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Synthesis and characterization of thermosensitive graft copolymer of *N*-isopropylacrylamide with biodegradable carboxymethylchitosan

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ABSTRACT

A novel thermosensitive and hydrogel was designed and synthesized by graft copolymerization of *N*-isopropylacrylamide (NIPAAm) with biodegradable carboxymethylchitosan (CMCS). The influence of the content of CMCS grafted on the properties of the resulted hydrogels was examined. The morphology of the hydrogels was observed by scanning electron microscopy (SEM), their thermal property was characterized by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and deswelling/swelling kinetics upon external temperature changes. In comparison with the conventional PNIPAAm hydrogels, the resulted hydrogels have improved thermosensitive properties, including enlarged water content at room temperature and faster deswelling/swelling rate upon heating. The strategy described here presents a potential alternative to the traditional synthesis techniques for thermosensitive hydrogels.

1. Introduction

In recent years, "intelligent" or "smart" hydrogels constitute a fast-growing area of polymer science because of their rapid response to environmental sitimuli, high water content and biocompatibility (Huang & Lowe, 2005; Langer & Peppas, 2003; Zhang, Wu, Sun, & Chu, 2003). Those hydrogels can control drug delivery (Ankareddi & Brazel, 2007) through responding to thermal stimulation by swelling and deswelling. Various thermosensitive and biodegrable hydrogels have been developed for drug delivery based on thermoresponsive polymer poly(*N*-isopropylacryamide) (PNIPAAm) due to its unique phase transition at a lower critical solution temperature (LCST) in water around 32 °C which is near the human body temperature (Guilherme, Silva, Girotto, Rubira, & Muniz, 2003; Guo & Gao, 2007; Han & Bae, 1998; Kumashiro, Huh, Ooya, & Yui, 2001; Lowe, Virtanen, & Tenhu, 1999; Yoo, Sung, Lee, & Cho, 2000; Yoshida, Aoyagi, Kokufuta, & Okano, 2003). Below LCST, the polymer expands and swells in water. In contrast, the polymer shrinks and collapses above the LCST. PNIPAAm-based polymers may allow aqueous loading of protein drugs, protecting the drug from a hostile environment (Ramkissoon-Ganorkar, Liu, Baudys, & Kim, 1999) and modulating drug release in response to temperature change (Zhang, Huang, Cheng, & Zhuo, 2004). However, many current thermoresponsive PNIPAAm hydrogels have problems in nonbiodegradability and nonsustained drug release under physiological conditions (Qiu & Park, 2001; Zhang et al., 2004).

Degradation of the hydrogel matrix can not only circumvent removal of empty device but also be used to modulate drug release for a long period of time (Van Dijk Wolthuis, Hoogeboom, vanSteenbergen, Tsang, & Hennink, 1997). Hydrogel composed of carboxymethylchitosan (CMCS) has been extensively studied in medical materials (Don, Hsu, & Chiu, 2001; Jenkins & Hudson, 2001; Joshi & Sinha, 2006; Kojima, Yoshikuni, & Suzuki, 1994; Pourjavadi & Mahdavinia, 2003; Shanthi & Panduranga, 2001). Carboxymethylchitosan, a natural amphoteric polyelectrolyte derived from chitosan, has attracted considerable interest in a wide range of biomedical applications especially in its biocompatibility such as wound dressings, artificial bone and skin, bacteriostatic agents and blood anticoagulants etc (Chen, Wu, & Mi, 2004; Huang, Nayak, & Lowe, 2004; Kim, Gil, & Lowe, 2006; Meyer, Shin, Kong, Dewhirst, & Chilkoti, 2001; Thanou, Nihot, & Jansen, 2001; Turk, Dincer, Yulug, & Piskin, 2004). In CMCS molecule, the degree of substitution (DS) of $-NH_2$ groups is 0.1–0.2, (Chen, Du, Wu, & Xiao, 2002; Chen & Park, 2003), so it has abundant $-NH_2$ groups to take part in other reactions.

In this paper, the graft copolymerization of *N*-isopropylacrylamide (NIPAAm) with carboxymethylchitosan (CMCS) was carried out (Scheme 1) and the hydrogels obtained were characterized. In comparison with poly(*N*-isopropylacrylamide) (PNIPAAm) gel, the graft copolymer hydrogels would provide many more advantages, such as increased water content, mechanical properties and improved thermosensitive properties. The porous structure within

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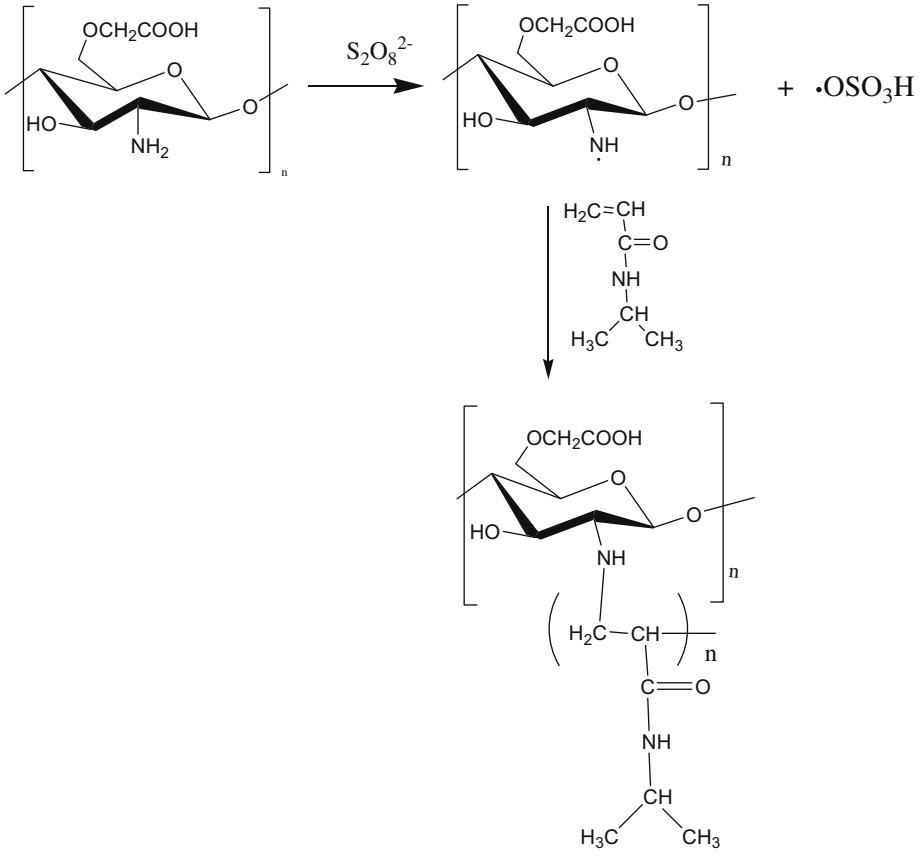

Scheme 1. Schematic illustration for the grafting of NPIAAm onto carboxymethylchitosan (CMCS).

the hydrogels observed by scanning electron microscopy (SEM) was formed and resulted in rapid deswelling in the presence of hydrophilic CMCS component during the graft copolymerization process, which is advantageous to the migration of water molecules out of the gel network.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAAm) (Acros, Belgium) was recrystallized from a 65:35(v/v) mixture of hexane and benzene and dried in vacuum. Carboxymethylchitosan (CMCS) was obtained from Nantong Xincheng Biological Industrial Co., Ltd. (Nantong, China). *N*,*N*′-Methylenebisacrylamide (BIS) (Acros, Belgium), ammonium persulfate (APS) and sodium pyrosulfite (SPS) are analytical reagents from Aldrich. All other reagents and solvents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Synthesis of poly(NIPAM-g-CMCS)

The synthesis of copolymers of poly(NIPAAm-g-CMCS) is illustrated below. A total of 1 g of NPIAAm and 0.12 g of carboxymethylchitosan (CMCS) were dissolved in 10 mL of deionized water under a nitrogen atmosphere for 30 min, then 0.015 g of ammonium persulfate (APS) as a initiator was added to the solution and the solution was stirred at 70.0 °C for 3 h (Wang, Yang, & Qiu, 1994). After the reaction was completed, the solution was dried in vacuum at 30.0 °C for 24 h. To remove the homopolymer (PNIPAAm), the rude dried hydrogel was immersed in excess acetone for 24 h and filtered to separate products. The homopolymer (PNIPAAm) was dissolved in acetone, but the poly(NIPAAm-g-CMCS) wasn't.

2.2.2. Grafting ratio and efficiency

The percentage and efficiency of grafting (%) were calculated by the difference of weights before and after grafting reaction according to the following formula:

$$\text{Grafting percentage (G\%)} = (W_g - W_c)/W_c \times 100\%;$$
$$\text{Grafting efficiency (GE\%)} = (W_g - W_c)/W_m \times 100\%;$$

where W_g, W_c and W_m denote weights of pure graft copolymer, carboxymethylchitosan and NIPAAm monomer, respectively.

2.2.3. Fourier-transformed infrared spectroscopy (FT-IR)

FT-IR spectra were recorded with AVATAR-360 FI-IR spectrometer (Nicolet, USA) and scanned from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1}, using KBr pellets at room temperature.

2.2.4. X-ray diffraction (XRD)

X-ray powder diffraction diagrams (XRD) by Ni-filtered Cu Ka radiation were generated at 30 kV and 30 mA as the X-ray source. Dried hydroxygel were whetted into small pieces when adhered to the matrix and scanned from 5 to 60° at a rate of 1°/min.

2.2.5. Thermogravimetric analysis (TGA)

The thermal properties of poly(NIPAAm-g-CMCS) and chitosan were measured by thermogravimetric ananlysis (TGA). Decomposition profiles of TGA were recorded with a heating rate of 10 °C/min in nitrogen between 0.0 °C and 800.0 °C.

2.2.6. Scanning electron microscopy (SEM)

The samples were quickly frozen in liquid nitrogen (−80 °C) and cold dried in vacuum for 24 h. After being covered with gold on an aluminium holder, the dried hydrogels were analyzed by scanning electron microscope with sub-accelerating voltage of 20,000 V.

2.2.7. Phase transition determination

2.2.7.1. Differential scanning calorimetry (DSC). The state of water in the hydrogels was investigated with a Perkin Elmer differential scanning calorimeter (DSC) (Diamond DSC) in the range of temperature from 20 to 50 °C with a heating rate of 2 °C/min under N_2 flow at 20 mL/min.

2.2.7.2. Temperature dependence of swelling ratio. The swelling ratio (SR) was determined by a general gravimetric method. The dry poly(NIPAAm-g-CMCS) hydrogels were immersed in distilled water of the desired temperature in sealed containers, and the swollen weight of the samples was recorded at regular period of time after the removal of excess surface water with a filter paper, weighed and returned to the same container until there was no further weight increase. The SR is calculated by the following equation:

$$SR = (W_t - W_0)/W_0 \times 100\%;$$

where W_0 represents the dry-state copolymer and W_t is the weight of the swollen copolymer at temperature t °C, respectively.

The characterization of PNIPAAm and poly(NIPAAm-g-CMCS) hydrogels with varied feeding ratios is shown in Table 1.

2.2.8. Deswelling kinetics at 50 °C

The deswelling kinetics of the swollen hydrogel were measured gravimetrically in distilled water at 50 °C. At predetermined time intervals, the samples were taken out of the hot water and weighed after removing the excess water on the surfaces with wet filter paper. Similarly, each sample was measured three times and the average value of three measurements was recorded. Water retention (WR) was defined as follows:

$$WR = [(W_t - W_0)/W_s] \times 100;$$

where W_t is the weight of the wet hydrogel at immersion time t at 50 °C, W_s is the water weight at 20 °C and the other symbols are the same as defined above.

2.2.9. Swelling kinetics at 18 °C

The dried sample was immersed in distilled water at room temperature (18 °C) and removed from the water at regular time intervals. After removing the water on the surface with wet filter paper, the average weight of three measurements was recorded.

The water uptake (WU) at time t, was defined as follows:

$$WU = [(W_t - W_0)/W_s] \times 100$$

where W_t is the weight of the wet hydrogel at time t at 20 °C and the other symbols are the same as defined above.

Table 1
Feed composition for the preparation of grafted copolymer hydrogels.

Sample ID	NIPAAm (g)	CMCS (mg)	APS (mg)	SPS (mg)	BIS (mg)	H_2O (ml)
PNIPAAm	1	0	15	15	60	10
PNC6	1	6	15	0	0	10
PNC12	1	12	15	0	0	10

3. Results and discussion

3.1. FT-IR characterization of poly(NIPAAm-graft-CMCS)

The chemical structure of CMCS, NIPAAm homopolymer (APS as a initiator) (Kim, Cho, & Lee, 2000) and poly(NIPAAm-g-CMCS) was characterized by FT-IR (Fig. 1). As shown in Fig. 1, Curve a shows signals of CMCS at 1633 and 1553 cm^{-1} for C=O stretching (amide) and N—H bending (amine), respectively.

Compared with FT-IR spectrum of CMCS, poly(NIPAAm-g-CMCS) has a new peak around 1653 cm^{-1} corresponding to –NHCO groups in the graft copolymer. The FT-IR spectrum of poly(NIPAAm-g-CMCS) also reveals a significant peak at 2965 cm^{-1}, which is assigned to methyl group of $-CH(CH_3)_2$ in NIPAAm. In Fig. 1, Curve b obtained from NIPAAm homopolymer shows the peaks at 1645 and 1545 cm^{-1} which can be attributed to the characteristic peaks of amide I and amide II, respectively. The FT-IR spectrum of poly(NIPAAm-g-CMCS) is similar to that of NIPAAm homopolymer, for the mass ratio of CMCS in graft copolymer is quite small.

3.2. Grafting ratio and efficiency

Fig. 2 illustrates that the CMCS amount in the feed significantly affect the grafting of NIPAAm onto CMCS. The grafting degree grad-

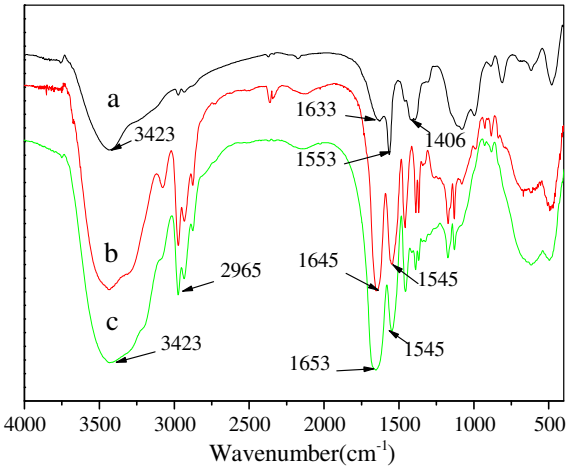

Fig. 1. FT-IR spectra of (a) CMCS, (b) PNIPAAm homopolymer and (c) poly(NIPAAm-g-CMCS).

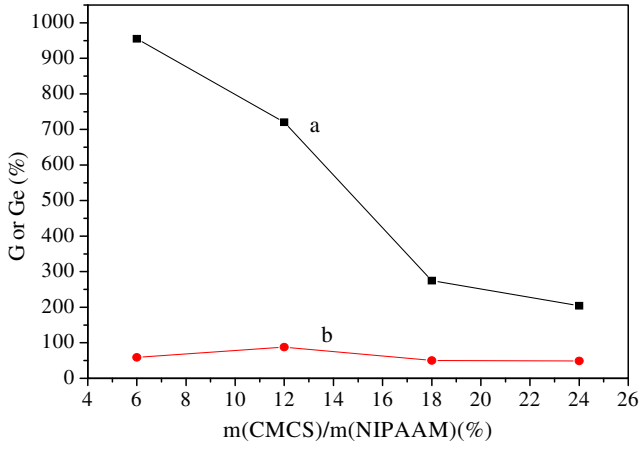

Fig. 2. Effect of monomer concentration on the grafting of NIPAAm onto CMCS: Grafting percentage (a), Grafting efficiency (b).

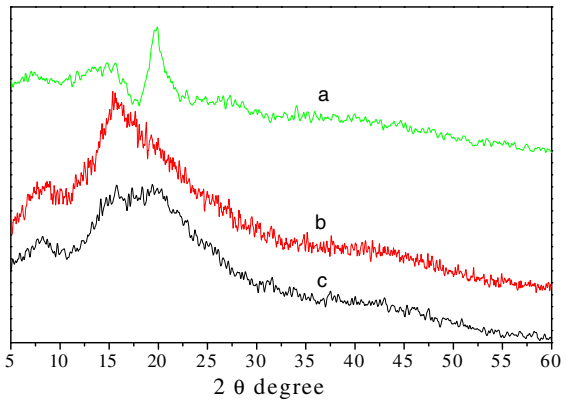

Fig. 3. XRD patterns of CMCS (a), PNC12 (b), PNC6 (c).

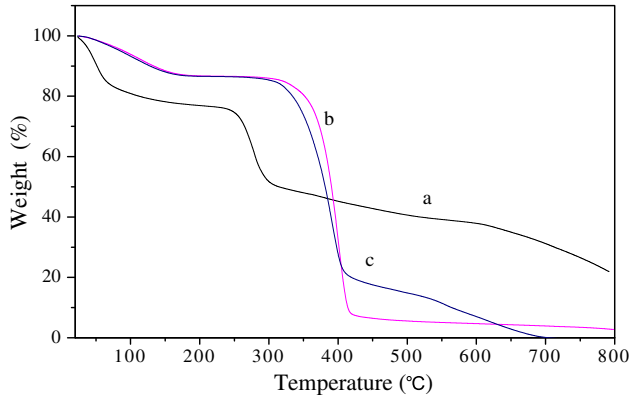

Fig. 4. TGA curves of CMCS (a), PNC12 (b), PNIPAAm (c).

ually decreases while grafting efficiency tended to raise first and then declined with the increase of CMCS content in the feed. The maximal grafting efficiency and degree are 87.9% and 955%, respectively. This is attributed to the abundant $-NH_2$ groups in the CMCS. However, the grafting degree sharply decreases with the NIPAM content reduction in literature. This can be attributed to the substantial amount of polymer grafted onto the substrate backbone, which inhibits the diffusion of APS and monomer into the CMCS for further grafting. As a result, this effect stimulates the formation of NIPAAm homopolymer, which increases with an increase in monomer concentration (Kim et al., 2000).

3.3. X-ray diffraction (XRD) patterns of dry hydrogel

The crystalline structure of poly(NIPAAm-g-CMCS) was studied by XRD analysis. The X-ray diffraction spectra of CMCS, PNC12 and PNC6 were shown as curves a, b and c in Fig. 3. Carboxymethylchitosan showed narrow bands at $2\theta = 20°$, demonstrating its crystalline structure, whereas in the grafted polymer this peak almost disappeared and a new peak appeared at about $2\theta = 15°$. These results indicate that after grafting with NIPAAm, the original crystalline structure of carboxymethylchitosan has been altered.

3.4. Thermogravimetric analysis (TGA)

The TGA thermograms of carboxymethylchitosan, poly(NIPAAm-g-CMCS) and PNIPAAm are presented in Fig. 4. The thermogram of the graft copolymer is different from those of carboxymetheylchitosan and PNIPAAm. It undergoes a two-step thermal degradation process. The first step of 25–150 °C is mainly attributed to water and the second of 368–420 °C to the decomposition of PNIPAAm. The grafted copolymers have stronger thermal capacity than that of carboxymethylchitosan. The curves of carboxymethylchitosan TGA are divided into two stages. The first stage of 25–90 °C is H_2O and small molecules decomposition and the second of 260–300 °C is central thermal decomposition of complex polysaccharides.

3.5. The SEM of poly(NIPAAm-g-CMCS) and PNIPAAm hydrogels

The SEM images exhibited in Fig. 5 illustrate the morphologies of fully swelling poly(NIPAAm-g-CMCS) hydrogel and traditional PNIPAAm hydrogel, respectively. The poly(NIPAAm-g-CMCS) hydrogel under swelling state exhibits abundant open and porous structure. This network structure shows a high level of porosity and connectivity, so that there is more surface area on the copolymer matrix. It can reduce the flow resistance of water molecules in or out of the hydrogel, enable the hydrogels to absorb large amount of water, and improve their temperature sensitivity. In contrast, the PNIPAAm hydrogel shows a smaller open and porous structure, and the surface area of the copolymer is less than that of PNC12.

3.6. Phase transition determination

3.6.1. Differential scanning calorimetry (DSC)

The LCST of the novel poly(NIPAAm-g-CMCS) hydrogels determined from the DSC thermodiagrams is displayed in Fig. 6(A). The data indicated that LCST of all the hydrogels was around 32 °C. That is to say, the CMCS almost had no apparent impact on the LCST of the hydrogel. A widely recognized mechanism to ex-

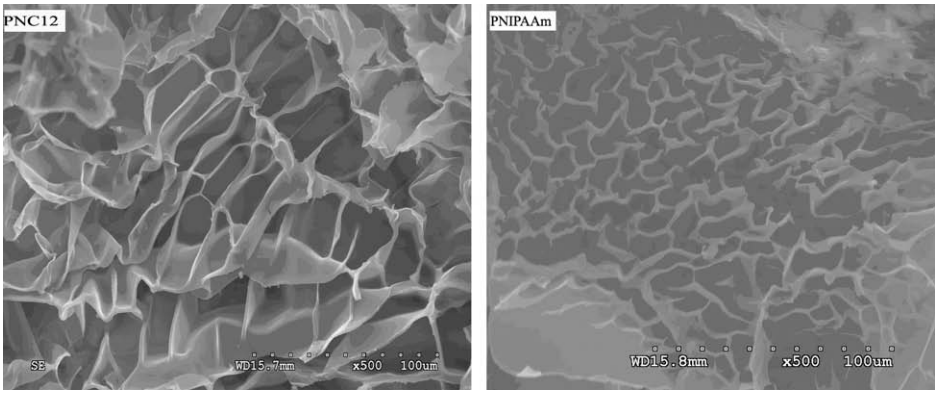

Fig. 5. The SEM image of PNC12 and PNIPAAm.

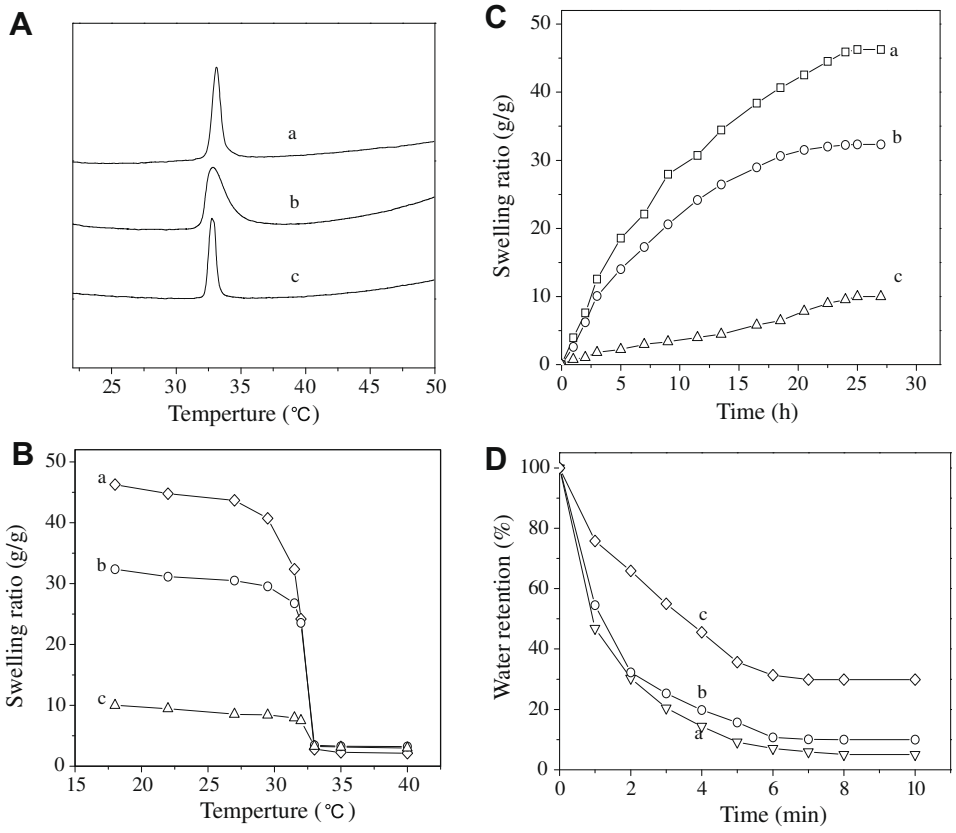

Fig. 6. Thermoresponsive properties of hydrogels: PNC12 (a), PNC6 (b), PNIPAAm (c). (A) DSC thermodiagrams of the hydrogels; (B) equilibrium swelling ratios as a function of temperature; (C) swelling kinetics of different hydrogels measured gravimetrically in distilled water at 18 °C; (D) time dependence of the swelling ratio of hydrogels when the temperature was rapidly increased from 18 to 50 °C.

plain the LCST of PNIPAAm hydrogel is that the phase transition is resulted from a balance between hydrophilicity and hydrophobicity in the polymeric backbone (Shibayama, Fujikawa, & Nomura, 1996). When a hydrophilic moiety was copolymerized into PNIPAAm hydrogel network, the hydrophilic/hydrophobic balance shifted to a more hydrophilic nature, and the corresponding LCST shifted to a higher temperature. In contrast, if a hydrophobic moiety was copolymerized into the polymeric chain, its LCST became lower. Herein, as shown in Scheme 1, CMCS chains were incorporated into PNIPAAm hydrogel network as pendant chains instead of backbone. Thus, the initial hydrophilic/hydrophobic balance of the backbone did not change. Consequently, LCST of the resulted hydrogels nearly had no change.

3.6.2. Temperature dependence of swelling ratio

The dry hydrogels were prepared in distilled water at 18 °C. After reaching the swelling equilibrium, these hydrogels were left at 22, 27, 29.5, 31.5, 32, 33, 35 and 40 °C in the thermostatic water bath for 2 h before measuring the swelling ratio change.

The temperature dependence of swelling ratio of poly(NIPAAm-g-CMCS) hydrogels was examined to evaluate their temperature sensitive properties. Fig. 6(B) represents the swelling ratio of PNIPAAm, PNC6 and PNC12 at different temperatures. As shown in Fig. 6(B), the hydrogels demonstrated a similar swelling profile, i.e. the swelling ratio decreased rapidly as the temperature increased toward LCST (32 °C). Traditionally, in terms of swelling ratio change, the phase separation temperature is regarded as the temperature at which the phase separation degree, swelling ratio change vs. temperature change ($\Delta SR/\Delta T$), is the greatest, or the temperature at which the swelling ratio of hydrogel decreases most dramatically. From Fig. 6(B), it was clear that LCST of the hydrogels was around 32 °C irrespective of the content of CMCS, which was in agreement with LCST determined from DSC.

Even though LCST of the hydrogels was not virtually affected by CMCS, the data in Fig. 6(B) indicated that, at a temperature below LCST, the equilibrium swelling ratio of hydrogel was improved via grafting CMCS. The higher swelling ratio of the PNIPAAm hydrogel with CMCS grafts was due to the macroporous network structure that could enhance the water holding capacity. It is interesting to note that, at the temperature above LCST, the content of CMCS has no influence on the swelling ratio of hydrogel, which suggested that, with or without CMCS, all the hydrogels would collapse into a similar structure at a temperature above their LCST.

3.7. Swelling kinetics at 18 °C

The shrinking rate at increased temperatures is critical for a temperature sensitive hydrogel. The swelling rate at a low temperature is also critical for its practical applications, especially when used as recyclable intelligent devices. Fig. 6(C) demonstrates the swelling behavior of PNC12, PNC6 and PNIPAAm hydrogels in distilled water at room temperature (18 °C). Of the hydrogels, PNC12 and PNC6 exhibited a faster swelling rate, reaching swelling ratios around 46 and 32 within 30 h, while that of PNIPAAm was less than 9 within the same time interval. This tendency was easily understood since a greater amount of CMCS as pore-forming agent enlarges the matrix space of PNC12 and PNC6 hydrogels.

3.8. Deswelling kinetics at 50 °C

The temperature dependence of the swelling ratio only demonstrated the equilibrium hydration state of PNC12 and PNC6 hydro-

gels at different temperatures. In practical applications, the response kinetics or deswelling kinetics in response to sudden alterations in temperature are of great importance. Fig. 6(D) shows that PNC12, PNC6 hydrogels were rapidly lost in water when the temperature rapidly increased from 18 to 50 °C. However, the shrinking rate was different. Specifically, the water retention of PNC12 and PNC6 decreased from 100% to 46% and 56% in 1 min, while that of PNIPAAm decreased from 100% to 30% within 10 min. The results indicate that the existence of the interconnected and uniform pore structures in copolymer (Fig. 5) also enhanced the deswelling rate of the graft copolymer hydrogels.

4. Conclusion

In this study, a novel type of hydrogel was synthesized by the graft copolymerization of NIPAAm and CMCS. The structure, morphologies and properties of the resulted hydrogels were examined with FT-IR, XRD, SEM, DSC, and TGA etc. The poly(NIPAAm-g-CMCS) hydrogel has a better temperature sensitivity and swelling ratio, compared to the poly(NIPAAm) hydrogel. This thermosensitive and biodegradable hydrogel may have the potential applications in controlled drug delivery system; they can also be used to separate and purify some biological materials such as proteins, enzymes and amylose.

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