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

Jung-Ying Tsao[a,b,c], Hsieh-Ho Tsai[d], Chien-Pang Wu[d], Pi-Yun Lin[e], Shan-Yu Su[a,b,c], Lieh-Der Chen[b], Fuu-Jen Tsai[a,b,f,g], Yuhsin Tsai[a,*]

[a] School of Chinese Medicine, China Medical University, No. 91 Hsueh-Shih Road, Taichung, Taiwan
[b] School of Post-Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan
[c] Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan
[d] Nano-Powder and Thin Film Technology Center, Industrial Technology Research Institute, Tainan, Taiwan
[e] Instrument Center of National Cheng Kung University, Tainan, Taiwan
[f] Department of Medical Genetics, Pediatrics and Medical Research, China Medical University Hospital, Taichung, Taiwan
[g] Department of Biotechnology and Bioinformatics, Asia University, Taichung, Taiwan

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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-

* Corresponding author. Tel.: +886 4 22053366x1019; fax: +886 4 22032295.
E-mail address: yhtsai@mail.cmu.edu.tw (Y. Tsai).

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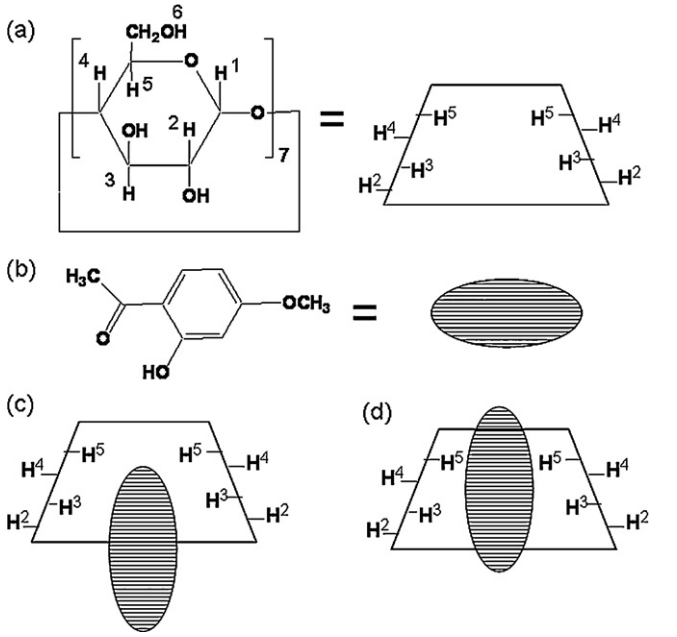

Fig. 1. Chemical structures: (a) β-CD and (b) PAE; and possible models of entry of PAE into β-CD cavity: (c) PAE partially inside β-CD cavity and (d) PAE deeply inside β-CD cavity.

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

days, which avoided damage to the hydrogel structure by high-rate drying. Finally, the hydrogels were placed in a vacuum-dried oven at 50 °C for 24 h.

2.5. LCST determination

The LCST behavior of the PNIPAAm hydrogel samples was determined using a differential scanning calorimeter (TA instruments Q2000 DSC). All hydrogel samples were immersed in distilled water at room temperature and allowed to swell to equilibrium before the DSC measurement. Thermal analysis was performed from 10 °C to 70 °C on the swollen hydrogel sample at a heating rate of 2 °C/min under dry nitrogen. Finally, LCST was determined using the onset point of the endothermal peak, as determined by the intersecting point of two tangent lines from the baseline and slope of the endothermal peak.

2.6. Sample preparation

The hydrogel samples for swelling, deswelling and PAE–β-CD release experiments were prepared by polishing a dry hydrogel disk to 200 mg by abrasive paper (#1500) for fixing the amount of absorption.

2.7. Swelling and deswelling properties of PNIPAAm hydrogels

Dried hydrogels were immersed in a phosphate buffer solution at 25 °C. The swelling ratios were obtained by weighing the initial and swollen samples at various time intervals. After the excess surface water was removed with filter paper, the weights of swollen samples were obtained and the swelling ratios were calculated from the following equation (Zhang et al., 2004):

$$\text{swelling ratio} = \frac{W_t - W_d}{W_d}$$

where W_t denotes the weight of the swollen hydrogels at a predetermined time and W_d denotes the weight of the dry hydrogel.

Before the deswelling measurement, the hydrogel samples were immersed in a phosphate buffer solution at 25 °C to reach equilibrium. The deswelling kinetics of the hydrogels were measured at 45 °C gravimetrically after wiping off water on the surfaces with filter paper. The weight changes of the hydrogels were recorded at regular time intervals during deswelling. Water retention is defined as follows (Zhang et al., 2004):

$$\text{Water retention}\,(\%) = \frac{W_t - W_d}{W_e - W_d} \times 100$$

where W_t and W_e denote the weights of the swollen hydrogel at a predetermined time and at equilibrium, respectively; W_d denotes the weight of the dry hydrogel.

2.8. In vitro release of PAE–β-CD complex from PNIPAAm hydrogels

The in vitro release experiments were performed at 25 °C and 45 °C to investigate how the thermo-sensitive property of PNIPAAm hydrogel affects the PAE–β-CD complex release. A PAE–β-CD complex solution (1250 μg/ml) was prepared by dissolving 0.25 g of PAE–β-CD complex in 200 ml of a phosphate buffered solution. PAE–β-CD complex-loaded hydrogel samples were obtained by swelling the dried hydrogel disks (200 mg) in 25 ml of PAE–β-CD complex solution at 25 °C for 4 days to reach equilibrium. Release experiments of PAE–β-CD complex were conducted by immersing the above loaded hydrogel samples into a 25 ml buffer solution. At a predetermined time after the in vitro release experiment began, 3 ml of the release buffer solution were moved into the quartz tube and the concentration of the PAE–β-CD complex in the release buffer was determined using a UV spectrophotometer (Perkin–Elmer Lambda 25 UV/VIS spectrometer) at 275 nm and a PAE–β-CD complex calibration curve. After the UV/vis measurement, 3 ml of the release buffer solution were poured back into the release buffer solution and the release experiment continued. All release studies were conducted in triplicate.

3. Results and discussion

3.1. H NMR spectroscopy analysis of PAE–β-CD complex

Since PAE is hydrophobic and has a low aqueous solubility, the driving force of forming PAE–β-CD complex in aqueous solution is that β-CD has a hydrophobic cavity which can encapsulate PAE by hydrophobic interaction to stabilize PAE (Brewster and Loftsson, 2007; Tsai et al., 2010; Van Axel Castelli et al., 2008; Vyas et al., 2008; Yuan et al., 2008). Therefore, H NMR spectroscopy can provide direct evidence of the inclusion of the guest molecule inside the CD cavity. Such evidence is based on the expectation that, if the inclusion occurs, the physical or chemical environment would be affected by hydrogens of the internal surface of the cavity (H3 and H5 from any glucose unit of the CD, Fig. 1(a)); however, that of the external surface (H1, H2 and H4) would not be affected. Therefore, changes in the chemical displacements of the protons H3 and H5 located inside the cavity of CD are observed.

Fig. 2(a) and (b) present partial H NMR spectra of β-CD and PAE–β-CD complex, respectively. A high intensity peak appearing at 3.827 ppm in PAE–β-CD complex, which was not observed in β-CD, was generated from acetyl moiety of PAE. The change in chemical shifts of H3 ($\Delta\delta$H3) and H5 ($\Delta\delta$H5) were 0.059 and 0.082 ppm, respectively; chemical shifts of H2 and H4 did not significantly change. Above results confirmed the inclusion of PAE into the β-CD cavity. According to Greatbanks and Pickford (1987), when $\Delta\delta H3 > \Delta\delta H5$, the inclusion of the guest inside the cavity is partial (as shown in Fig. (c)); when $\Delta\delta H3 \leq \Delta\delta H5$, the guest is included deeply inside the cavity. Since $\Delta\delta H3 \leq \Delta\delta H5$ for PAE–β-CD complex, we posited the feasibility of penetrating PAE into β-CD (as shown in Fig. (d))

3.2. DSC analysis of PAE–β-CD complex

DSC analysis is often performed to characterize inclusion compounds with β-CD by comparing the thermal behaviors of the individual components, their physical mixtures and inclusion compounds (Bilensoy et al., 2007; Calderini and Pessine, 2008; Cevher

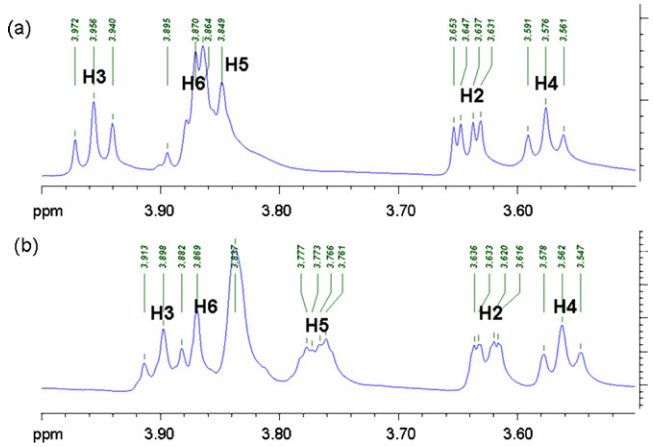

Fig. 2. Partial H NMR spectra of (a) β-CD and (b) PAE–β-CD complex.

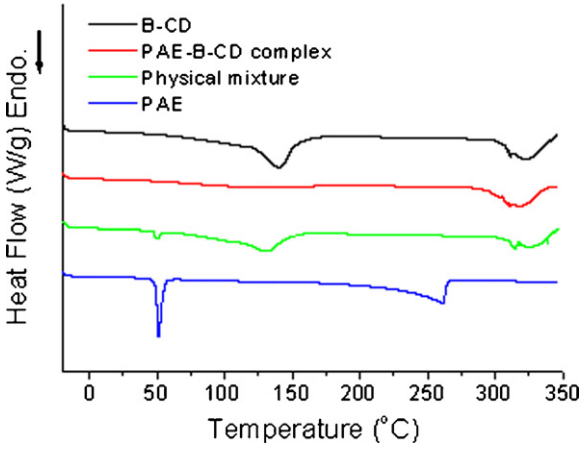

Fig. 3. DSC curves of PAE, β-CD, PAE–β-CD complex and physical mixture of PAE and β-CD.

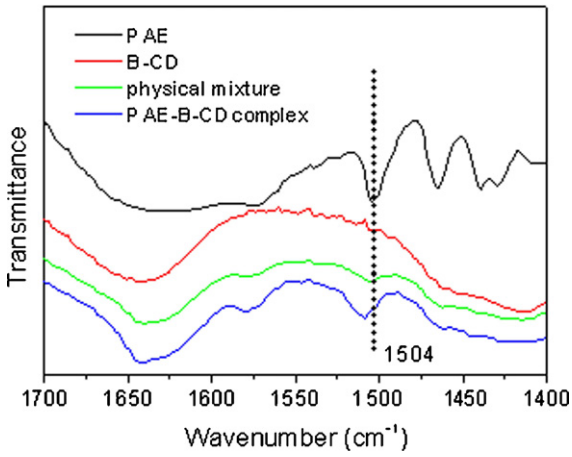

Fig. 5. FTIR spectra of PAE, β-CD, PAE–β-CD complex and the physical mixture of PAE and β-CD.

et al., 2008). Fig. 3 shows the DSC curves of PAE, β-CD, PAE–β-CD complex and the physical mixture of PAE and β-CD. The dehydration and the decomposition of β-CD occurred around 120 °C and 310 °C, respectively. PAE was a crystalline compound, in which an endothermic peak appeared at its melting temperature (around 50 °C), followed by decomposition at 280 °C. The physical mixture of PAE and β-CD had the same endothermic peak as PAE. However, this characteristic peak of PAE melt was not observed for PAE–β-CD complex, indicating that the interaction of PAE molecules to form a crystal structure was destroyed by β-CD in the PAE–β-CD complex. These DSC results confirmed that PAE was no longer present as a crystalline material and its β-CD solid complexes existed in the amorphous state because a β-CD molecule encapsulated each PAE molecule (Calderini and Pessine, 2008).

3.3. XRD analysis of PAE–β-CD complex

The powder X-ray diffraction spectra confirm the formation of the inclusion complex (Calderini and Pessine, 2008; Cevher et al., 2008). Fig. 4 shows the XRD patterns of PAE, β-CD, PAE–β-CD complex and the physical mixture of PAE and β-CD. In the X-ray diffractogram of PAE powder, sharp peaks appeared at a diffraction angle of 2θ at 16, 17, 21, 24 and 26°, suggesting that PAE is a crystalline material. Crystallinity peaks of PAE still appeared in the physical mixture of PAE and β-CD (Calderini and Pessine, 2008). In contrast, these characteristic peaks disappeared for PAE–β-CD complex, revealing that PAE was into the cavity of β-CD and total amorphization of PAE was obtained in PAE–β-CD complex. These XRD results were consistent with those from DSC.

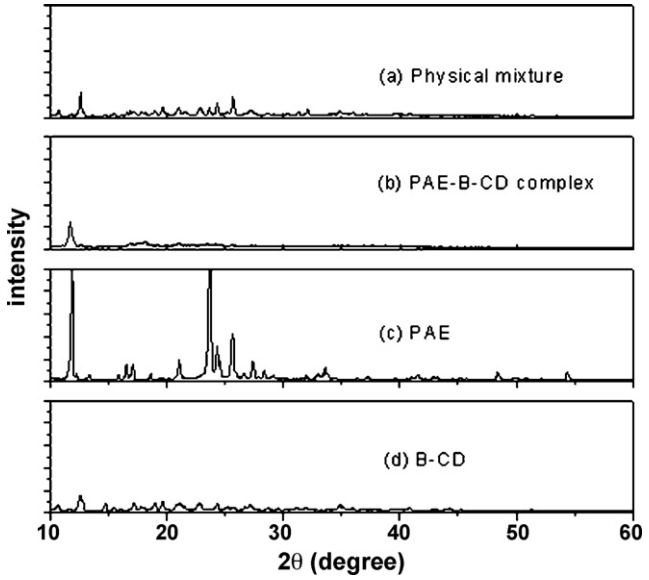

Fig. 4. XRD patterns of (a) physical mixture of PAE and β-CD, (b) PAE–β-CD complex, (c) PAE and (d) β-CD.

3.4. FTIR analysis of PAE–β-CD complex

Fig. 5 shows the FTIR spectra of PAE, β-CD, PAE–β-CD complex and the physical mixture of PAE and β-CD. The FTIR spectra of PAE–β-CD complex and the physical mixture of PAE and β-CD are similar to that of β-CD, due to the low quantity of PAE. However, several variations were found in the spectra, caused by interactions between the encapsulated PAE and the inner core of β-CD. The characteristic aromatic C=C stretching band of PAE at 1504 cm^{-1} apparently shifted to 1509 cm^{-1} for the inclusion complex, but not for the physical mixture of PAE and β-CD (Fig. 5). Variations in the characteristic bands of PAE indicated the complex as a new compound with different spectroscopic bands (Fernandez et al., 2008).

3.5. Swelling/deswelling properties of PNIPAAm hydrogel

Fig. 6(a) presents the swelling properties of PNIPAAm hydrogels containing various cross-linker contents (1.5 and 3 mmol), as determined by immersing the dried and pre-weighted hydrogel into deionized water at 25 °C. Both samples reached the maximum swelling ratio after about 360 min (6 h). Additionally, the hydrogel with a high cross-linker content had a lower maximum swelling ratio than the hydrogel with a low cross-linker content, indicating that the hydrogel compositions affected its swelling properties. Above observations are consistent with the literature (Zhang et al., 2004; Lee and Yeh, 2005), since increasing the cross-linker content increases the cross-linking density of the resulting hydrogel and restricts its chain motion of the hydrogel in water, ultimately decreasing the swelling ratio of the hydrogel.

The deswelling kinetics of these samples was analyzed at temperatures exceeding their LCST, as determined by using a DSC. When the temperature reached LCST, the polymer–water interaction was suppressed and the polymer–polymer interaction increased, leading to volume shrinkage of the hydrogel and the beginning of deswelling. Fig. 7 plots a typical DSC trace, in which an endothermic signal appeared at the temperature of LCST. Both

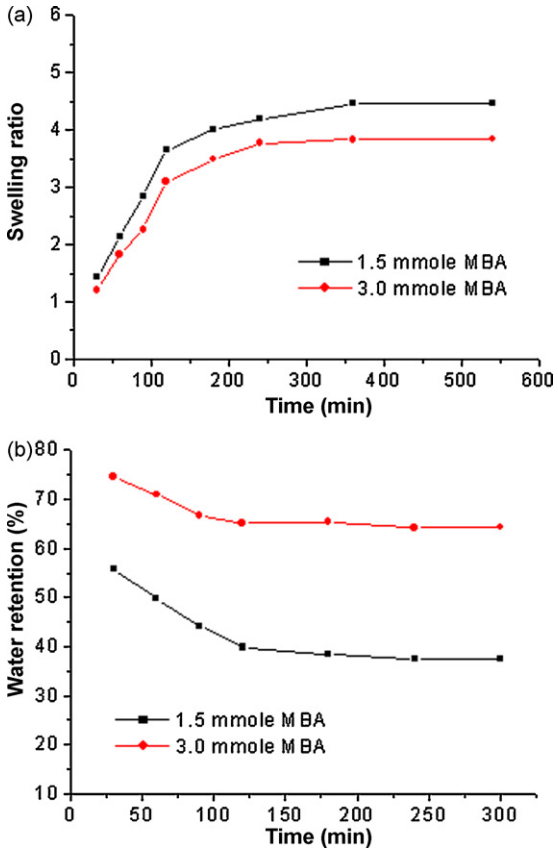

Fig. 6. (a) Swelling and (b) deswelling properties of PNIPAAm hydrogel.

samples in this study had a LCST at around 33 °C (Lee and Yuan, 2002; Zhang et al., 2004; Lee and Yeh, 2005).

The deswelling study of hydrogels was performed at the temperature of 45 °C, which exceeded the LCST of PNIPAAm. Fig. 6(b) summarizes the experimental results. Similar to the swelling profiles, the cross-linker content also influenced the deswelling behaviors of hydrogels. According to this figure, the water retention increased with an increasing cross-linker content, revealing that the cross-linking structures hindered the network contraction at a temperature higher than LCST and suppressed the free water from diffusing from the hydrogel (Zhang et al., 2004; Lee and Yeh, 2005).

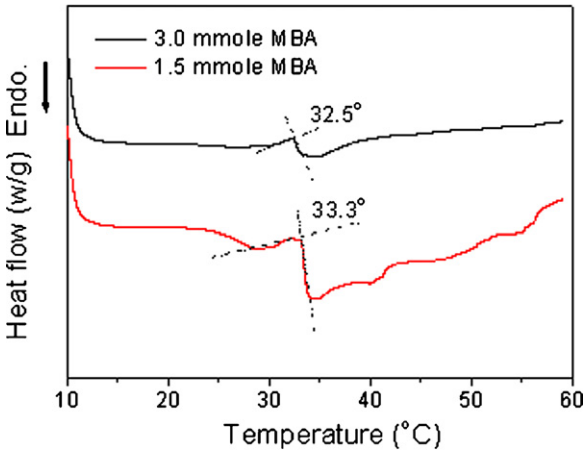

Fig. 7. LCSTs of hydrogels.

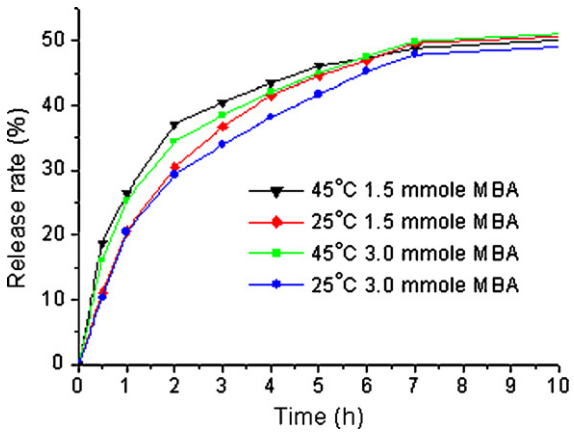

Fig. 8. PAE–β-CD complex released from hydrogels.

3.6. PAE–β-CD complex release

PAE–β-CD complex release experiments were conducted by immersing the PAE–β-CD complex-preloaded hydrogel samples into a phosphate buffer solution at 25 °C (below LCST) and 45 °C (above LCST), and monitored by using a UV spectrophotometer at 275 nm with a PAE–β-CD complex standard calibration curve. Fig. 8 presents the PAE–β-CD complex release from hydrogels at 25 °C and 45 °C. Both hydrogels had higher PAE–β-CD complex release rates at 45 °C than those at 25 °C, indicating that PAE–β-CD complex were squeezed out of the hydrogel with the pressure caused by a drastic decrease in volume at 45 °C (Lee and Yuan, 2002). Additionally, hydrogel with a high cross-linker content had a lower PAE–β-CD complex release rate than that with a low cross-linker content. These cross-linker effects on release correlated with deswelling results, in which increasing the cross-linker content of the hydrogel reduced the deswelling capacity and increased water retention. These results of PAE–β-CD complex release experiments indicated that the release of PAE–β-CD complex is related to the volume contraction of PNIPAAm hydrogel, which was affected by PNIPAAm hydrogel compositions and release temperatures.

4. Conclusion

PAE–β-CD complex was prepared via coprecipitation and characterized by using H NMR, DSC, XRD and FTIR. Analytical results confirmed the inclusion of PAE into the β-CD cavity. Two PNIPAAm hydrogels containing different cross-linker contents (1.5 and 3 mmol) were synthesized. Experimental results of swelling and deswelling indicated that increasing the cross-linker content of the PNIPAAm hydrogel decreased the swelling ratio and increased the water retention. Experimental results of PAE–β-CD complex release revealed that the release rate at 45 °C (>LCST) was higher than at 25 °C (<LCST). Moreover, a lower cross-linker content of the hydrogel contained implies a higher rate of PAE–β-CD complex release. Above release results indicated that the release of PAE–β-CD complex is related to the volume contraction of the hydrogel, which was affected by hydrogel compositions and release temperatures.

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