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Modulate the phase transition temperature of hydrogels with both thermosensitivity and biodegradability

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ABSTRACT

The synthesis and characterization of thermoresponsive hydrogels on the basis of *N*-isoproplyarylamide (NIPAAm) and acrylamide (AAm) copolymers crosslinked with a novel biodegradable crosslinker (PEG-co-PLA) were carried out in this study. Swelling measurement results demonstrated that four gels of PNAM5, PNAM10, PNAM12 and PNAM15 are thermoresponsive. The equilibrium swelling ratio and degradation of the hydrogels strongly depend on hydrogels composition. The morphology of the hydrogels was observed by scanning electron microscopy (SEM), and their thermal property was characterized by differential scanning calorimetry (DSC). The results show that the proportion of AAm in the copolymer has notable effect on the low critical solution temperature (LCST) of the hydrogel. When the molar ratio of AAm to NIPAAm was increased from 1:10 to 3:10 the LCST of the copolymer increased from 39.7 to 64.2 °C. The compression modulus of PNAM15 is of the highest among other hydrogels, because PNAM15 hydrogel has a more compact structure.

1. Introduction

Hydrogels are crosslinked and three-dimensional hydrophilic polymer networks, which cannot dissolve when they hold a large amount of water and biochemical fluid. Among them, stimuli-responsive hydrogels, which undergo a volume change in response to external conditions such as temperature, pH, ionic strength and electric field, have received much attention over the past decade (Chen & Hoffman, 1995; Kim, Lee, Kim, & Lee, 2002; Li, Wu, & Liu, 2008; Shibayama, Fujikawa, & Nomura, 1996; Shibayama, Mizutani, & Nomura, 1996; Shibayama, Kawakubo, & Norisuye, 1998; You, Teruo, & Sung, 1990). In particular, temperature-sensitive hydrogels, which demonstrate a good solubility in aqueous solutions at low temperatures but separate from solution when the temperature is raised above the lower critical solution temperature (LCST), have been investigated for applications in such as controlled drug delivery and solute separation (Benee, Snowden, & Chowdhry, 2002; Hoffman, 2002; Ipsita & Munishwar, 2004; Joseph & Robert, 2001; Li et al., 2009; Neradovic, Hinrichs, Kettenes-van, van Nostrum, & Hennick, 2001; Peppas, Bures, Leobandung, & Ichikawa, 2000; Robert & Nicholas, 2003; Zhang, Zhuo, Cui, & Zhang, 2002). An important and useful feature of thermo-sensitive hydrogels is the possibility of controlling their LCST by various means, in particular by varying the monomer composition (Barker et al., 2003; Brazel & Peppas, 1995; Feil, Bae, Feijen, & Kim, 1993; Lowe, Virtanen, & Tenhu, 1999; Shibayama & Fujikawa et al., 1996; Shibayama & Mizutani et al., 1996; Zhang, Cheng, Huang, & Zhou, 2003). However, the mechanisms of the temperature-induced phase separation and the influence of comonomers on the LCST are not fully understood.

In general, the balance of hydrophilicity/hydrophobicity in the thermo-sensitive hydrogels structure is responsible for exhibiting the LCST phenomenon (Bokias, Hourdet, Iliopoulos, Staikos, & Audebert, 1997; Cho, Lee, & Cho, 2003; Zhang, Yang, Chung, & Ma, 2001). The incorporation of hydrophobic comonomers leads to a lower LCST and hydrophilic comonomers to a higher LCST. It is well-known that poly(*N*-isopropylacrylamide) (PNIPAAm) exhibits an abrupt volume change at its LCST of approximately 32 °C in aqueous solution. At a temperature below LCST, the hydrophilic moieties (–CONH–) may interact with water molecules through hydrogen bonds, which lead to water uptaking of the PNIPAAm hydrogel. At a higher temperature, the hydrogen bonding interactions become weakened or destroyed. Thus the hydrophobic interactions among the hydrophobic moieties [$-CH(CH_3)_2$] grow stronger, which induces the freeing of the entrapped water molecules from the network. The chemical modifications of poly(NIPAAm) are usually

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performed to introduce the addition of hydrophilic or hydrophobic comonomers that can control its LCST (Kaneo et al., 1995, 1998; Yoshida et al., 1995; Yuzo, Ryo, Kiyotaka, Yasuhisa, & Teruo, 1995). It is possible to increase the functionality of hydrogels based on PNIPAAm by finding the right balance of hydrophobic and hydrophilic comonomers or tuned to a desired temperature range by copolymerization with a more hydrophilic comonomer (which raises the LCST) or a more hydrophobic comonomer (which lowers the LCST) (Aslam, 2007; Eriko & Toyoichi, 1988; Guilherme, Silva, Girotto, Rubira, & Muniz, 2003; You, Teruo, & Sung, 1988).

In view of these aspects, a promising strategy for designing novel hydrogels is to combine the merits of both thermoresponsive and enzymatic degradation properties, and modulate the component of hydrogel to a desired lower critical solution temperature range. In this work, we synthesized thermoresponsive hydrogels on the basis of *N*-isoproplyarylamide (NIPAAm) and acrylamide (AAm) copolymers crosslinked with a novel crosslinker containing multiple hydrolytically degradable oligolactate in phosphate-buffered saline (PBS) (pH 7.4) solutions. The hydrophilic/hydrophobic balance of resulted hydrogels has been altered by incorporation of varying amounts of acrylamide (AAm) through copolymerization. We have shown that increasing the AAm content of a poly(NIPAAm-co-AAm) hydrogel raises its LCST (relative to that of PNIPAAm) to an extent that depends on the amount of the more hydrophilic comonomer (AAm) present in the system. The experiments demonstrated that increasing the hydrophilic (AAm) content of poly(NIPAAm-co-AAm) hydrogels results in production of increasingly expanded and flexible globules above the LCST of the hydrogels. The results indicate that increasing the hydrophilic content of the copolymer reduces the change in enthalpy which is observed upon passing through its LCST.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAAm) monomer (Acros) was recrystallized from a 65:35 (v/v) mixture of hexane and benzene and dried in vacuum. Acrylamide (AAm) was obtained from Shanghai Lingfeng Chemical Reagent Co. Ltd. Poly(ethylene glycol) (PEG) with molecular weight of 600 PEG(600) was purchased from Belgium. PEG(600) was dried under vacuum for 4 h and DL-Lactide was purified twice by recrystallization from ethyl acetate prior to use. Sodium pyrosulfite as an accelerator was used as received. Ammonium peraulfate (APS) as an initiator was further purified by recrystallization. All other chemicals used were of reagent grade and were used without further purification.

2.2. Syntheses of PEG-co-PLA copolymers as crosslinker

The synthesis of PEG 600 copolymer with DL-Lactide is illustrated below. At first, 6 g of dry PEG600 (10 mmol) was placed into a dried 100 ml three-necked flask equipped with a magnetic stirrer. Then, the flask was heated to 130 °C with nitrogen. Under nitrogen, DL-Lactide (7.2 g, 50 mmol) was charged into the flask. After the solid was molten, stannous octoate (20 mg, 0.05 mol) was subsequently charged into the flask. The reaction mixture was extensively stirred under nitrogen at 150 °C for 8 h and was subsequently cooled to room temperature. The copolymer of PEG-co-PLA was dissolved in dichloromethane, precipitated with anhydrous ether, filtered, and vacuum dried at 40 °C.

A total of 12 g above-described PEG-co-PLA copolymer was dissolved in 80 ml of dichloromethane in a 250 ml round bottomed flask. The solution was added 3.5 ml of triethylamine and cooled to 0 °C in an ice bath. Then, a total of 4.5 ml of acryloyl chloride was dissolved in 40 ml dichloromethane and added dropwise to the flask for 1 h, the reaction mixture was stirred for 12 h at 0 °C and 12 h at room temperature. The rude product was filtered and further refined by dichloromethane and hexane, and dried in vacuum at 50 °C for 24 h. This PEG macro-monomer is called PEL600 and its chemical structure is represented in Fig. 1.

2.3. Synthesis of thermoresponsive and biodegradable hydrogels

To synthesize a poly(NIPAAm-co-AAm) hydrogel, NIPAAm, AAm and degradable crosslinker PEL600 (Table 1) were dissolved in 5 mL of deionized water under a nitrogen atmosphere for 30 min to remove dissolved oxygen. Then 10 mg of ammonium persulfate (APS) and 10 mg sodium pyrosulfite were added to the solution, and the mixture was incubated at 20 °C for 24 h. After the completion of gelation, disk-shaped hydrogels were then cut off and washed in an excess amount of deionized water for 1 day to remove the residual unreacted monomers. Swollen hydrogels were dried in a vacuum for 2 days at 60 °C till no further weight loss occurred. After that, the dry hydrogel was approximately 10 mm in diameter and 4 mm in thickness.

2.4. Phase transition determination

2.4.1. Differential scanning calorimetry (DSC)

The LCST of a hydrogel was investigated with a Perkin-Elmer differential scanning calorimeter (DSC) (Diamond DSC). The hydrogel sample was immersed in distilled water at room temperature and allowed to reach the equilibrium state. Then the swollen sample was placed in a hermetic sample pan and sealed. The thermal analysis was performed in the range of temperature from 25 to

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

Sample	Content in		Crosslinker PEL600 (g)	APS (mg)	H_2O (mL)
	NIPAAm (g)	AAm (g)			
PNAM5	0.5	0.05	0.18	10	5
PNAM10	0.5	0.10	0.18	10	5
PNAM12	0.5	0.12	0.18	10	5
PNAM15	0.5	0.15	0.18	10	5

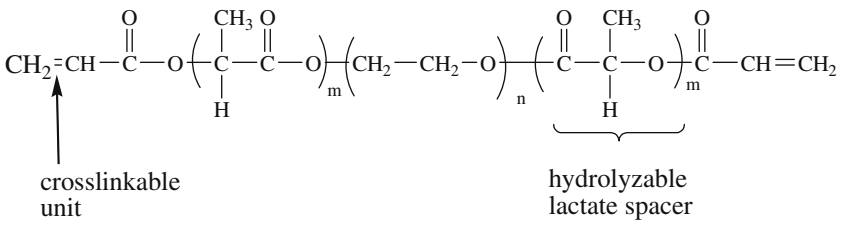

Fig. 1. The chemical structure of degradable crosslinker.

80 °C with a heating rate of 3 °C/min on the swollen sample under a dry nitrogen atmosphere with a flow rate of 50 ml/min.

2.4.2. Temperature dependence of the swelling ratio

The dried gels were immersed in deionized water at different temperatures. The swelling ratio (SR) was determined by the gravimetric method. The dry hydrogels were immersed in distilled water of the desired temperature in sealed containers, and the swollen weight for sample 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 swelling ratio (Q) is calculated by the following equation:

$$\text{Swelling ratio } (Q) = [(W_t - W_0)/W_0] \times 100\%; \quad (1)$$

where W_0 represents the weight of the dry hydrogel and W_t the weight of the swollen hydrogel at temperature t, respectively.

2.4.3. Swelling kinetics at 20 °C

A hydrogel sample, after equilibrated in distilled water at room temperature, was quickly frozen in liquid nitrogen and then freeze-dried under vacuum at −40 °C for 30 h. Then the freeze-dried sample was immersed in the distilled water at room temperature (20 °C) and removed from 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:

$$\text{WU} = [(W_t - W_0)/W_s] \times 100\%, \quad (2)$$

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

2.5. Morphology of hydrogel

The swollen samples, after reaching an equilibrium state in deionized water, 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 morphologies of the dried hydrogels were analyzed by scanning electron microscope (SEM) with sub-accelerating voltage of 20,000 V.

2.6. Compression experiment

The swollen gels were tested by gel strength instrument (LLOYD LRX). The following equation can be used to calculate the compression module of gels (Yao, Peng, Feng, & He, 1994)

$$\lambda = F/A_0 = G(\sigma - \sigma^{-2}), \quad (3)$$

where λ is the compression stress; F, the compression load; A_0, the undeformed cross-sectional area of the swollen gels; σ, the compression strain (L/L_0); L_0, the undeformed sample length; G, compression modulus. The effective crosslinking density (ρ_x) can then be calculated from the compression modulus and polymer swelling ratio (Q) as follows (Lee & Chen, 2001):

$$\rho_x = GQ^{1/3}/RT, \quad (4)$$

where R is the gas constant (8.48×10^4 g cm mol^{-1} K^{-1}) and T, the absolute temperature (293 K).

2.7. Degradation properties

The degradation of the synthesized hydrogels should depend on crosslinker due to the existence of poly(DL-Lactide). In this paper we focused on degradation study under normal physiological conditions by using PBS (pH 7.4) as degradation medium. The samples were equilibrated in PBS (pH 7.4) at 37 °C. At various time intervals, samples were removed from the solvents, dried in air overnight, and weighed to determine weight loss. After weighing, the samples were placed back in PBS (pH 7.4) for continuous degradation. PBS solvent was replaced every day.

3. Results and discussion

3.1. Thermoresponsive properties of hydrogels

3.1.1. Differential scanning calorimetry (DSC)

The LCST of the novel poly(NIPAAm-co-AAm) hydrogels determined from the DSC thermodiagrams is displayed in Fig. 2(A). It is well-known that PNIPAAm hydrogels have LCST behavior. At temperature below the LCST, hydrogen bonds between water molecules and hydrophilic groups give the hydrogels a good solubility. When the external temperature is increased to the LCST, the hydrogen bonds are overwhelmed by the hydrophobic interactions among the hydrophobic groups, causing a phase separation and shrinkage of the gel matrix. Similar to the PNIPAAm hydrogels, the prepared poly(NIPAAm-co-AAm) gels became swollen at temperatures below the LCST, but underwent a deswelling process when the external temperature was increased. Some steep falls in hydrogel swelling were observed when the temperatures were close to the relative LCSTs observed around 39.7, 53.5, 59.3, 64.2 °C for PNAM5, PNAM10, PNAM12, PNAM15 hydrogels (Table 2), respectively. The increasing AAm content caused the increase of LCST of the hydrogels, as a higher temperature was needed to drive the disruption of hydrogen bonds strengthened by the increased hydrophilicity. The interactions between the hydrophobic groups in response to temperature changes could not easily overwhelm the stronger hydrogen bonds between the hydrophilic amino groups of AAm units, and thus the LCST were increased.

3.1.2. Temperature dependence of swelling ratio

The dry hydrogels were dispersed in distilled water at 20 °C. After reaching the swelling equilibrium, these hydrogels were left at 20, 27.3, 34.2, 38.1, 42.8, 48.6, 55.3, 60.5, 63.8, 65.4, 68.3, 70.5 and 72.3 °C in the thermostatic water bath for 2 h before measuring the swelling ratio change.

The temperature dependence of swelling ratio of poly(NIPAAm-co-AAm) hydrogels was examined to evaluate their temperature sensitive properties. Fig. 2(B) represents the swelling ratio of PNAM5, PNAM10 PNAM12 and PNAM15 at different temperatures. The swelling ratio decreased rapidly as the temperature increased toward LCST. 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. 2(B), it is clear that LCST of the hydrogels increases with the increase of AAm content, which is consistent with LCST determined from DSC.

3.1.3. Reswelling kinetics at 20 °C

The swelling ratio at a low temperature is also critical for its practical applications, especially when used as recyclable intelligent devices. Fig. 2(C) demonstrates the swelling behavior of PNAM5, PNAM10, PNAM12 and PNAM15 hydrogels in distilled water at room temperature (20 °C). The poly(NIPAAm-co-AAm) gels have a large equilibrium swelling ratio at 20 °C. During the hydration process, water penetration rate was a crucial step to determine the swelling kinetics of hydrogel. In the case of PNAM5, its hydration was much slower from the beginning of swelling process and the corresponding water uptake reached 39.5% within 6 h

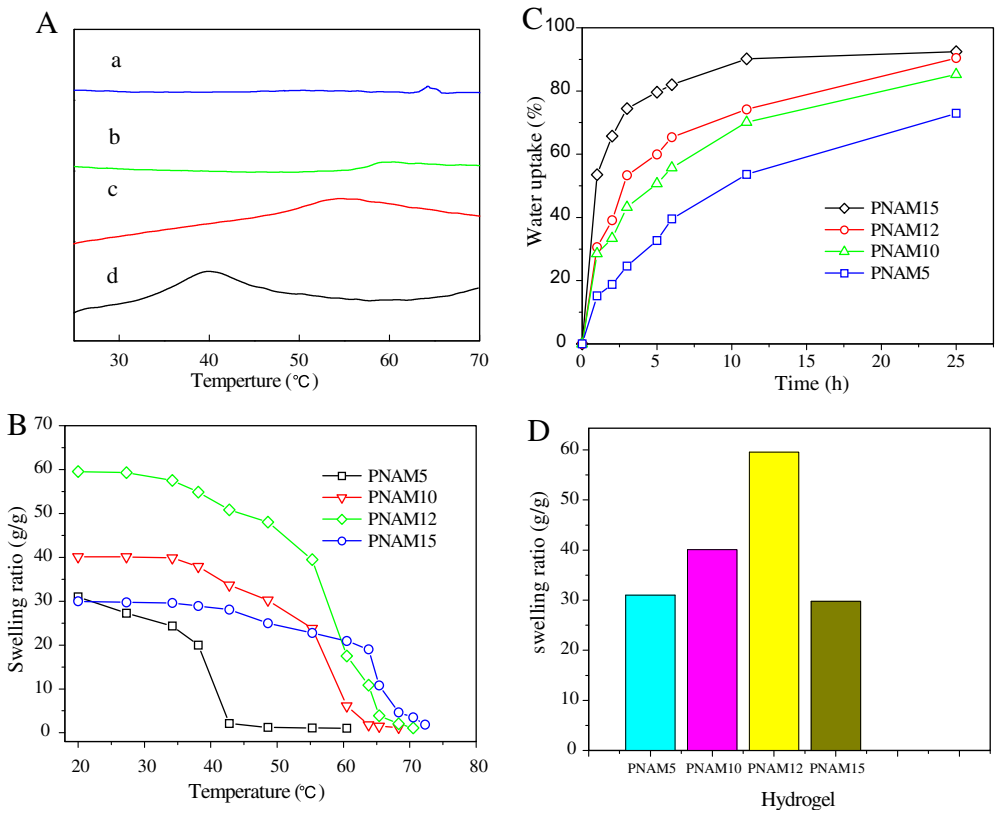

Fig. 2. Thermoresponsive properties of hydrogels: DSC thermodiagrams of the PNAM5 (a), PNAM10 (b), PNAM12 (c), PNAM15 (d) (A). Equilibrium swelling ratios as a function of temperature for PNAM5, PNAM10, PNAM12, PNAM15 (B). Swelling kinetics of different hydrogels measured gravimetrically in distilled water at 20 °C: PNAM5, PNAM10, PNAM12, PNAM15 (C). Equilibrium swelling ratio of PNAM5, PNAM10, PNAM12, PNAM5 hydrogels in water at room temperature (20 °C) (D).

Table 2
LSCT of the hydrogels of poly(NIPAAm-co-AAm) and PNIPAAm.

Sample ID	PNAM5	PNAM10	PNAM12	PNAM15
LSCT	39.7	53.5	59.3	64.2

and 53.6% within 11 h. However, the water uptake of PNAM15, PNAM12, and PNAM10 reached 81.98%, 65.35%, 55.7% within 6 h and 90.12%, 74.15%, 70.1% within 11 h, respectively.

The faster hydration/water uptake of the PNAM15 hydrogel was also due to the hydrophilic nature of the AAm. The increasing hydrogen bonds between water molecules and amino groups resulted in a faster water uptake of the PNAM15 hydrogels. But with the AAm content increasing, the swelling ratio of the hydrogel tended to increase first and then declined (Fig. 2(D)).

3.2. Effect of gel strength and crosslinking densities

The crosslinking densities of the hydrogels, calculated from Eq. (4), are listed in Table 3. The sample of PNAM15 has a higher G value than that of the other hydrogels, which is a result of higher crosslinking density. As shown in Table 3, the compression moduli of the hydrogels increase with the increasing crosslinking densities of the hydrogels. This can be explained in Eq. (4). Eq. (4) can be adjusted into:

$$G = \rho_x RT/Q^{1/3}, \quad (5)$$

i.e., the compression modulus depends on the crosslinking density and the swelling ratio. Because the values of $Q^{1/3}$ are nearly equal to 3 for all the gels (see Table 3), the compression moduli of the gels are directly proportional to their crosslinking densities. Hence, the crosslinking densities of the poly(NIPAAm-co-AAm) gels play a crucial role in determining their compression moduli.

Table 3
Effective crosslinking densities of poly(NIPAAm-co-AAm) hydrogels.

Sample no.	Swelling ratio Q (g/g) 20 °C	$Q^{1/3}$	Compression modulus G (g cm^{-2})	Crosslinking density $\rho_x \times 10^6$ (mol cm^{-3})
PNAM5	30.9	3.13	48.5	6.11
PNAM10	40.1	3.42	55.7	7.67
PNAM12	59.6	3.91	76.9	12.10
PNAM15	29.8	3.10	122.3	15.26

3.3. Morphology of hydrogel

The SEM micrographs of the novel poly(NIPAAm-co-AAm) hydrogels, as displayed in Fig. 3, illustrated the influence of the hydrogel composition on the morphology. Fig. 3 represents microphotographs of fractured surfaces of PNAM5, PNAM10, PNAM12, PNAM15, respectively. All of the hydrogels prepared have a similar porous network structure. However, the average pore size of the four hydrogels decreased in the order of PNAM5 < PNAM10 < PNAM12 > PNAM15. It can be seen that PNAM15 hydrogel has a relatively dense structure, while the PNAM5 hydrogels show a porous network structure. The results show that the pore sizes of poly(NIPAAm-co-AAm) increased first and then declined with the increasing content of AAm.

3.4. Degradation properties of hydrogels

At 37 °C, gels PNAM5, PNAM10, PNAM12, PNAM15 were observed to disintegrate after incubation in PBS (pH 7.4), as shown

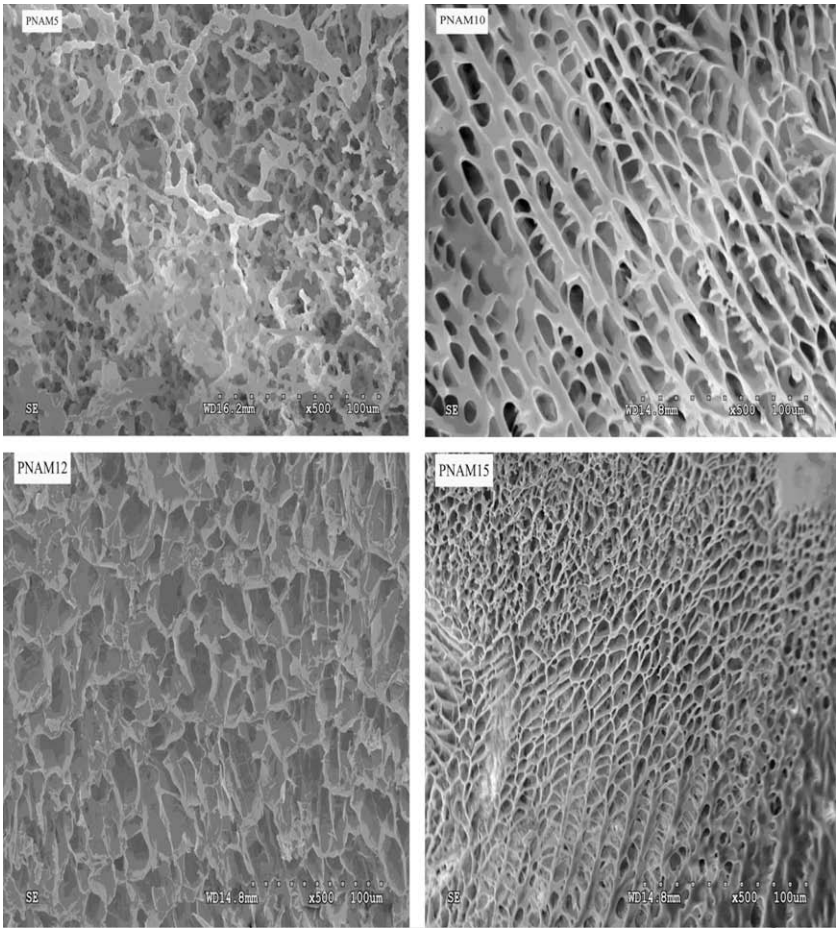

Fig. 3. The SEM image of PNAM5, PNAM10, PNAM12, PNAM15.

in Fig. 4. They then gradually dissolved within 2 months. As discussed in the previous section, all the hydrogels were highly hydrophilic and swollen at temperatures below the LCST. Therefore, abundant water could easily access and hydrolyze the ester bonds of oligolactate cross-links so that the hydrogels were broken down after a certain time. As the crosslinkers broke down, the liner poly(NIPAAm-co-AAm) chains and the degraded cross-links were freed up and diffused into the buffer solutions. There was observable disintegration of the hydrogels during 30 days of incubation in PBS (pH 7.4) solvent at 37 °C. The weight of PNAM5, PNAM10 hydrogels decreased more than 10% within 9 days. After 22 days the weight loss of the four hydrogels leveled off, and the weight losses of PNAM5, PNAM10, PNAM12 and PNAM15 were 21.2%, 19%, 16.9%, 12.3%, respectively. Fig. 4 also demonstrated that the weight losses and degradation rates of the four hydrogels decreased in the order PNAM5 > PNAM10 > PNAM12 > PNAM15 with the increase of AAm component, which is due to the increasing crosslinking density in the gels.

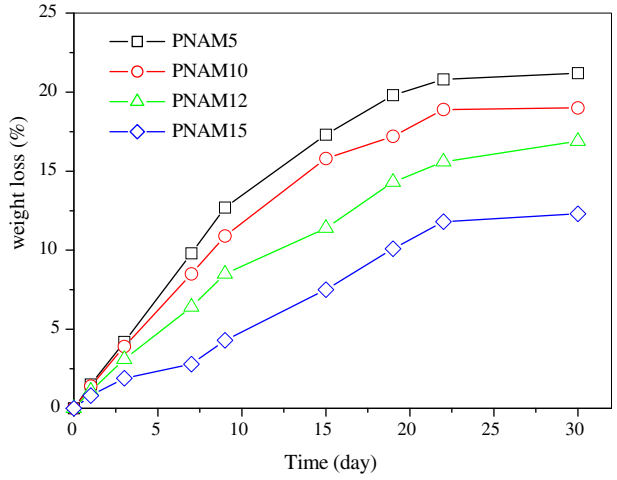

Fig. 4. Weight loss of PNAM5, PNAM10, PNAM12 and PNAM15 in PBS (pH 7.4) solvent at 37 °C.

4. Conclusion

In this study, a novel type of hydrogel was synthesized by the copolymerization of NIPAAm and AAm with PEG-co-PLA copolymer as a crosslinker. The structure, morphologies and properties of the resulted hydrogels were examined with SEM and DSC. The LCST of the hydrogel was increased and rapid responses to temperature were obtained as a result of the introduction of AAm into the polymer framework.

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