typical ones aiming at improving water solubility via disrupting the intermolecular hydrogen bond between the secondary hydroxyl groups of parent CDs. Such hydrogen bonds are mainly responsible for their low aqueous solubility [1]. However, the increase of water solubility does not necessarily result in lowering toxicity [4, 5]. On the other hand, cyclodextrin-based polymers are of interest due to their merits compared to parent CDs, such as high solubility in water and capability to solubilize a number of drugs [6]. In these polymers numerous types of cross linker have been tried to synthesize the polymers of different properties [7].

N. S. Saindane · D. M. Bramhane · P. R. Vavia (✉)
Department of Pharmaceutical Sciences, Institute of Chemical Technology, N. P. Marg, Mumbai 400019, India
e-mail: vaviapradeep@yahoo.com

Also to improve the water solubility of complexes, complexation ability and to impart the cell internalization property, amphiphilic star polymers can be synthesized by conjugating the macromolecules on the cyclodextrin.

In the present work, we have tried to synthesize the amphiphilic star polymer by conjugating polypropylene oxide with the beta cyclodextrin. The typical star shape and amphiphilic nature of polymer may improve the complexing and solubilizing ability. The 21-armed amphiphilic star polymer of β-CD was synthesized by reacting it with propylene oxide.

To evaluate the synthesized polymer as a drug carrier, Ibuprofen was selected as model drug [8]. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID), when delivered by conventional route, Ibuprofen always shows the significant side effects like GIT hemorrhage and renal damage. Therefore to avoid this, parenteral route would be good option, however poor solubility restricts its use [7]. Different techniques of improving solubility could be used, however they are not sufficient to achieve this goal. Therefore a system which improves the solubility and provides controlled release would enhance its therapeutic efficacy.

Materials and methods

Materials

Beta cyclodextrin, USP and propylene oxide (99% pure) was purchased from Roquette, France and Fisher Scientific respectively. Ibuprofen was obtained from Sekhasaria Chemical ltd, India. Dialysis membrane of molecular weight cut-off of 3.5 kDa was obtained from Spectrum Labs. All other chemicals used were of analytical grade.

Methods

Synthesis of star copolymer

Amphiphilic star copolymer of beta-cyclodextrin and polypropylene oxide was synthesized according to the procedure described as follows [9]: Dry β-CD (dried initially at 80 °C for 12 h, 1 mol) was slurried with propylene oxide (42 mol). The solution of sodium hydroxide (7% w/v of β-CD) in methanol was prepared and added slowly to the reaction mixture and this reaction mixture was placed in the reaction autoclave. The interior of autoclave was purged with nitrogen to remove oxygen and autoclave temperature was raised slowly to 120 °C and respective pressure was monitored. The reaction mixture was kept under continuous stirring for period of 12 h. At conclusion of reaction, tartaric acid was added to neutralize basic catalyst and then polymer was extracted with dichloromethane and filtered under vacuum. This product was stripped under reduced pressure to remove dichloromethane, unreacted propylene oxide and volatile byproducts to get viscous brown colored polymer. The higher molecular weight polymers were synthesized by subsequent addition propylene oxide and catalyst solution and repeating the procedure as above.

Characterization of star copolymer

NMR, FTIR and DSC ^{1}H NMR and ^{13}C NMR spectra were recorded in D_2O solution, on a Bruker (500 MHz) instrument and ^{13}C NMR spectra was recorded on the same instrument. IR measurements were performed using a Perkin Elmer 320 FT-IR. Differential scanning calorimeter diagrams were recorded using a Shimadzu DSC 60 apparatus.

Average molecular weight and molecular weight distribution Approximate molecular weight of polymer was determined by hydroxyl number determination [9]. Hydroxyl number of the reaction product was determined experimentally in terms of amount of KOH required to back titrate after reacting the product with acetic anhydride. The amount of KOH used in the titration will be conventionally expressed as mg of KOH/g of product. Then from hydroxyl number average molecular weight of an anhydroglucose unit can be determined from the following formula,

$$\text{Avg. molecular weight of an anhydroglugose unit} = \frac{1,000 \times 56.1 \times F}{\text{Hydroxyl number}}$$

where F = number of hydroxyl group of basic structure (in an anhydroglucose unit i.e. 3), 56.1 = molecular weight of KOH.

Approx. 2 g sample was added into an Erlenmeyer flask with standard ground-glass joint and dissolved in 20.0 mL 1 M acetic anhydride solution in anhydrous pyridine and to this flask, reflux condenser was attached and heated up to 130 °C for 45 min. After cooling down, condenser was rinsed three times with 10 mL pyridine each, and then three times with 10 mL distilled water each into the Erlenmeyer flask. Formed acetic acid was titrated with 1 M NaOH. In order to determine the titrant consumption for the reaction solution and solvents, a blank sample was treated and titrated in exactly the same way as the actual sample. This blank consumption was stored as common variable B in the titrator. The titrations were carried out with a start volume that is approx. 20 mL for the sample and approx. 35 mL for the blank.

$$\text{Hydroxyl number} = \frac{(B - T) \times M}{\text{Sample weight}}$$

where T = titrant consumption in mL, $M = 56.1$ [=c (NaOH) in mol/L * M (KOH) in g/mol], B = blank consumption in mL.

The molecular weight distributions were determined by size exclusion chromatography (SEC) using Knauer System connected to a refractometer index detector with the mobile phase 0.1 M phosphate buffer pH adjusted to 7.5 with NaOH at 28 °C [10].

Solubility of star polymer To determine the amphiphilicity of the polymer, the solubility was checked in the polar and non-polar solvents.

Loading capacity of the star polymer

Star polymer (0.5 g) and excess Ibuprofen were dissolved in 10 mL of chloroform and it was stirred for 24 h on magnetic stirrer. Clear polymer solution was obtained after filtration and quantity of Ibuprofen was determined by UV spectrophotometry at 220 nm after proper dilutions. The formed complex of star polymer and Ibuprofen was characterized by ^{1}H NMR and FTIR as described above.

Phase solubility study of the star polymer

The polymer solutions in water (10 mL) of different concentrations (0, 1, 2, 4, 6, 10% w/v) were added in the vials. To these vials, excess Ibuprofen was added and kept on the mechanical shaker for period of 24 h. These solutions were filtered and quantity of Ibuprofen was determined by UV spectrophotometry at 220 nm after proper dilutions.

Drug release study

The drug release from the polymer–drug complex was evaluated in 100.0 mL 7.4 pH phosphate buffer. The known amount of drug–polymer complex was filled in the dialysis bag (*spectrum dialysis bag molecular weight cutoff 3.5 kDa*) and dialysis bag was placed in the dissolution medium at 37.0 °C with slow magnetic stirring under sink condition in the 7.4 pH phosphate buffer for period of 12 h. The drug concentration was determined by UV spectrophotometry at 220 nm.

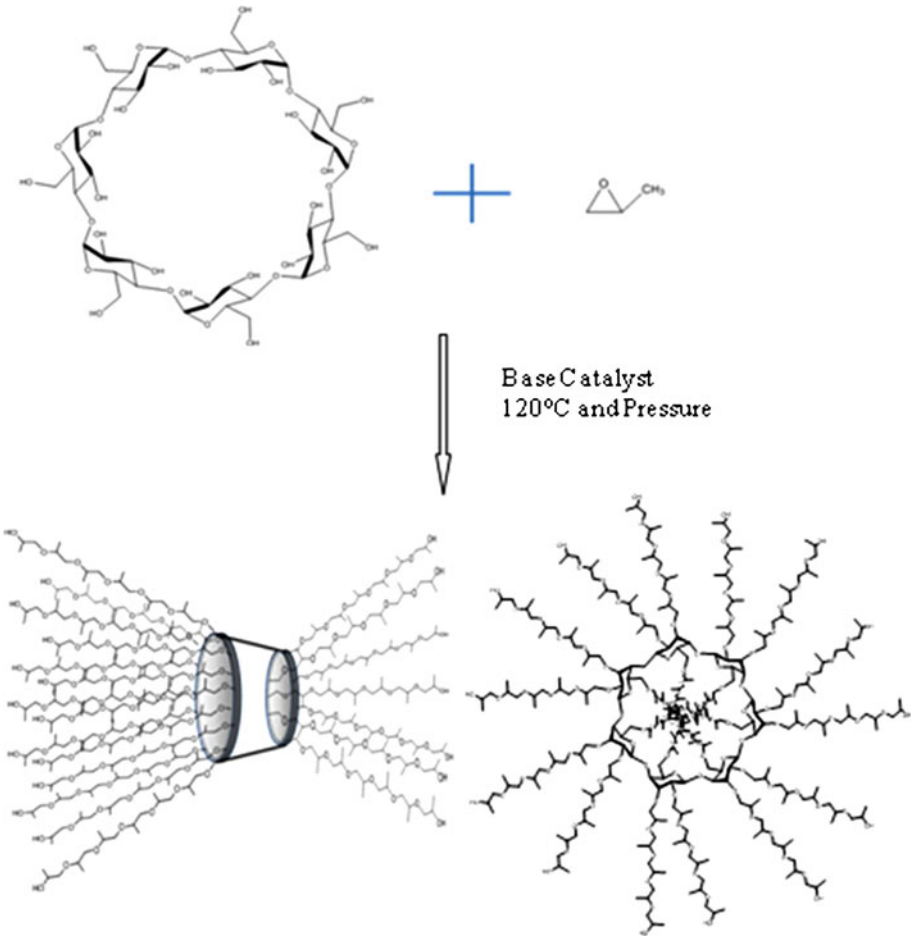

Fig. 1 Scheme of synthesis of star copolymer of β-CD and polypropylene oxide

Haemolysis study [11]

Haemolysis studies were carried out on star polymer and β-CD at higher and lower concentration. Freshly collected human blood was washed three times with an isotonic 0.1 M phosphate buffer saline (PBS) solution (pH 7.4) by centrifugation at 2,800 rpm for 5 min. Star polymer, β-CD and drug polymer complex were diluted with 0.1 M PBS up to a concentration 0.5 and 2.0 mg/mL for each sample. The RBC suspension (0.1 mL) was added to 0.9 mL of each sample. After incubation at 37 °C for 30 min, the samples were centrifuged at 3,000 rpm for 10 min, the supernatant was collected and analyzed for haemoglobin release by spectrophotometric determinations at 416 nm. To obtain 0 and 100% haemolysis, 0.1 mL of RBC suspension was added to 0.9 mL of PBS and distilled water, respectively.

The degree of haemolysis was calculated by the following equation:

$$\%\,\text{Haemolysis} = \frac{(\text{ABS} - \text{ABS}_0)}{(\text{ABS}_{100} - \text{ABS}_0)} \times 100$$

where ABS_{100} and ABS_0 are the absorbances of the solution at 100 and 0% haemolysis, respectively.

Table 1 Optimization of reaction condition for the synthesis of star copolymer of BCD and polypropylene oxide

Trial no.	Mole of β-cyclodextrin	Mole of propylene oxide	Temperature (°C)	Concentration of catalyst (% w/v of β-cyclodextrin)	Remark
I	1	42	80	3	No reaction
II	1	42	80	5	No reaction
III	1	42	80	7	Viscous product was observed with unreacted β-CD
IV	1	42	100	7	Viscous product was observed with unreacted β-CD
V	1	42	120	7	Viscous product was observed with no unreacted β-CD

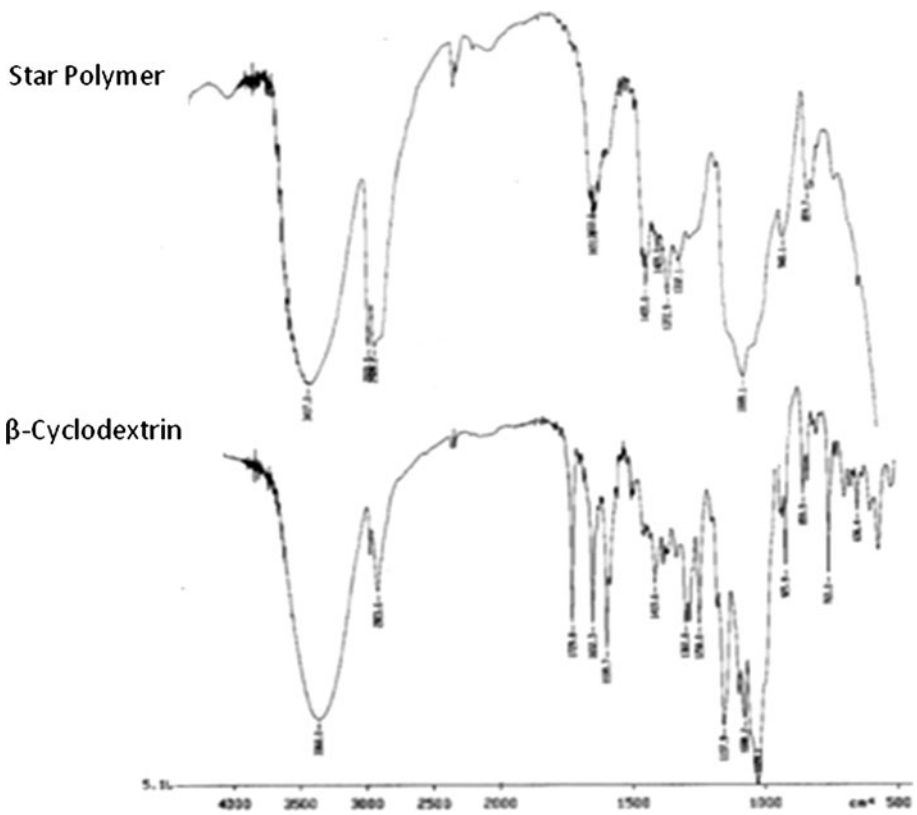

Fig. 2 FTIR spectra of star polymer (B-IV) and β-CD

Result and discussion

Synthesis of star copolymer

The star amphiphilic polymers of β-CD and polypropylene oxide were synthesized successfully by ring opening polymerization of propylene oxide as shown in the Fig. 1. In the reaction each hydroxyl group of β-CD reacts with the propylene oxide to give 21-armed star polymer of β-CD at high temperature and pressure, catalyzed by base. The molecular weight of the star polymer was increased by repeating same polymerization cycle. Table 1 gives the optimized reaction conditions involved in the synthesis of star polymers.

Characterization of star copolymer

NMR, FTIR and DSC

Figure 2 shows the FTIR spectra of β-CD and star polymer respectively. The characteristic peaks at 3417.0, 2970.5, 2928.2, 1651.9 and 1601.9 cm^{-1} were observed for the OH stretch, CH stretch of CH_3, CH stretch of CH_2, C–O stretch of ring, C–O stretch of chain respectively when compared β-CD spectra.

In the ^{1}H NMR of star polymer (Fig. 3), prominent overlapped signal for the CH_3 proton of polypropylene oxide chain was observed at 1.097 ppm indicating the long chain length of polypropylene oxide. The other characteristics signals were observed at 3.36, 3.37, 3.39, 3.42, 3.44, 3.48, 3.50, 3.89, 5.2 ppm for the protons H^2 H^3, $–CH_2–$ of Chain, $–CH_2–$ of Chain, Terminal $–CH_2–$, Terminal $–CH_2–$, Terminal $–CH_2–$, H^3,H^5,H^4 respectively.

In the ^{13}C NMR of star polymer (Fig. 4) shows the prominent signals for the CH_3 and terminal CH_3 of polypropylene oxide chain at 15.87 and 18.44 ppm respectively. The other signal related to $–CH_2–$ of chain, $–C^1–$ of ring, $–C^2–$ of ring, $–C^3–$ $–C^4–$ of ring, $–C^5–$ of ring were observed at 58.41, 164.68, 72.13, 77.41, 72.11 ppm respectively.

The DSC thermogram (Fig. 5) of star polymer showed an endothermic peak which corresponds to melting point of the star polymer.

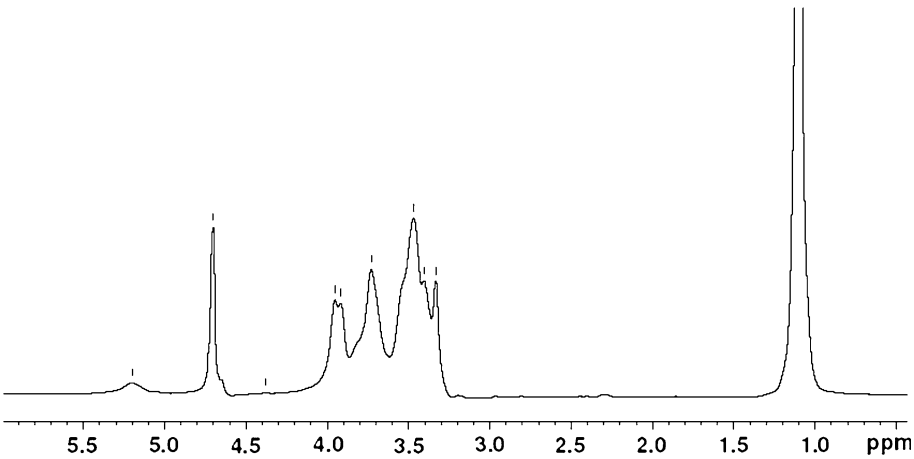

Fig. 3 1H spectra of star polymer (B-IV)

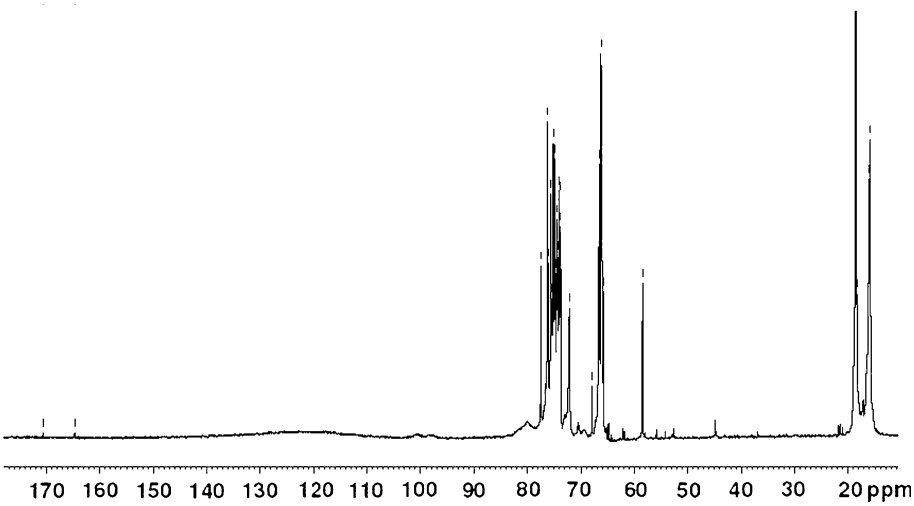

Fig. 4 13C spectra of star polymer (B-IV)

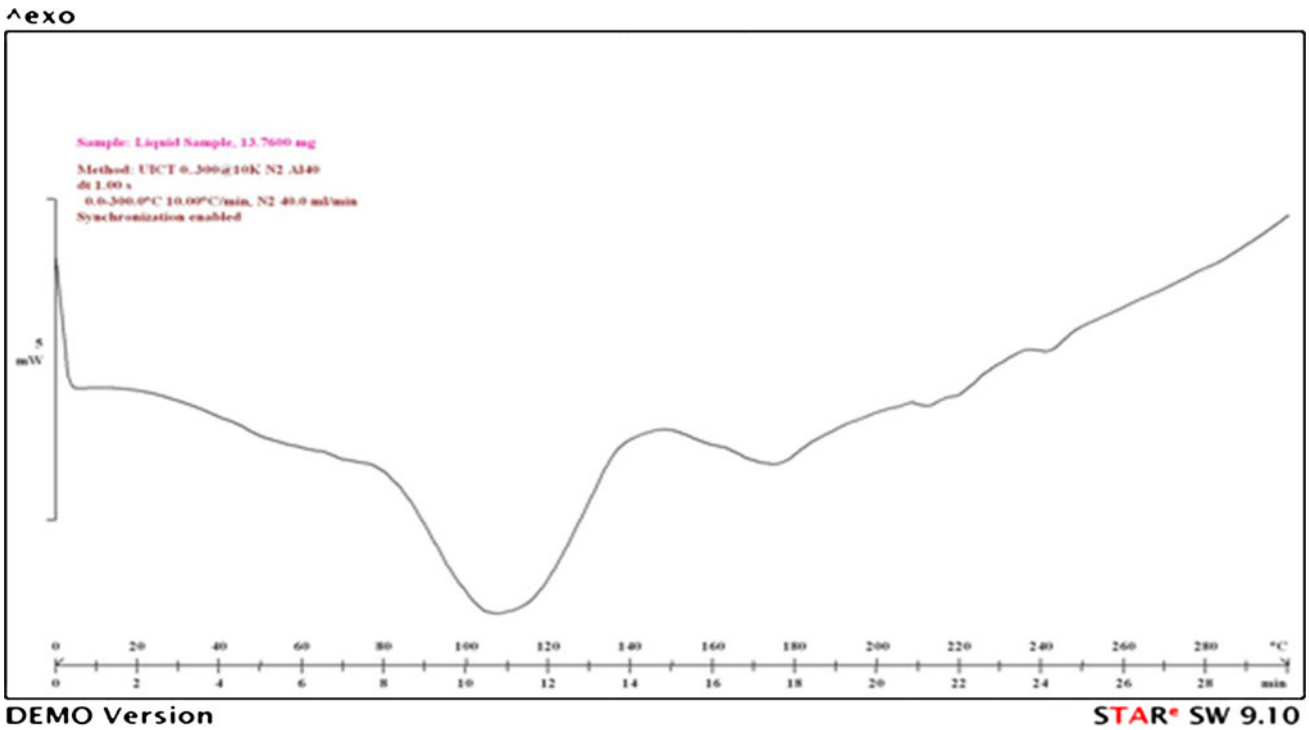

Fig. 5 DSC thermogram of star polymer (B-IV)

Table 2 Average molecular weight star polymers

Polymer sample	Hydroxyl number (mg of KOH/g of sample)	Molecular wt. of an anhydroglucose unit	Average molecular wt. of polymer	Avg. no. of propylene oxide per anhydroglucose unit
B-II	154.257	1090.90	7636	16.01
B-III	124.54	1351.37	9459.61	20.50
B-IV	56.66	2918.83	20431.81	48.92

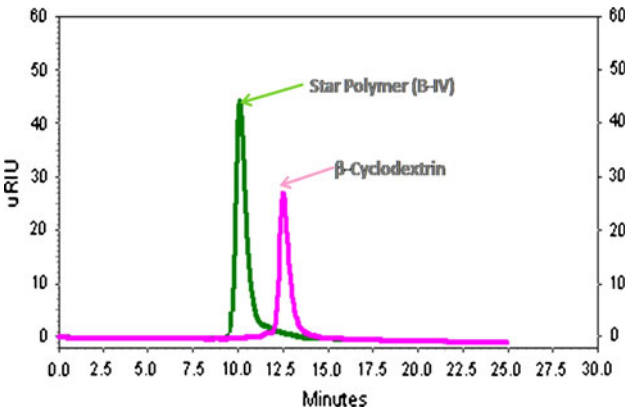

Fig. 6 Size exclusion chromatogram of star polymer (B-IV)

Table 3 Solubility of star polymers in different solvents

Solvent	Polymer B-IV	Polymer B-III	Polymer B-II
Water	[a]	[a]	[a]
Methanol	[a]	[a]	[a]
Ethanol	[a]	[a]	[a]
DMSO	[a]	[a]	[a]
DMF	[a]	[a]	[a]
Diethyl ether	[a]	[a]	[a]
Chloroform	[a]	[a]	[a]
Dichloromethane	[a]	[a]	[a]
Cyclohexane	[a]	[a]	[a]

[a] Soluble

Average molecular weight and molecular weight distribution

The approximate molecular weight of star polymers was determined by hydroxyl value determination. Table 2 gives molecular weight of an anhydroglucose unit, average molecular weight of polymer and average number of propylene oxide per anhydroglucose unit of three different star polymers.

Monomodality of size exclusion of chromatogram (Fig. 6) shows that formed star polymer was free from homopolymers and other impurities and uniform molecular weight distribution [12].

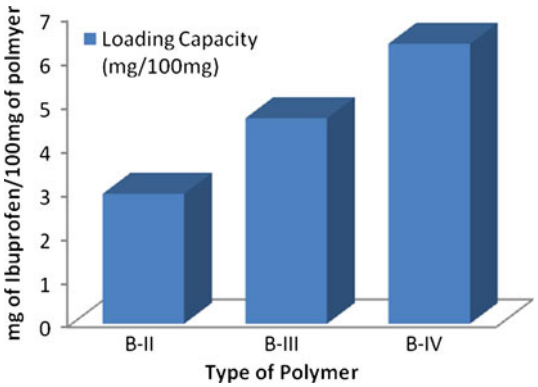

Fig. 7 Loading capacity of star polymers

Solubility of the star polymer

The star polymer of different molecular weight showed the solubility in the both polar as well as non polar solvents, this confirms the amphiphilic nature of star polymers (Table 3), it is due to hydrophobic nature of propylene oxide chain and hydrophilic nature of cyclodextrin.

Loading capacity of the star polymer

When the loading capacity of star polymers of different molecular weight was studied, it was observed that with increase in the molecular weight, increase in the loading capacity of polymers was observed (Fig. 7), probably groups of polypropylene oxide chains may be involved in the complexation of star polymer and Ibuprofen. The formed Ibuprofen and star polymer complex was characterized by FTIR and ^{1}H NMR. The FTIR spectra of drug (Fig. 8) showed the characteristics peaks of OH-str

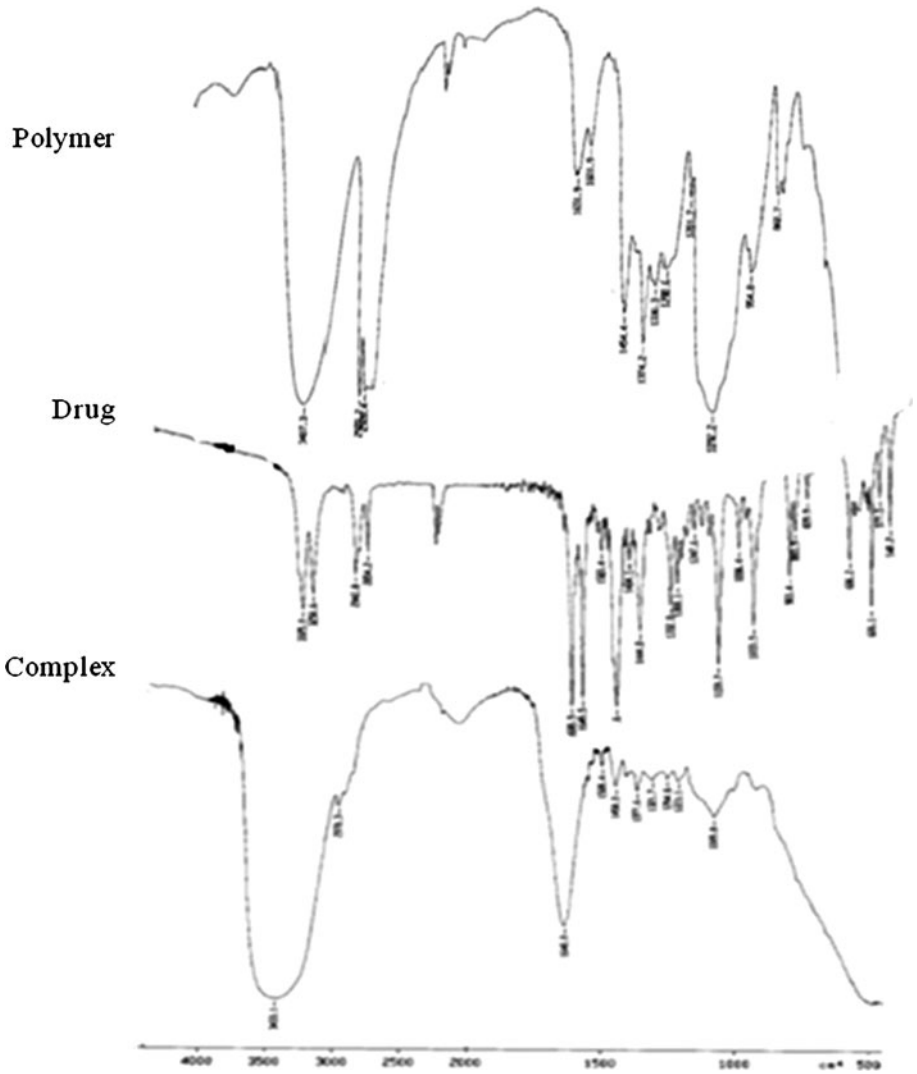

Fig. 8 FTIR spectra of star polymer (B-IV), Ibuprofen and complex

(3,434.9) of carboxyl acid group, CH-str (2955.9, 2921.2, 2869.3) of CH, CH_2, CH_3 of alkyl chain and C=O-str (1,719.8) of carboxyl group. The FTIR spectra of complex (Fig. 8) showed that the most of peaks of Ibuprofen-polymer complex has been masked by the peaks of polymer indicating the formation of complex but one new peak was observed which is may due to asymmetric stretching of COO^- of Ibuprofen. The NMR of drug showed peaks corresponding CH_3 of alkyl chain (0.818, 0.808), CH_3 of acid chain (1.331, 1.321), CH of alkyl chain (1.796, 1.786, 1.777, 1.767, 1.758), CH_2 of alkyl chain (2.422, 2.412), CH of acid chain (3.566, 3.556, 3.546, 3.536) and phenyl protons (7.212, 7.201, 7.158, 7.147). In the ^1H NMR study, signal corresponding to CH_3 (0.05), CH_3 (0.02), CH_2 (0.01), Ph-CH (0.1) of Ibuprofen shows shifting in the δ-values indicating involvement of groups in the complexation (Fig. 9).

Phase solubility study of the star polymer

In the phase solubility study, it was observed that as molecular weight of star polymers increases the solubilizing ability of polymer increases (Fig. 10). This is due to the entrapment of Ibuprofen molecules within chains of polypropylene oxide. All graphs of phase solubility shows positive deviation from linearity indicating AP-type of

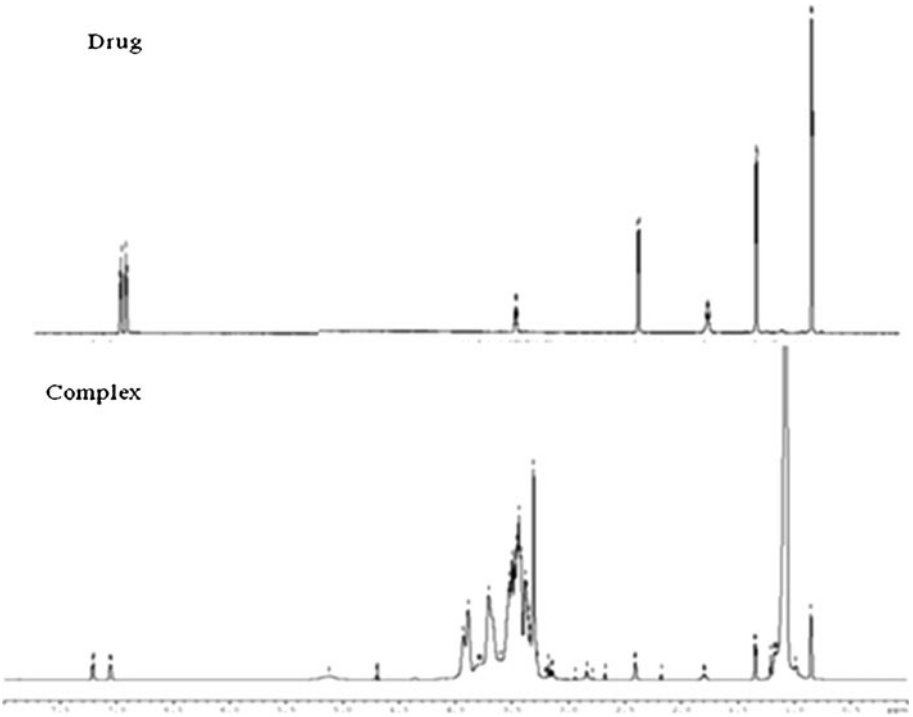

Fig. 9 1H spectra of Ibuprofen and complex

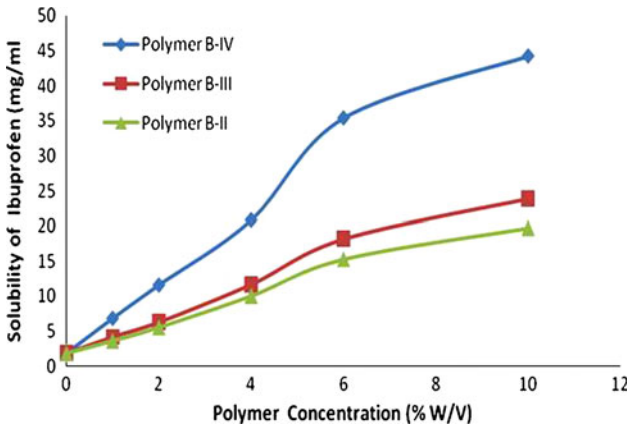

Fig. 10 Phase solubility profile of star polymer

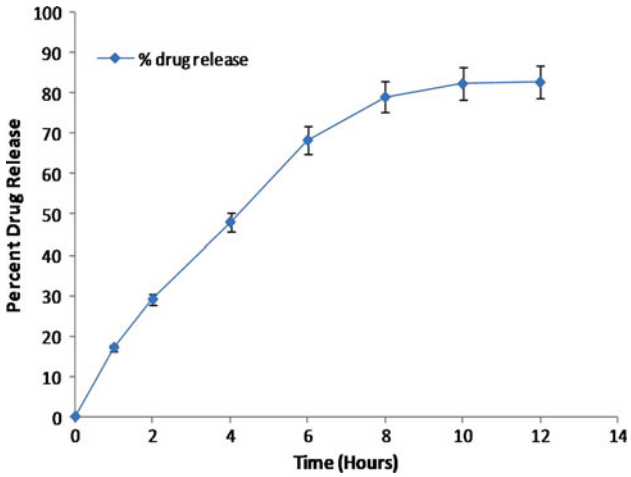

Fig. 11 Drug release profile of star polymer (B-IV)

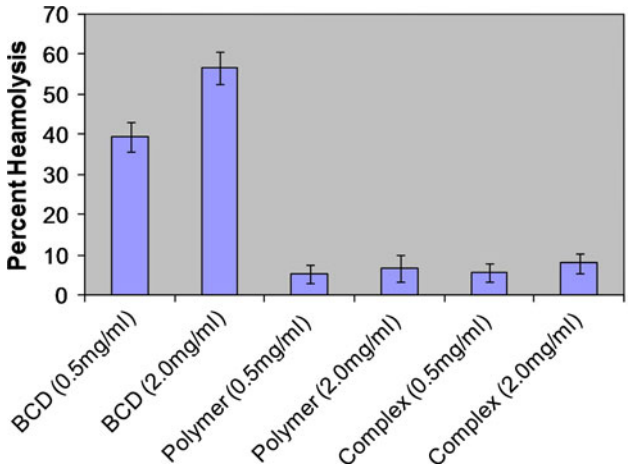

Fig. 12 Haemolytic activity of star polymer (B-IV) and Complex

solubility of profile suggestive of exclusion complexation or higher order complexation [13].

Drug release study

The release of drug from the drug–polymer complex was evaluated at 7.4 pH. The controlled drug release was observed for the period of 12 h (Fig. 11). In the 12 h period, more than 80% drug release was observed. The polypropylene oxide chains of star polymer prevent the rapid release of complexed drug. In the 12 h period, more than 80% drug release was observed. This may be useful for the parenteral administration of Ibuprofen.

Haemolysis study

To evaluate the haemolytic activity, haemolysis test was performed at lower and higher concentration of star polymer and compared with β-CD. It was observed that star polymer and polymer–drug complex were non-toxic at low as well as higher concentration as compared to β-CD (Fig. 12).

Conclusion

The amphiphilic star copolymers of β-cyclodextrin and polypropylene oxide can be synthesized by ring opening polymerization with desired molecular weight distribution. Synthesized star polymers are biocompatible and able to form complex with guest molecules, provides enhancement in solubility with controlled release. These features of star polymer could be beneficial as nanocarrier in cancer therapy.

References

1. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
2. Hedges, A.R.: Industrial application of cyclodextrins. Chem. Rev. **98**, 2035–2044 (1998)
3. Szente, L., Szejtli, J.: Highly soluble cyclodextrin derivatives, chemistry, properties, and trends in development. Adv. Drug Deliv. Rev. **36**, 17–28 (1999)
4. Macarak, E.J., Kumor, K., Weisz, P.B.: Sulfation and hemolytic activity of cyclodextrin. Biochem. Pharmacol. **42**, 1502–1503 (1991)
5. Shiotani, K., Uehata, K., Irie, T., Uekama, K., Thompson, D., Stella, V.J.: Differential effects of sulfate and sulfobutyl ether of β-cyclodextrin on erythrocyte membranes in vitro. Pharm. Res. **12**, 78–84 (1995)
6. Szeman, J., Ueda, H., Szejtli, J., Fenyvesi, E., Machida, Y., Nagai, T.: Complexation of several drugs with water-soluble cyclodextrin polymer. Chem. Pharm. Bull. **35**, 282–288 (1987)
7. Fenyvesi, E.: Cyclodextrin polymers in the pharmaceutical industry. J. Incl. Phenom. **6**, 537–545 (1988)
8. Fabrice, L., Fabienne, P., Myriam, M.: Binding of ketoprofen enantiomers in various human albumin preparations. J. Pharm. Biomed. Anal. **23**, 793–802 (2000)

9. Roberta, E.G., Ronald, J.K.: Cyclodextrin polyethers and their production. US Patent 3,459,731 (1969)
10. Jones, S.A., Brown, M., Martin, G.P.: Determination of polyvinyl alcohol using gel filtration liquid chromatography. Chromatography **59**, 43–46 (2004)
11. Quaglia, F., Ostacolo, L., Mazzaglia, A., Villari, V., Zaccaria, D.: The intracellular effects of non-ionic amphiphilic cyclodextrin nanoparticles in the delivery of anticancer drugs. Biomaterials **30**, 374–382 (2009)
12. Adeli, M., Zarnegar, Z., Kabiri, R.: Amphiphilic star polymers containing cyclodextrin core and their application as nanocarrier. Eur. Polym. J. **44**, 1921–1930 (2008)
13. Loftsson, T., Duchene, D.: Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. **329**, 1–11 (2007)