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Study of branched cationic β-cyclodextrin polymer/indomethacin complex and its release profile from alginate hydrogel

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ABSTRACT

A series of branched cationic β-cyclodextrin polymers (CPβCDs) with designed chemical structures were synthesized from β-cyclodextrin (β-CD), epichlorohydrin (EP) and choline chloride (CC). Indomethacin (IDM), an anionic drug, was chosen as a model drug to evaluate the drug loading capacities of CPβCDs. The formation of IDM-CPβCD complex was confirmed by ^{1}H NMR and DSC. Phase solubility studies and Job plots indicated that CPβCDs can solubilize IDM up to 100 times of its intrinsic solubility in a 1:1 complexation form. Mechanism studies with the help of adamantane revealed that the effective complexation is a combination of inclusion complexation, charge interaction and hydrophobic interaction. In addition, IDM-CPβCDs loaded alginate hydrogels were prepared and obtained controllable release profile in dissolution tests. The tunable structures of CPβCDs make them promising drug carriers with superior drug loading capacities and controllable drug release abilities.

1. Introduction

Cyclodextrins (CDs) consist of 6 (α-), 7 (β-) or 8 (γ-) glucopyranose units and their internal cavities could form inclusion complexes with hydrophobic guest molecules (Szejtli, 1998). CDs are widely used in pharmaceutics to enhance drug solubility, stability and bioavailability, reduce volatility and mask unfavorable effects of the drugs. They could also modify drug release profile from many controlled release drug delivery systems (Loftsson and Brewster, 1996; Hedges, 1998; Hirayama and Uekama, 1999; Davis and Brewster, 2004).

Various CD derivatives have been developed during the past decades, which extensively expanded the applications of CDs and overcome the serious drawbacks of parent CDs such as low water solubility and toxicity (Irie and Uekama, 1997). For instance, substitution of their hydroxyl groups may disrupt the hydrogen bonds and significantly increase their aqueous solubility (Szejtli, 1998; Loftsson and Duchêne, 2007). Among them, methylated β-CD, hydroxypropylated β-CD and sulfobutyl ether β-CD are the most familiar CD derivatives with commercially available products (Davis and Brewster, 2004).

Two categories of CD derivatives are of special interest, i.e., charged CDs and CD polymers. Sulfate and sulfobutyl ether β-CDs are anionic derivatives, which possess significantly lower membrane disrupting ability than the native β-CD (Shiotani et al., 1995). Meanwhile, cationic derivatives such as quaternary ammonium β-CDs has been reported to induce selective extraction of lipid other than cholesterol from cell membrane(Zhong et al., 2001), and additional introduction of alkyl chain could further diminish their toxicity (Binkowski-Machut et al., 2006). Charged CDs have different complexation and solubilization abilities compared with other CD derivatives. The ionic interactions outside the cavities and the hydrophobic interactions inside the cavities could both contribute to the drug solubilization. They may form stronger complexes with oppositely charged drugs but weaker complexes with drugs carrying the same type of charge (Másson et al., 1998). On the other hand, CD polymers exhibit superior aqueous solubility and could enhance drug bioavailability (Fenyvesi, 1988). A kind of β-CD polymer crosslinked by epichlorohydrin (EP) with designable molecular weight is more efficient in binding larger guest molecules with the help of the hydrophobic EP fractions or adjacent CD units (Harada et al., 1981; Szeman et al., 1987; Renard et al., 1997). In addition, cationic CD polymers have been extensively researched as effective nonviral vector in gene delivery systems (Yang et al., 2007; Li and Loh, 2008; Li et al., 2010).

In order to combine the predominances of charged CDs and CD polymers, a series of cationic cyclodextrin polymers (CPβCDs) were synthesized in our former work (Li et al., 2004). The CPβCDs utilize choline chloride (CC) to provide cationic groups and epichlorohydrin (EP) to constitute polymer chains. Hemolysis test (Li et al., 2004) and MTT assay (Gil et al., 2009) reveal that CPβCDs exhibit good hemocompatibility to human erythrocytes and no cytotoxic-

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ity to bovine brain microvessel endothelial cell at a concentration up to 500 μg/mL, respectively. Also, CPβCDs have been approved effective to enhance drug bioavailability and acquire targeted drug delivery, such as across the blood–brain barrier (Qian et al., 2008; Gil et al., 2009).

It is well known that CDs and their derivatives could modify drug release behaviors through different administration routes (Uekama et al., 1998; Hirayama and Uekama, 1999). The hydrophilic and ionizable CDs can serve as potent drug carriers in the immediate and delayed release formulations, respectively, while the release rate of water soluble drugs can be retarded by hydrophobic CDs (Hirayama and Uekama, 1999). In addition, they are often used to modulate drug release behaviors from polymeric matrices, which have exhibited various advantages in controlling the drug release behaviors (Bibby et al., 2000). Hydrogel is one kind of crosslinked polymeric network that has much higher water-uptake capacities than normal hydrophilic polymeric matrices. It could also be designed as stimuli-responsive, pulsed or targeted drug delivery systems (Lin and Metters, 2006). For example, poly(*N*-isopropyl acrylamide)-based hydrogels are thermal-responsive thus are able to establish stimuli-regulated pulsed drug release system (Kikuchi and Okano, 2002). And another work has demonstrated a supermolecular hydrogel system via cooperation effect of complexation of PEO segments with α-cyclodextrin and the hydrophobic interaction between PHB blocks, which is thixotropic and could be utilized as injectable drug delivery system (Li et al., 2006). Drug release behaviors from the polymeric matrices are governed by various mechanisms such as diffusion, swelling or erosion (Arifin et al., 2006). Accordingly, CDs additives in these polymeric matrices could modulate the drug release profiles by: their channeling effect could promote erosion of the polymeric matrices, whereas the form of drug complexation may restrict drug diffusivity (Rosca and Vergnaud, 2008).

Indomethacin (IDM) is a nonsteroidal anti-inflammatory drug that is widely used as anodyne in the treatment of rheumatoid arthritis, as well as in other degenerative joint diseases. IDM is negatively charged and has a poor aqueous solubility. In this work, a series of IDM-CPβCDs complexes were prepared and the effect of CPβCD structure on IDM loading ability was inspected. Moreover, CPβCDs are cationic polymeric excipients, they may exert complicated interactions with the matrices and the drug than parent β-CD when applied in hydrogel systems. In order to investigate these interactions, calcium alginate (CaAlg) hydrogels containing different IDM-CPβCDs complexes were also prepared and evaluated by *in vitro* dissolution studies.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD) and epichlorohydrin (EP) were purchased from Tianjin Bodi Chemical Co., Ltd., China. Choline chloride (CC) and sodium alginate (Alg) were obtained from Chengdu Kelong Chemical Reagent Plant, China. Indomethacin (IDM) was provided by Wuhan Hezhong Bio-Chemical Co., Ltd., China. Adamantane (AD) was purchased from Aladdin Reagent (China) Co., Ltd. All the other reagents and solvents were of AR grade and used as received without further purification. Distilled water was used throughout.

2.2. Syntheses and characterizations of CPβCDs

2.2.1. Syntheses of CPβCDs

CPβCDs were synthesized following the procedure reported in a previous work (Li et al., 2004). In this work, the molar feeding ratios of β-CD/EP/CC were 1/15, 1/15/4, 1/15/6 and 1/15/10, respectively. A typical synthesis procedure of CPβCD1/15/4 is described below: 1.0 g NaOH was dissolved in 20 mL of water, and then 5.675 g β-CD were dissolved in the sodium hydroxide solution. The solution was electromagnetically stirred at 25 °C for 24 h in a water bath. After that, 2.792 g CC were fed into the solution rapidly then 6.940 g EP were added dropwise at a flow rate of 0.1 mL/min. After the completion of EP feeding, the mixture was heated to 60 °C and kept for 2 h. The reaction was quenched by neutralization with an aqueous hydrochloride acid solution (3N). The solution obtained was dialyzed for 24 h with a dialysis membrane of molecular weight cut-off 1000. The solution obtained was evaporated and the solid was pulverized into fine powder.

2.2.2. Characterizations of CPβCDs

Proton nuclear magnetic resonance (^{1}H NMR) spectra were conducted in D_2O using a Bruker AV II-400 spectrometer operated at 400 MHz. The mass fractions of β-CD, EP and CC in the polymers were calculated from the integral areas of protons (Table 1).

The apparent average hydrodynamic radii (*Rh*) of CPβCDs in phosphate buffer solution (PBS) (pH = 7.4) at a concentration of 50 mg/mL at room temperature were measured by a dynamic lightscattering (DLS) using a Nanosizer (Zetasizer Nano ZS, Malvern, UK). The zeta potential was measured by Malvern zetasizer and Particle Analyzer (Malvern Instruments). The CPβCDs were dissolved in deionized water before measurement without pH adjustation. The value was recorded as the average of five measurements.

2.3. Preparation and characterization of IDM-CPβCDs complexes

2.3.1. Preparation of IDM-CPβCDs complexes

The IDM-CPβCDs complexes were prepared by kneading method according to the procedures described by Lin et al. (1991) and Arias et al. (1997). Equal quantity of IDM and CPβCD were separately triturated for 1 min using a mortar with a pestle, then mixed together and manually ground for 40 min. During the process, an appropriate amount of water was added to maintain suitable consistency. The obtained paste was dried in a vacuum oven at 37 °C for 24 h and then washed with adequate amount of cold acetone. Then, the complex was dried again and pulverized into fine powder.

2.3.2. Loading efficiency

The obtained IDM-CPβCDs complexes were assayed for drug loading efficiencies (LE) (Table 1). Proper quantities of complexes

Table 1
Properties and IDM loading capacities of CPβCDs.

Sample	CD content[a] (%)	EP content[a] (%)	CC content[a] (%)	*Rh*[b] (nm)	Zeta potential[c] (ζ, mV)	LE, exp (%)	LE, th[d] (%)
CPβCD1/15	77.66	22.34	–	70.4 ± 3.3	−13.8 ± 1.2	27.97	19.7
CPβCD1/15/4	77.79	19.91	2.30	64.2 ± 2.1	6.3 ± 3.5	35.23	19.7
CPβCD1/15/6	47.99	41.32	10.69	86.1 ± 4.2	12.8 ± 3.6	35.29	13.1
CPβCD1/15/10	52.43	28.27	19.30	81.2 ± 3.6	15.1 ± 2.4	36.65	14.2

[a] The CD, EP and CC contents of each polymer was calculated according to the ^{1}H NMR data.
[b] Hydrodynamic Radii, measured by DLS, $N = 5$, ± S.D.
[c] $N = 5$, ± S.D.
[d] Calculated assuming that only the CD cavities of CPβCDs contribute to the load efficiency of IDM by forming 1:1 complex.

were dissolved in excessive hydroalcoholic solution (1:1, v/v), and were submitted to ultraviolet analysis after a complete dissolution. The LE is defined as:

$$\mathrm{LE} = \frac{W_d}{W_t} \times 100\%$$

where W_d and W_t represent the quantity of drug in the complex and the total complex, respectively.

2.3.3. NMR studies

^{1}H NMR spectra of IDM-CPβCDs complexes were conducted following the conditions described previously. The spectrum of IDM was recorded using NaOD/D_2O as a solvent.

2.3.4. DSC analyses

CPβCDs, IDM and IDM-CPβCDs complexes were analyzed by differential scanning calorimetry (DSC) to confirm complexation via a TA Q20 instrument. Samples were dried under vacuum at 60 °C for 24 h before analysis. 4–5 mg of each powder was filled into an aluminum pan and was heated at a scanning rate of 10 °C/min from 50 °C to 250 °C.

2.4. Interactions between IDM and CPβCD in solution

2.4.1. Phase solubility studies of IDM-CPβCDs complexes

Aqueous solution of CPβCD was prepared as CD concentration ranging from 1 to 7%, w/v (calculated according to the ^{1}H NMR data as shown in Table 1). An excess of IDM was added into 2 mL of each CPβCD solution, and then the vessels were sealed and mechanically shaken for 3 d in a 25 °C water bath. The pH values of the solution were 6.0 ± 0.3. Afterwards, the mixture was filtered and the filtrate was diluted for ultraviolet analysis at 320 nm to determine the IDM concentration. All experiments were performed in duplicate. The apparent stability constants ($K_{1:1}$) of the complexes were calculated from the phase solubility diagram according to Higuchi and Connors (1965) method:

$$K_{1:1} = \frac{slope}{S_0(1 - slope)}$$

where S_0 represents the intrinsic solubility of IDM without CPβCDs, and *slope* is the slope of the solubility curve.

2.4.2. Effects of different units of CPβCDs on solubilization of IDM

The inclusion complexation and non-inclusion interactions could be distinguished by adding an excess of AD, which can predominately occupy the CD cavities before inclusion formed between IDM and CPβCDs. An excess of IDM was fed into 10% (w/v) CPβCDs aqueous solution (with or without adding excessive AD previously) and then was mechanically shaken for 3 d. After that, the solutions were filtered and properly diluted for ultraviolet analysis.

2.4.3. Determination of complex stoichiometry (Job plot)

The continuous variation (Job plot) method (Job, 1928) was employed since it could provide a reliable determination of the complex stoichiometry. An equal molar concentration of IDM and CPβCD aqueous solutions ($M = 5 \times 10^{-5}$ mol/L) were mixed, in which the total molar concentration was kept as a constant (i.e., $[\mathrm{IDM}]_t + [\mathrm{CP\beta CD}]_t = \mathrm{M}$), while the molar fraction of IDM (r) varies from 0.1 to 0.9. The solution was mechanically shaken for at least 10 min to form complexes (Dotsikas et al., 2000). Afterwards, ultraviolet analysis was conducted for the absorbance of the solutions in the absence (A_0) and presence (A) of the corresponding concentrations of CPβCD. The product of the absorbance difference ($\Delta A = A_0 - A$) and the total concentration of IDM ($[\mathrm{IDM}]_t$) was plotted against the molar fraction of IDM (r). The stoichiometry of the complex is indicated by the r value corresponding to the maximum of the plot.

2.4.4. Dissolution of IDM-CPβCDs complexes

Dissolution of the IDM-CPβCDs complexes were performed according to the dispersed amount method (Mura et al., 1999): 100 mg of IDM or IDM-CPβCDs complexes containing equivalent drug were added to 300 mL of PBS (pH = 7.4), each solution was stirred with a paddle at 100 rpm and kept at 37 ± 1 °C. 5 ml of each sample was withdrawn and filtrated at fixed time intervals, and was assayed by ultraviolet analysis for IDM concentration. Equal volume of fresh medium was fed back into the solution and the correction for the cumulative dilution was calculated. All experiments were run in triplicate.

2.5. Effects of CPβCDs on the release profile of IDM from the hydrogel system

2.5.1. Preparation of IDM-CPβCD loaded calcium alginate (CaAlg) hydrogel

CaAlg hydrogels containing IDM-CPβCD1/15/6 complex, IDM-CPβCD1/15/4 complex, IDM-β-CD complex or IDM alone were prepared. The drug-carrier complexes were obtained following the kneading procedure (see above) and the quantity of the excipients were about 3 times that of IDM. In the case of IDM alone, it was also ground for a same period of time before mix. Afterwards, 40 mg of IDM or IDM complexes containing equivalent drug were mixed with 1 mL of 2% Alg solution in a mold with a diameter of 17 mm and a height of 10 mm. The mixture was stirred homogeneously and then was sonicated for 30 min to remove air bubbles. Then the mold was slowly filled with 5% $CaCl_2$ solution before covered with a lid to proceed gelation. The mixture was allowed to gelate for 1 h and the $CaCl_2$ solution was refreshed every 20 min. Afterwards, the resulting hydrogel tablets were washed by water and acetone and the surface moisture was removed by air-flow. The tablets had an average diameter of 9.5 mm and an average height of 6.0 mm ($P \leq 0.05$). They were uniform in shape and no disfigurement. The composition of each hydrogel tablet is shown in Table 2.

2.5.2. Characterizations of IDM-CPβCD loaded CaAlg hydrogel

Water content tests were performed by completely desiccate the tablets and evaluate the change of the mass, six repeats were conducted. Entrapment efficiency (EE) was determined by completely dissolve the hydrogel in PBS and then was analyzed by

Table 2
Composition and characterization of IDM loaded CaAlg hydrogel.

Hydrogel with	IDM (mg)	β-CD (mg)	CPβCDs (mg)	Weight[a] (mg)	Water content[a] (%)	EE[b] (%)	Lag time[b] (h)	Release rate[c] (%/h)
IDM	40	–	–	524.9 ± 11.5	88.4 ± 0.6	87.9 ± 3.2	16.4 ± 0.3	9.81 ± 0.03
IDM-β-CD	40	128	–	594.8 ± 15.0	78.2 ± 2.5	81.3 ± 0.2	5.8 ± 0.1	7.38 ± 0.05
IDM-CPβCD1/15/4	40	–	128	680.3 ± 22.3	83.4 ± 0.2	78.3 ± 0.4	15.6 ± 0.3	7.53 ± 0.02
IDM-CPβCD1/15/6	40	–	128	647.6 ± 20.5	86.7 ± 0.5	74.9 ± 0.2	9.3 ± 0.2	8.71 ± 0.09

[a] $N = 6$, ± S.D.
[b] $N = 3$, ± S.D.
[c] IDM release rate following the zero order kinetics (i.e., k_1), $N = 3$, ± S.D.

ultraviolet method. Three parallel samples were performed and an average EE value was calculated as:

$$EE = \frac{AQ}{TQ} \times 100\%$$

where AQ and TQ are the actual and theoretical quantity of drug present in the hydrogel, respectively.

2.5.3. In vitro dissolution studies

Drug release from CaAlg hydrogels was performed in simulated gastrointestinal fluid (according to USP32, without enzymes) under sink conditions. A single hydrogel tablet containing IDM, IDM-β-CD complex or IDM-CPβCD complex was immersed into 500 mL of simulated gastric fluid (SGF) for 2 h and then into simulated intestinal fluid (SIF) for another 22 h. The system was maintained at 37 °C and was mechanically stirred with a paddle at 100 rpm. 5 mL of samples were withdrawn every hour for ultraviolet analysis, and a 5 mL aliquots of corresponding fresh medium were fed back into the dissolution vessel. All dissolution tests were run in triplicate.

3. Results and discussion

3.1. Physicochemical characterizations of CPβCDs

The ^{1}H NMR spectrum of CPβCD1/15/6 (Fig. 1a) shows one peak around 5 ppm assigned to the C-l proton of the glucose unit, two broadened peaks between 3 and 4 ppm corresponding to protons from C-2, 3, 4, 5 and 6 of the pyranose rings of CD, respectively. The peak occurs at around 3.1 ppm is assigned to the methyl groups of CC, indicating the successful introduction of quaternary ammonium group. Based on the integral area of ^{1}H NMR spectra, β-CD, EP and CC contents of each polymer were calculated as shown in Table 1. When CC/β-CD feeding ratio is increased from 4 to 10, the CC content of CPβCDs increases from 2.3% to 19.3% and the zeta potential increases from 6.3 ± 3.5 to 15.1 ± 2.4 accordingly. In general, parent β-CD and neutral CPβCD1/15 have intrinsic negative charge property. Therefore, the cationic charge effect of quaternary ammonium groups of CC outperforms the intrinsic negative charge effect on the charge property of the CPβCDs. On the other hand, since CC links to CD through EP, the total amount of CD content and EP content decrease with the increase of CC feeding ratio. Thus, the chemical structure and charge property of CPβCDs are tunable by varying feeding ratios. Since CPβCDs are branched cationic polymers with CD units, they can complex drugs by both inclusion complexation (CD cavity) and electrostatic interactions (polymeric chain with cationic group), which make them to be potential excipients in pharmaceutical applications.

Table 1 also exhibits the particle size of CPβCDs. The hydrodynamic radii *Rh* of CPβCDs 1/15, 1/15/4, 1/15/6 and 1/15/10 in PBS (pH = 7.4) measured by dynamic light scattering technique at 90° angle were 70.4 ± 3.3, 64.2 ± 2.1, 86.1 ± 4.2, and 81.2 ± 3.6, respectively. Their hydrodynamic sizes do not have significant difference, which may due to the aggregation of several molecules. We also found their hydrodynamic size might decrease gradually in a 6-d period until to a stable state (all around 20 nm, data not shown). Thus, the aggregate of several molecules could be the form of CPβCDs while they are applied as drug carrier in aqueous solution.

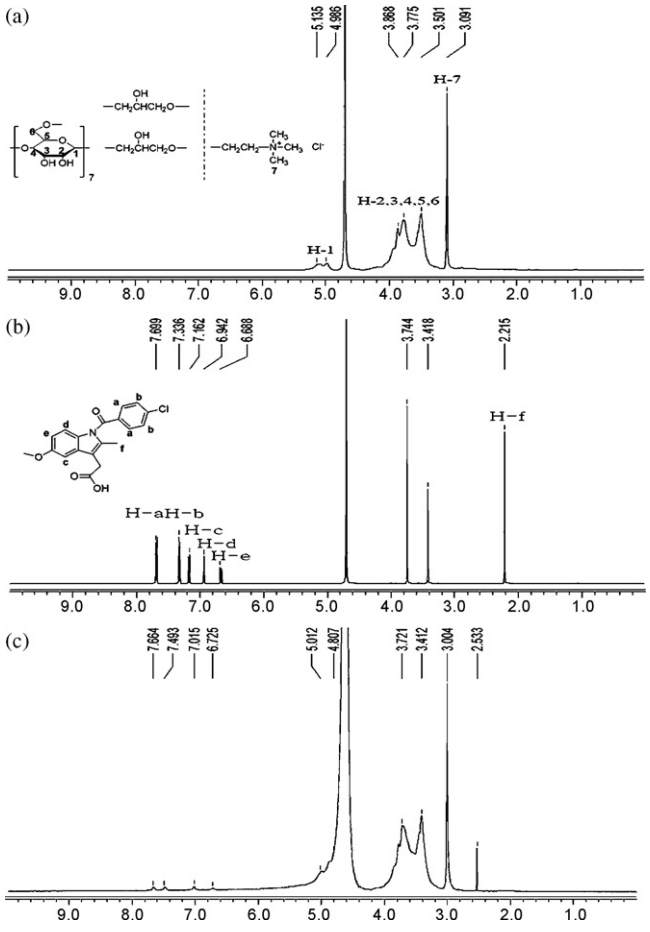

Fig. 1. ^{1}H NMR spectra of CPβCD1/15/6 (a), IDM (b), and IDM-CPβCD1/15/6 complex (c). The spectrum of IDM was conducted using D_2O/NaOD as a solvent.

3.2. IDM-CPβCDs complexes

3.2.1. Characterizations of IDM-CPβCDs complexes

The LE values of complexes with different CPβCDs are shown in Table 1. The theoretical loading efficiencies (LE, th) are calculated assuming that only the CD cavities of CPβCDs are effective in binding IDM molecules by forming 1:1 complex. As for all the four samples, the experimental loading efficiencies (LE, exp) are remarkably higher than the theoretical ones, which confirm that the polymeric chain and/or cationic group make CPβCDs more efficient in drug loading, both by forming inclusion and non-inclusion complexes. In compare with neutral PβCD1/15, the other three samples with cationic groups have much higher drug loading capacities due to additional electrostatic interactions. However, although the increase of CC content from CPβCD15/4 to 15/10 could increase the electrostatic interaction, their experimental LEs do not have significant difference (all around 35–36%). This could because of the decrease of CD and EP content from CPβCD15/4 to 15/10, resulting in fewer inclusion complexation and polymeric chain assistance to drug.

To confirm the presence of drug complex, the ^{1}H NMR spectra of IDM, CPβCD1/15/6 and their complex are shown in Fig. 1. Slightly downfield chemical shifts of protons in CPβCD1/15/6 can be observed, whereas the chemical shifts of protons in IDM are increased, resulting from the complexation of IDM and CPβCD. Also, proportions of integral areas of protons assigned to different part of IDM have been changed. The integral area proportions of protons assigned to the methyl group (H-f, 3H) to that assigned to the p-chlorobenzoyl ring (H-a and H-b, 4H) and the indole ring (H-d and H-e, 2H) are changed from 3:4 and 3:2 to 3:1.7 and 3:1.3, respectively. In addition, the peak of H-c is almost disappeared in the complex (Fig. 1c). These results suggest that both the indole ring and the p-chlorobenzoyl ring are to some extent shielded by CPβCD. It is known that parent CDs are more likely to form inclusion complexes with the p-chlorobenzoyl ring of IDM (Fronza et al., 1996). Thus, the shielding of the indole ring may be attributed to the non-inclusion complexation of hydrophobic EP chains.

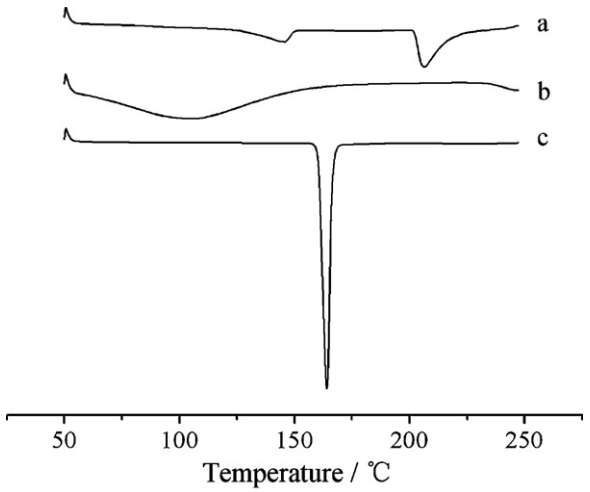

Fig. 2. DSC curves of IDM-CPβCD1/15/6 complex (a), CPβCD1/15/6 (b), and IDM (c).

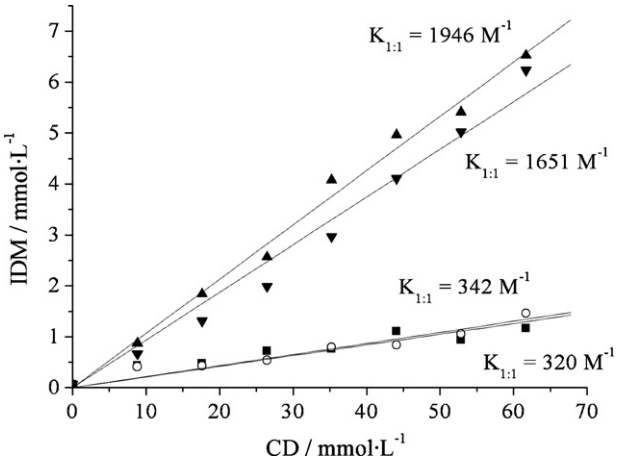

Fig. 3. Phase-solubility diagrams of IDM in aqueous PβCD1/15 (■); CPβCD1/15/4 (○); CPβCD1/15/6 (▲); and CPβCD1/15/10 (▼) solutions. The concentration of CD units in CPβCDs is used as the abscissa.

The DSC curve of IDM-CPβCD complex reveals a complete change compared with CPβCD or IDM alone. As shown in Fig. 2b, an endothermal transition of CPβCD1/15/6 was observed in the range of 57–150 °C, with a peak value at 106.2 °C. This is similar to that of β-CD, which may be attributed to the dehydration of the CD cavities. It is notable that there is no obvious degradation occurs in CPβCD alone within the temperature range scanned. As for the complex, there is no obvious dehydration (Fig. 2a). It indicates that the great mass of CPβCD has formed binary system with IDM. As can be seen, the endothermic melting peak of IDM corresponding to Form I polymorph at around 162.0 °C is disappeared in the curve of IDM-CPβCD complex. It proves that the crystal structure of IDM has been completely destroyed by forming inclusion or non-inclusion complexation with CPβCD. In addition, two endothermal peaks are observed for the IDM-CPβCD complex, one at 145.6 °C and another at 206.0 °C, to which there are no corresponding peaks presented neither for CPβCD nor for IDM alone. The former peak may be attributed to the endothermal transition of the non-inclusion complexes of CPβCD and IDM, and the latter peak is ascribed to the degradation of CPβCD in the complex. The complexation process may have an influence on the physical structure of CPβCD, which brought forward the degradation of CPβCD in the complex. All above information indicate that CPβCDs have formed effective interactions with IDM, which is in accordance with the loading efficiency and ^{1}H NMR studies.

3.2.2. Phase solubility of IDM-CPβCDs complexes

Phase solubility diagrams of IDM in aqueous CPβCDs solutions were prepared using the concentration of CD units in CPβCDs as the abscissa (Fig. 3). The apparent stability constants ($K_{1:1}$) were calculated from the intrinsic solubility (S_0) of IDM.

The $K_{1:1}$ values of IDM in PβCD1/15 and CPβCD1/15/4 are 320 and 342 M^{-1}, respectively, which are lower than that in the native β-CD aqueous solutions under the same pH value, which was reported as 366 M^{-1} (Salústio et al., 2009). It looks in contrast with the findings reported by Harada et al. (1981) that the stability of the inclusion complexes of CD polymers with substrates of two guest parts is much higher than that of β-CD, which is due to the cooperation in binding of the adjacent two CD units on a polymer chain. In our work, the molecular structures of CPβCDs are different from the CD polymers studied by Harada et al. (1981). The branched chains consist of EP may shield the CD cavities through a "self-inclusion" process, similar to the effect of hydrophobic alkyl chains attached on β-CDs (Binkowski-Machut et al., 2006). In addition, the attached quaternary ammonium groups increase the steric hindrance to block the inclusion of IDM. Thus, PβCD1/15 and CPβCD1/15/4 are less effective in binding IDM compared with native β-CD. However, the cationic ammonium groups can solubilize anionic drug molecules through charge interactions, so the apparent stability constant of CPβCD1/15/4 is higher than that of PβCD1/15. When the CC/β-CD feeding ratio is further increased to 6 and 10, the quantity of EP and CC in CPβCDs both increase remarkably. It induces a significant augmentation of the $K_{1:1}$ value, i.e., 1946 M^{-1} for CPβCD1/15/6 and 1651 M^{-1} for CPβCD1/15/10, respectively, nearly 5 times higher than that of parent β-CD. The quaternary ammonium group of CC can form charge interactions with the anionic drug, leading to stronger binding abilities. However, charge interaction is not the only determinant, otherwise the $K_{1:1}$ value of CPβCD1/15/10 should be the highest one. As a matter of fact, CPβCD1/15/6 exhibits the maximum of $K_{1:1}$ value. It might be determined by multiple factors, e.g. inclusion by CD units, hydrophobic interactions by EP chains and charge interactions by cationic groups. Considering that IDM has relatively large molecular size, its inclusion-complexation stability in CD cavities might be relatively low. Nevertheless, the hydrophobic EP chains can enhance the binding abilities of CPβCDs to IDM by stabilizing drug/CD complex. In conclusion, CPβCD1/15/6 possesses the largest total content of EP and CC thus has the highest apparent stability constant.

3.2.3. Solubilization effects of CPβCDs

As shown in Fig. 4, the aqueous solubility of IDM (0.0615 mmol/L) can be significantly improved by complexation with CPβCDs. For example, the solubility of IDM increases to 6.5 mmol/L, which is around 100 times higher than its intrinsic aqueous solubility, while being complexed with CPβCD1/15/6 at a concentration of 10% (w/v). The order of the solubilization ability of different CPβCDs is in accordance with that of the stability constant ($K_{1:1}$).

CPβCDs may solubilize IDM in solution by forming inclusion complexes or decreasing the interfacial tension between drug and water. In order to distinguish the solubilization effect of CD units from that of the cationic CC groups and the hydrophobic EP chains, we introduce adamantane (AD) before complexation process. AD could form very stable complex with β-CD and block its hydrophobic cavity (Miyauchi and Harada, 2004). The IDM solubility in the presence of AD is decreased by 40% in the case of PβCD1/15 and 10–15% in the case of other cationic species (Fig. 4). The larger decrease of the solubilization ability of PβCD1/15 is because that there is no charge interaction present in the neutral β-CD poly-

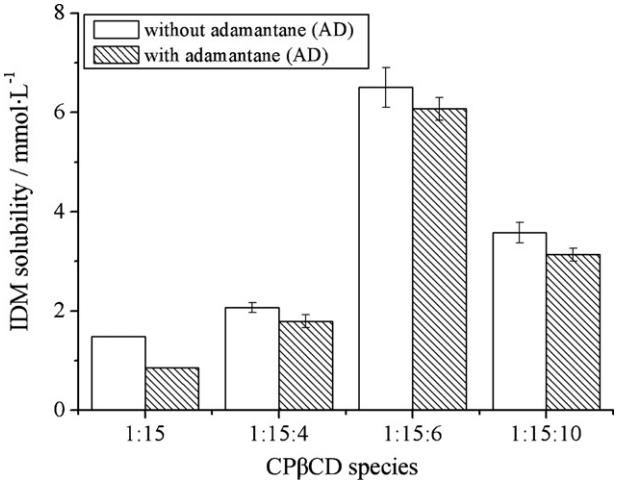

Fig. 4. The solublization effects of CPβCDs.

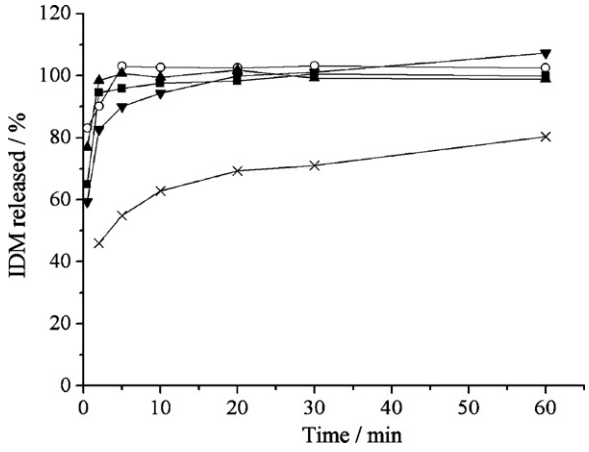

Fig. 6. Dissolution of IDM alone (×) or from PβCD1/15 (■); CPβCD1/15/4 (○); CPβCD1/15/6 (▲); and CPβCD1/15/10 (▼) complex in pH = 7.4 buffer.

mer and the CD content took a relatively large proportion, which can be blocked by AD. As for the other cationic species, the 10-15% decrease of solubilization ability indicates that CD units only play a small portion in solubilizing IDM. Thus, the increase of the solubility of IDM is dominantly due to the non-inclusion complexations formed by charge and/or hydrophobic interactions contributed by the CC and EP units of CPβCDs. However, it is notable that AD is not likely to occupy all CD cavities in solution and the residual CD cavities may still contribute to the solubilization ability of CPβCD, thus the effect of CD cavities on solubilizing IDM is to some extent underestimated.

3.2.4. Determination of complex stoichiometry

Complex stoichiometry is determined by the continuous variation plot. If a parameter ($\Delta A[IDM]_t$) directly related to the concentration of the complex in solution, and is then plotted against the molar fraction of IDM (r), the maximum value of this parameter will occur at $r = m/(m+n)$, where m and n represent the proportion of IDM and CD in the complex (IDM_mCD_n). This indicate that if the stoichiometry of the complex is 1:1, the maximum value of the parameter will be achieved at $r = 0.5$, and if the stoichiometry is 1:2, the corresponding r value will be around 0.3. As shown in Fig. 5, the maximum value of the parameter of IDM-CPβCDs is reached at $r = 0.5$, indicating that the complex stoichiometry is 1:1. A possible explanation is the adjacent CD units of CPβCDs are not likely to bind the same drug molecule because the steric hindrance from CPβCDs' branched polymeric chain prevents them to sustain such interactions. Meanwhile, although the hydrophobic EP chains may form non-inclusion interactions with IDM molecules, it would not influence the continuous variation plot.

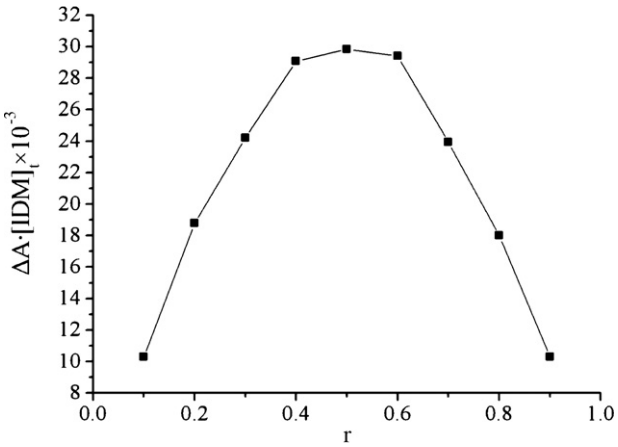

Fig. 5. Continuous variation plot (Job plot) of IDM-CPβCD1/15/6.

3.2.5. Dissolution studies of IDM-CPβCDs complexes

Dissolution studies of IDM complexes were performed under the physiological pH value of intestine where the drug is absorbed (Fig. 6). It is found that about 90% of IDM in the complexes was dissolved within 5 min. This is mainly due to the highly hydrophilic characteristics of CPβCDs. The polymer dissolved much more rapidly than the drug. It could act on the hydrodynamic layer surrounding the complexes, decreasing the interfacial tension between drug and water and resulting in a rapid drug dissolution. The ground procedure may further accelerate the dissolution of IDM, by increasing the total interface area between drug and CPβCD or decreasing drug crystallinity (Mura et al., 2002). In contrast, the pure crystalline IDM dissolved much slower and can not achieve dissolution equilibrium within the time scale. The dissolution studies confirm that CPβCDs are effective excipients in improving IDM dissolution.

According to all above investigations both in solution and solid forms, CPβCD with proper charge density, particular CPβCD1/15/6 in this work, exhibits superior drug loading capacity and IDM drug release profile than parent β-CD and neutral β-CD polymer. The tunable chemical structures of CPβCDs may help them to be promising drug carriers.

3.3. Effects of CPβCD on the release profile of IDM from CaAlg hydrogel

3.3.1. Characterizations of IDM-CPβCDs loaded CaAlg hydrogel

CaAlg hydrogel tablets were prepared to evaluate the effects of CPβCD in controlled release systems. Usually, the gelation rate and resulting shape of CaAlg gels are difficult to control, so they are often used in the form of bead. In the present work, optimized gelating conditions were chosen to obtain tablets with uniform structure. For example, the Alg concentration was fixed at 2% because at a higher concentration the drug release is dramatically restrained, and it is difficult to obtain uniform tablet at a lower concentration (data not shown). The inherent moisture of the hydrogels were not removed intentionally to keep a uniform shape.

The average weight and the water content of different hydrogel tablets are shown in Table 2. The weight difference of IDM-β-CD tablets and IDM tablets is less than 128 mg, which is the weight of β-CD added into the tablet. This shows that part of the drug complexes were lost in the gelating process, which would induce a decrease

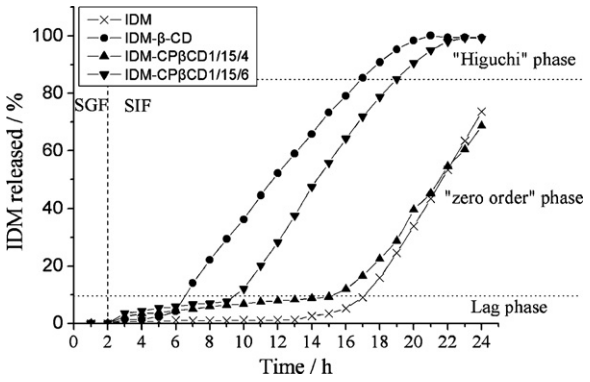

Fig. 7. Dissolution of IDM from CaAlg hydrogels containing IDM alone(×), IDM-β-CD complex (●), IDM-CPβCD1/15/4 complex (▲), and IDM-CPβCD1/15/6 complex (▼).

of the entrapment efficiency. On the other hand, it can be seen that water makes up the largest proportion of the hydrogel tablet. Under a suitable pH condition, the water can easily diffuse IDM to the surface of the tablet (accompanied by a color change from white to yellowish green), thus the following dissolution test should be performed as soon as possible, especially for those containing β-CD or CPβCD excipients.

The entrapment efficiency (EE) of different hydrogel tablets is IDM hydrogel > IDM-β-CD hydrogel > IDM-CPβCD1/15/4 hydrogel > IDM-CPβCD1/15/6 hydrogel. Although the IDM-saturated calcium chloride solution was used for all the samples, CD additives might dissolve into the solution during the gelating process and increase IDM solubility, which induced a decrease of drug content reserved in the hydrogel. Since CPβCDs exhibit better aqueous solubility than β-CD and are more effective in solubilizing IDM, so the corresponding EE value are even lower.

3.3.2. In vitro release profile of IDM-CPβCDs loaded CaAlg hydrogel

CaAlg hydrogels are widely applied in drug controlled release systems due to their swellable and erosion-sensitive characteristics. *In vitro* dissolution of CaAlg hydrogels (Fig. 7) were performed in simulated gastrointestinal fluid in order to study the influence of CPβCDs on the drug release behaviors from polymeric systems.

Drug release mechanisms from the CaAlg hydrogels are modulated by the swelling/erosion process. The hydrogel tablets performed pH dependent swelling behaviors, thus neither hydrogel swelling nor drug release was observed in the first 2 h in SGF. However, an effective drug release was achieved in the following 22 h in SIF. Since the CaAlg hydrogels have superior adhesive ability to mucosal tissues (Gombotz and Wee, 1998), this system could be promising in prolonged intestinal drug administration.

It is notable that an initial lag phase is present for all hydrogels, which is much longer than that of the CaAlg gels in bead or microdisc forms (Puttipipatkhachorn et al., 2005). This is because of the slow disintegration of the tablets' "core-shell" structure. The IDM hydrogel had a lag time of 16.4 ± 0.3 h, of which the IDM-β-CD, IDM-CPβCD1/15/4 and IDM-CPβCD1/15/6 species are shortened to 5.8 ± 0.1 h, 15.6 ± 0.3 h and 9.3 ± 0.2 h, respectively. The CD additives make the "shells" more hydrophilic and vulnerable, thus the lag phase is shortened. The IDM-CPβCD loaded hydrogels exhibit a longer lag phase than the IDM-β-CD species because the branched polymeric structure of CPβCD could cumber the disentanglement of alginate chains.

The lag time data suggest that CPβCDs are reliable in retaining the shape of CaAlg hydrogels and achieving controllable drug release. Moreover, a very slow increase of tablet diameter and drug release was observed during the lag phase. The IDM-β-CD and IDM-CPβCD species displayed a relatively strong swelling ability than the IDM species in the lag phase. And the IDM-CPβCD species swell even faster than the IDM-β-CD one. It may be attributed to the hydrophilic character of CPβCDs that can significantly enhance water uptake into the hydrogel.

When the "shell" of the hydrogel was totally destroyed, a much faster increase of tablet diameter could be seen and an effective IDM release was taken place. Dissolution tests were carried out under sink conditions, so the drug release rate in this period is governed by hydrogel swelling rate, as well as the diffusion of IDM or IDM complexes. A complete swelling of the hydrogel was achieved after 14–16 h for the IDM-β-CD and IDM-CPβCD1/15/6 hydrogels, or 20–22 h for the IDM and IDM-CPβCD1/15/4 species. Afterwards, hydrogel erosion would take place and become the dominant impact factor of drug release. At the same time, the size of the hydrogel began to decrease till disappearance.

Dissolution profiles of the above systems after the lag phase could be described by two types of kinetics (Costa and Lobo, 2001): (1) Zero order kinetics: $Q = k_1 t$; and (2) Square root of time kinetics (Higuchi kinetics): $Q = k_2\sqrt{t}$, where Q is the percentage of drug released at a given time t, k_1 and k_2 are the corresponding rate constants. The dissolution data of IDM can be well fitted into the two kinetics ($R^2 > 0.99$).

The drug release rate is greatly increased during the "zero order" phase (k_1 in Table 2, $R^2 > 0.99$). CD additives may influence drug release rate in two different manners: their "channelling" effect would accelerate disintegration of the polymeric matrices and increase drug release (Bibby et al., 2000), whereas the complexation effect would increase the molecular size of the drug thus decrease diffusivity. Hydrogels containing CD excipients release IDM slower than that containing IDM alone, indicating that the complexation effect is the dominative factor. On the other hand, CPβCDs have larger molecular size thus their "channelling" effect is stronger than that of β-CD. In addition, CPβCDs are more effective on enhancing swelling than β-CDs due to their hydrophilic characteristics, so the drug release from the IDM-CPβCD hydrogels is faster than from the IDM-β-CD species.

And for the IDM-β-CD and IDM-CPβCD1/15/6 hydrogels, a Higuchi phase emerged after 80% of the total drug content was released. The release rate gradually decreased until a final completion of drug release. The completion of drug release is attained at $t = 20$ h and 21 h for the IDM-β-CD species and the IDM-CPβCD1/15/6 species, respectively. However, considering the lag time of the IDM-CPβCD species is 3 h longer than that of the IDM-β-CD one, the average release rate of IDM from IDM-CPβCD hydrogels is faster.

Polymeric matrices are widely applied in controlled release drug delivery systems in multiple dosage forms thus the drug release profile becomes adjustable by various mechanisms such as diffusion, swelling or erosion (Arifin et al., 2006). Excipients like CDs have been used in controlled release systems aiming at modulating the drug release profile (Sangalli et al., 2001). In this work, CPβCDs with appropriate chemical structures are proved to be effective in modulating drug release behaviors, such as the lag time and release rate. Further studies will focus on detailed investigations on the structure-performance relationship of CPβCDs in various controlled release systems.

4. Conclusions

CPβCDs are polymeric β-CD derivatives consisting of CD units, hydrophobic chains (EP) and cationic groups (CC). Their chemical structures are controllable mainly through varying feeding ratios in synthesis. The drug loading capacity is influenced by different units of the polymer via inclusion or non-inclusion complexa-

tions such as hydrophobic and charge interactions, particular for anionic drugs. Investigations on IDM-CPβCDs complexes both in solution and solid forms suggest that CPβCD1/15/6 exhibits highest IDM loading capacity. On the other hand, *in vitro* dissolution studies from CaAlg hydrogels indicate that CPβCDs can modulate IDM release profile from polymeric matrices through interactions among CPβCDs, drug molecules and the matrices. The tunable structure and properties of CPβCDs make them to be promising drug excipients in controlled release drug delivery systems.

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