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Release of paeonol-β-CD complex from thermo-sensitive poly(N-isopropylacrylamide) hydrogels

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ABSTRACT

By preparing an inclusion complex of paeonol (PAE) with β-cyclodextrin (β-CD), this study investigated its release behavior from thermo-sensitive poly(N-isopropylacrylamide) (PNIPAAm) hydrogels. The PAE–β-CD complex was prepared via coprecipitation. According to differential scanning calorimeter (DSC) and X-ray diffraction (XRD) results, the solid PAE–β-CD complex was found in the amorphous state, indicating that each PAE molecule was encapsulated by a β-CD molecule. The change of chemical shifts of H3 and H5 in proton nuclear magnetic resonance (H NMR) spectra indicated that PAE was inside the CD cavity. PNIPAAm hydrogels containing different cross-linker contents were then synthesized and had a similar lowest critical solution temperature (LCST) of around 33 °C. Experimental results of swelling and deswelling indicated that increasing the cross-linker content of the hydrogel decreased the swelling ratio and increased the water retention. According to experimental results of PAE–β-CD complex release, the release rate at 45 °C (>LCST) was higher than at 25 °C (<LCST). Moreover a lower cross-linker content the hydrogel contained implies a higher rate of PAE–β-CD complex release. Above results suggest that the release of PAE–β-CD complex is related to the volume contraction of the hydrogel, which is affected by hydrogel compositions and release temperatures.

1. Introduction

As three-dimensional, cross-linked networks of water-soluble polymers, hydrogels can be made from a water-soluble polymer, encompassing a wide range of chemical compositions and bulk physical properties (Osada et al., 2004; Emileh et al., 2007). Consequently, hydrogels are commonly used in clinical practice and experimental medicine for drug delivery applications (Hoffman, 2002; Hoare and Kohane, 2008). However, hydrogels are limited in terms of loading of hydrophobic drugs due to their hydrophilic nature.

Recent studies have synthesized many hydrogels covalently bonded with cyclodextrin (CD) moiety to increase the loading of hydrophobic drugs by copolymerizing CD-containing vinyl monomer with water soluble monomers (Andrade-Vivero et al., 2007; Rosa dos Santos et al., 2008; Zawko et al., 2008; Zhang et al., 2008), grafting CD to the hydrogel (Liu and Fan, 2005) or cross-linking directly using CD and digylcidylethers to form a hydrogel (Rosa dos Santos et al., 2007, 2009; Rodriguez-Tenreiro et al., 2007). This is owing to that CDs, cyclic oligosaccharides whose molecules have hydrophilic outer surfaces and a hydrophobic cavity at the center (Fig. 1(a)), can function as host molecules to include hydrophobic drugs (quest molecules) to form water-soluble CD-drug complexes (Brewster and Loftsson, 2007; Van Axel Castelli et al., 2008; Vyas et al., 2008; Yuan et al., 2008).

In addition to these chemically CD-modified hydrogels, Kanjickal et al. (2005) and Quaglia et al. (2001) simply loaded the CD-drug complexes into the polyethylene glycol hydrogels for drug release applications. Other studies adopted the same method for mucoadhesive gels (Bilensoy et al., 2007; Cevher et al., 2008) and nanoparticles (Sajeesh and Sharma, 2006; Trapani et al., 2008). The feasibility of this method to increase hydrophobic drug loading in hydrogels is of interest because directly loading CD-drug complexes into the hydrogels is versatile and flexible for practical applications.

Paeonol (2′-hydroxy-4′-methoxyacetophenone, PAE), as shown in Fig. 1(b), is the main active compound of the Paeonia lacti-

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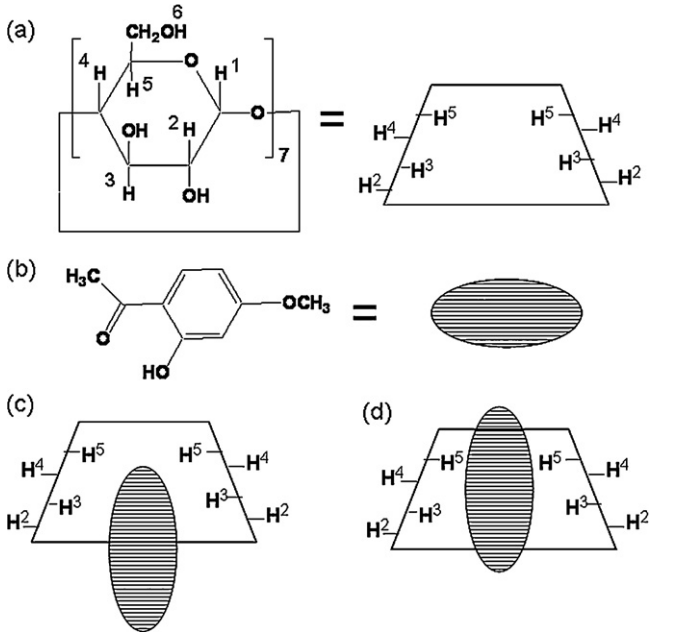

Fig. 1. Chemical structures: (a) β-CD and (b) PAE; and possible models of entry of PAE into β-CD cavity: (c) PAE partially inside β-CD cavity and (d) PAE deeply inside β-CD cavity.

flora Pallas, a traditional Chinese herb used commonly in Asia and Europe. PAE has antioxidant and anti-inflammatory activity as well as the ability to suppress tumor formation (Chung, 1999; Chou, 2003; Nizamutdinova et al., 2007). PAE can also inhibit melanin synthesis and down-regulate melanin transfer (Xie et al., 2007), whose effects can be exploited in cosmetic applications. Despite its many features that make it appropriate for potential medical uses, PAE is hydrophobic and has a low aqueous solubility, possibly limiting its range of applications by using hydrogels for delivery.

In this study, a new delivery system for hydrophobic PAE is prepared, in which PAE is complexed with β-CD to increase its solubility, followed by direct loading to a thermo-sensitive poly(N-isopropylacrylamide) (PNIPAAm)-based hydrogel. By exhibiting the lowest critical solution temperature (LCST) at around 33 °C (Lee and Yuan, 2002; Eeckman et al., 2004; Zhang et al., 2004; Lee and Yeh, 2005; Salehi et al., 2009), this hydrogel is expected to control the release behavior of PAE–β-CD complex at body temperature by its drastic shrinkage in response to thermal stimuli. Therefore, the preparation and characterization of the PAE–β-CD complex, swelling–deswelling properties of hydrogel and release of PAE–β-CD complex from these hydrogels are investigated in this study.

2. Materials and methods

2.1. Materials

PAE, β-CD, sodium phosphate dibasic (Na_2HPO_4), potassium phosphate monobasic (KH_2PO_4), NIPAAm, ammonium peroxydisulfate (APS), N,N,N′,N′-tetramethylethylene diamine (TEMED), N,N′-methylene bisacrylamide (MBA) were of analytical grade and purchased from Aldrich (St. Louis, MO, USA). Ethanol (95%, v/v) was purchased from Merck Co. (Santa Ana, CA, USA). Phosphate buffer solution (PBS) (pH = 7.4) was prepared by adding 3.4 g of KH_2PO_4 and 3.55 g of Na_2HPO_4 to 1000 mL of water, then adjusted to pH = 7.4 by 0.1 M of NaOH. The water was doubly distilled and deionized.

2.2. Preparation of PAE–β-CD complexes and physical mixtures

PAE–β-CD complex was prepared by coprecipitation. β-CD (3.5 g) was dissolved in distilled water (43.75 g) at 70 °C in an oil bath for 1 h. PAE (0.5 g) in ethanol (3.46 g) was slowly added to the β-CD solution with continuous agitation. The molar ratio of PAE to β-CD was 1:1. Next, the vessel was sealed stirred continuously for 6 h. Additionally, 4 ml of ethanol was added dropwisely to regulate the solubility of the hydrophobic solute in β-CD solution. The final solution was refrigerated overnight at 4 °C. The precipitated PAE–β-CD complex was recovered by filtration and washed with ethanol to remove unencapsulated PAE. This residue was dried in a vacuum oven at room temperature for 48 h to prevent the sublimation of PAE from the inclusion complex. The final powder was stored at 4 °C in an airtight bottle.

A physical mixture of β-CD and PAE in the same molar ratio as the PAE–β-CD inclusion complex was prepared using a mortar and pestle for 2 min to obtain a homogeneous physical mixture.

2.3. Characterization of PAE–β-CD complex

Proton nuclear magnetic resonance (H NMR) spectroscopy. H NMR spectrum was performed using a Bruker 600NMR spectrometer at 600 MHz and D_2O as a solvent. The chemical shifts (δ) were reported as ppm and referenced to the residual water signal (4.75 ppm) for H NMR experiments.

Differential scanning calorimeter (DSC). Thermal analyses were performed with a DSC TA Q2000. Samples of 10 mg of PAE, β-CD, PAE–β-CD complex and the physical mixture of PAE and β-CD were sealed into aluminum pans. Samples were heated over a temperature range of −20 to 350 °C at a heating rate of 10 °C min^{-1} under nitrogen gas flow.

X-ray diffraction (XRD). X-ray powder diffraction patterns were recorded on a Rigaku-D/MAX-IIIV diffractometer using Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a 300 mA current. The scanning rate was 0.02° s^{-1} over a 2θ range of 10–60°.

Fourier transform infrared spectroscopy (FTIR). The PAE, β-CD, PAE–β-CD complex and the physical mixture of PAE and β-CD were analyzed by FTIR (Varian 2000 FT-IR) in a region ranging from 400 to 4000 cm^{-1}. The samples (about 0.1 g) were mixed with KBr (0.1 g) and pressed into a tablet form. The FTIR spectrum was then recorded.

2.4. Synthesis of PNIPAAm hydrogels

PNIPAAm hydrogels were synthesized by adding the desired amount NIPAAm and MBA into 26 ml of APS solution (16.8 mM) with continuous agitation under nitrogen atmosphere. After the monomer dissolved in the solution, 200 μl of TEMED were added with agitation; the mixture was transferred to a 10 mm diameter plastic syringe and sealed. Table 1 summarizes the feeding compositions. After 4 h of polymerization at room temperature, the resulting hydrogels were gently pushed out of the syringe and cut to 10 mm thick disks. The cut hydrogels were then immersed in deionized water at room temperature for 1 week to remove unreacted chemicals, during which deionized water was renewed daily. The purified hydrogels were air-dried at room temperature for 3

Table 1
The feeding compositions of PNIPAAm hydrogels.

Sample ID	MBA (g)	NIPAAm (g)	APS (g)	Water (ml)	TEMED(μl)
1.5 mmole MBA	0.23	6.3318	0.1	26	200
3.0 mmole MBA	0.46	6.3318	0.1	26	200