Study on Physicochemical Properties of Thermosensitive Hydrogels Constructed Using Graft-Copolymers of Poly(*N*-isopropylacrylamide) and Guar Gum

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ABSTRACT: A copolymer, poly (*N*-isopropylacrylamide)-*g*-Guar gum (PNIAAm-*g*-GG, GPNA), was synthesized using *N*-isopropylacrylamide (IPAAm) and Guar gum (GG). Thermosensitive hydrogels were obtained by crosslinking GPNA with glutaraldehyde (GA). The hydrogels were pseudoplastic fluid at the temperature below lower critical solution temperature (LCST), but they were dilatant fluid at temperature above LCST. With an increase in GA, hydrogels changed from dilatant fluid to pseudoplastic fluid. The state and content of water in GPNA hydrogels were studied by differential scanning calorimetry (DSC). The results indicated the existence of freezing bound water and free water when water content of the hydrogel was more than 98.4%. The melting enthalpy of freezing bound water was higher than that of free water, but the melting temperature was lower. The content of freezing bound water was more than that of nonfreezing water. At 40°C, above the LCST of hydrogel, water loss of the GPNA hydrogel was slow at the beginning, and then became more rapid with disorganization in hydrogel structure. At 35°C below LCST of hydrogel, amount of water loss in hydrogels increased with square root of time. The drug was released slowly from the gel at 40°C, and released more rapidly at 35°C. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3473–3479, 2008

Key words: drug delivery system; hydrogels; graft copolymer; thermosensitivity; poly(*N*-isopropylacrylamide)-*g*-Guar gum

INTRODUCTION

Hydrogels are composed of the three-dimensional network polymers and have properties of both solid and liquid. In the swollen state, hydrogels are soft and rubbery, and some hydrogels are similar to living tissue and possess excellent biocompatibility. Among polymers that can form hydrogels, natural polymers are often preferred to synthetic materials because of their nontoxicity, low cost, free availability, and biocompatibility. Besides, they can be copolymerized with some monomers to obtain copolymers with special function, such as thermosensitive or pH-sensitive copolymers. The copolymers are of great interest as drug carriers, food additives, and so on.

Guar gum (GG), a plant polysaccharide, has many advantages including low cost, widespread availability, and biodegradability. GG-based formulations developed as controlled release dosage forms have been successful in clinical trials.[1–4] Poly (*N*-isopropylacrylamide) (PNIAAm) in aqueous media exhibits a thermoresponsive phase transition at 32°C, which is known as lower critical solution temperature (LCST).[5] PNIAAm is hydrophilic in aqueous media with extended chain conformation at the temperature below LCST. When the temperature is above LCST, the polymer undergoes a phase transition and changes into an insoluble and hydrophobic aggregate. The thermoresponsive transition is reversible occurring within a narrow temperature range and has been used in pharmaceutical applications such as on–off release drug delivery systems.[6–7] However, one problem associated with PNIAAm gel is its inability to maintain shape as it swells in the presence of water. To increase gel strength, PNIAAm has been copolymerized with various compounds including acrylic acid and butyl methacrylate, but most of these products are neither biodegradable nor easy to synthesize.

Thermosensitive hydrogels were first synthesized using *N*-isopropylacrylamide and Guar gum (GG) and crosslinked with glutaraldehyde (GA) in this work.[8] Various parameters such as rheology, loss water rate, water content in hydrogels, and *in vitro* drug release were discussed in terms of the nature of the polymer.

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EXPERIMENTAL

Materials

N-Isopropylacryamide (IPAAm) was donated by Kohjin Co., Ltd. (Shenzhen, China) and used after recrystallization from hexane. GG (M = 200,000) and glutaraldehyde (GA) (25% w/v) were purchased from Sigma Chemicals (USA), and GG was purified with ether before used. Sinomenine hydrochloride (SH-HCl) was obtained from ShanChuan Bio-technique (Xi-an, China). All other chemicals were of reagent grade and used without further purification.

Synthesis of poly(*N*-isopropylacrylamide)-*g*-guar gum[8]

Poly(*N*-isopropylacrylamide)-*g*-guar gum (PNIAAm-*g*-GG) was synthesized by free radical polymerization as follows: 2 g of GG was dispersed in 150 mL of water and allowed to hydrate over 12 h in a 250-mL round-bottomed flask; 4 g PNIAAm was dissolved in 20 mL water and added to the GG solution followed by 1-h continuous mixing; 10 mL of 0.05 mol/L ceric ammonium nitrate was added to the solution. Polymerization was carried out at 60°C under continuous nitrogen atmosphere flowing and stirring for 6 h. After polymerization, a sufficient amount of acetone was used as an antisolvent to precipitate the graft copolymer. The copolymer was dried under vacuum (60 mmHg) at 40°C for 12 h.

Preparation of GPNA hydrogels

Twenty milliliter of 2.5% (w/v) GPNA solution were acidified with 5 mL dilute sulfuric acid. 1, 3, or 5 mL of a 25% (w/v) GA solution was added as the crosslinking agent. Crosslinking reaction was carried out for 24 h at room temperature with a constantly stirring rate of 300 r/min. The crosslinked hydrogel was washed with 5 mL of water for three times to remove unreacted GA.

Rheology study

Rheology study on copolymers was performed using a Brookfield Rheometer (Ostwald viscosimeter 1 × 140 mm) with a UL adopter at the rotation speed of 5–100 r/min. Experiments were carried out at different temperatures with circulating water into the jacket.

Analysis of water state

The water state in GPNA hydrogels was studied by differential scanning calorimetry (DSC) (DSC-7 Differential Scanning Calorimeter, Perkin–Elmer, Norwark). For DSC, the samples about 2–3 mg were heated from −20 to 100 °C at a heating rate of 10°C/min under argon atmosphere flowing (50 mL/min).

Shrinking capacity

The shrinking capacity of GPNA hydrogels was investigated by drying the cylinder hydrogels in oven at different temperature. The hydrogels were weighed until their weights did not change any more. The rate of water loss was gained by calculating the mass of losing water at per second interval.

In vitro drug release

The dried hydrogels were soaked in purified water until their weights stopped increasing. Hydrogels loaded with SH-HCl by soaking in a 4% (w/v) SH-HCl solution for 3 days at 37°C to achieve equilibration. The drug-loaded hydrogels were then dried and stored in a desiccator.

In vitro drug release from the GPNA hydrogels was conducted using USP Apparatus 2 with a paddle speed of 100 r/m. At regular time intervals, samples were withdrawn and the concentration of SH-HCl was determined using a UV–visible spectrophotometer at a wavelength of 264 nm.

RESULTS AND DISCUSSION

Rheology of GPNA hydrogels

The GPNA hydrogels exhibited rather complicated rheological behavior. Figure 1 shows apparent viscosity versus shear rate for the hydrogels at different temperature. The LCST determined by viscometry (Ostwald viscosimeter 1 × 140 mm) was ~ 37.5°C.[8] At 30 and 35°C, below LCST, the viscosity of GPNA

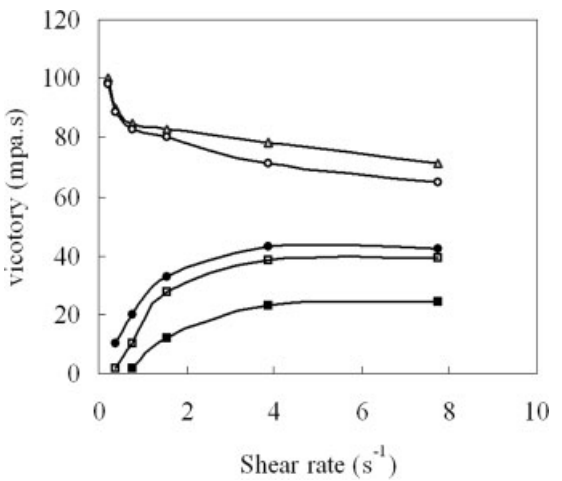

Figure 1 The viscosity of 2% (w/v) GPNA hydrogels with 20% GA at temperature (Δ) 30°C, (○) 35°C, (●) 38°C, (□) 40°C, and (■) 45°C (n = 3).

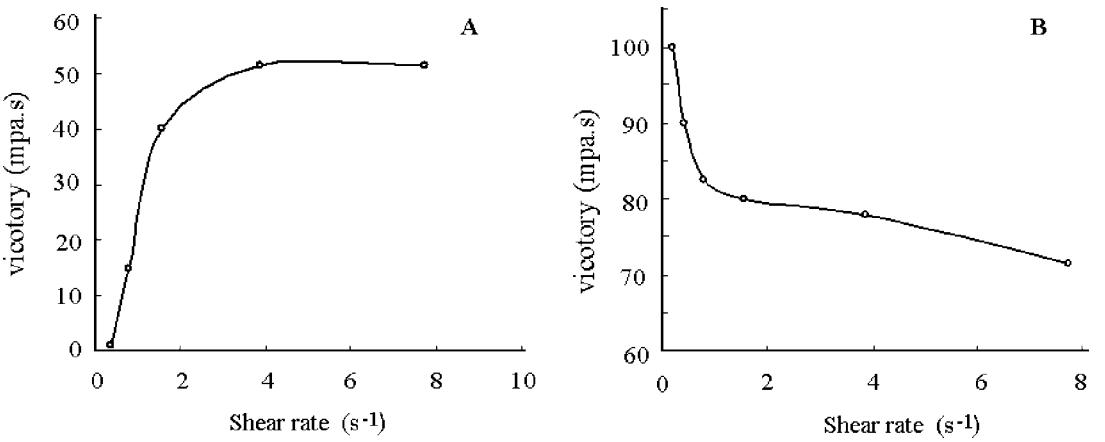

Figure 2 The viscosity of 2% (w/v) GPNA hydrogels at 30°C with GA of (A) 0 and (B) 10% (n = 3).

hydrogels decreased with an increase in shear rates, so GPNA hydrogels should be pseudoplastic fluid. On the contrary, at 38, 40, and 45°C, above LCST, the viscosity of GPNA hydrogels increased with an increase in shear rates, GPNA hydrogels belonged to dilatant fluid.

At the temperature below LCST, the polymer chains in hydrogels were asymmetric and in different direction. During shear test, long axis of polymer chain was turned and kept in a direction identical with the rotating direction of rotator. The faster the shear rate was, the stronger was the directionality effect. Thus, the flow resistance and viscosity of hydrogels decreased with an increase in shear rate. Additionally, hydrated sheath of gel particle could deform under the effect of shear stress, which lowered the flow resistance and viscosity of hydrogels system. At the temperature above LCST, the polymers changed into insoluble and hydrophobic aggregates, discontinuous phase generated, and the density of particles in the system rapidly increased. In static state, although the particles were disorderly and unsystematic, the lubrication of hydrated layer could reduce the flow resistance and viscosity. When the hydrogels were stirred, the particles were rearranged. Many particles collided and twined together, which could depress the original lubrication and enhance the flow resistance. So the flow resistance and viscosity increased with an increase in shear rate.

Figure 2 displays, at 30°C below LCST, the hydrogels change from dilatant fluid (0% GA) to pseudoplastic fluid (10% GA). Figure 2(A) shows that the viscosity of uncross-linked polymer hydrogels increases with the increase in shear rate. In uncrosslinked polymer hydrogels system, gel particles were mobile and their density was higher. During shear test, particles rearranged and formed disorderly space frame, which increased the flow resistance and viscosity. When the polymers undergo a crosslinked reaction [see Fig. 2(B)], the ability of particles to move in crosslinked hydrogels weakened. The increase of shear rate only changed the orientation of particles, enhanced a directionality effect, and decreased the flow resistance, so the viscosity decreased.

The state of water in hydrogels[9–14]

To understand the relationship between the hydrophobicity and microscopic structure, it is necessary to investigate the state of water in gels.[9] The water in hydrogels can be generally classified into three species: free water, freezing bound water, and nonfreezing bound water. Nonfreezing bound water, linking with hydrophilic group of the polymer network by hydrogen bond, does not freeze even at extra low temperature. Generally, its phase transition does not happen at the temperature range of 170–320 K. The free water can diffuse freely in hydrogels. The interaction between free water and polymer networks is the weakest, and its thermodynamic behavior accords with pure water. The most evident characteristic of freezing bound water is that melting temperature of freezing bound water is lower than that of pure water or free water. It is difficult to distinguish the melting peaks of freezing bound water and free water, so they are calculated together.[9] The water states in GPNA hydrogels were studied by DSC.

With an increase in water content, the split of melting peak becomes more apparent in the DSC curves, which suggests there are two states of water in the hydrogels, freezing bound water, and free water. It is already known that the freezing bound water exhibits lower melting temperature than the free water.[10] Thus, the melting peak in the lower temperature part was attributed to freezing bound water and the peak in the higher temperature part belonged to free water. In Figure 3, with an increase

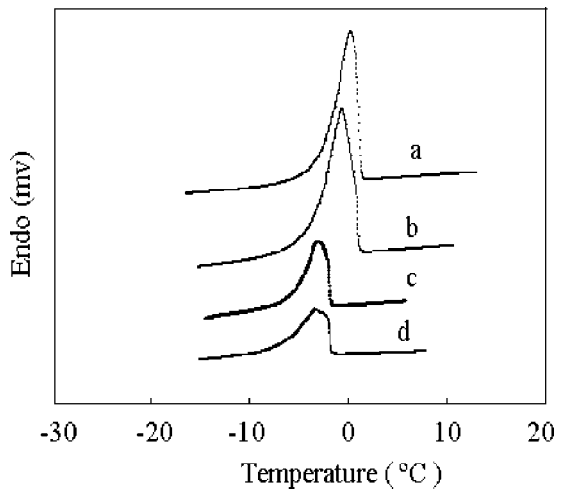

Figure 3 DSC curves of the GPNA hydrogels with different water contents (w%). (a) 99.5%, (b) 98.5%, (c) 96%, and (d) 94%.

in the total water content of GPNA hydrogels, the peak shape become narrow, shoulder peak disappears, and the peak position moves toward high temperature, which indicate the free water portion become more distinct. The content of freezing bound water is higher than that of nonfreezing bound water in hydrogels (see Table I). In the initial swelling process, the water content of hydrogels was low, intermolecular hydrogen bonds of hydrone ruptured, the new hydrogen bonds between hydrone, and hydrophilic group of polymers formed, and then the water molecules were bound to the polymers. The water thought as nonfreezing water can disperse uniformly throughout the hydrogels and cannot move freely.[11] Being saturated by nonfreezing bound water, the superfluous water preferentially oriented around the polymer framework and nonfreezing bound water as a secondary or tertiary layer of hydration. These structures resulted from the tendency of water molecules to form the maximum amount of hydrogen bonds in the limited space.[12] This kind of water was named freezing bound water. With the increase in water content, free water would be discovered in hydrogels. The content of freezing bound water, Q_f, was calculated as:

$$Q_f(\%) = \frac{\Delta H}{\Delta H_0} \times 100$$

where ΔH is the melting enthalpy that can be evaluated from the thermogram area with one polymer unit; ΔH_0 is the melting enthalpy of purified water with one water unit.

The molar content of water in hydrogels (X) was calculated as follows[12–13]:

$$X = \frac{x}{x + y}$$

where x is the total molar of water in hydrogels, y is the total molar of polymer unit in hydrogels. The total molar of polymer unit and hydrone in hydrogels was 1, then the total molar of hydrone in hydrogels equals to its molar content. That is for polymer unit, expressed as follows: $X = x$, $y = 1 - X$.

X_0 was the critical value of water content of the freezing bound water appearing, and X_1 was the critical value of the free water appearing. As water content in hydrogels was over X_0, but below X_1, the water in hydrogels included nonfreezing bound water and freezing bound water. The average molar enthalpy (ΔH_{mol}) equaled approximately to the enthalpy of freezing bound water. The total molar of freezing bound water was calculated as follows:[13]

$$x - n_0(1 - X) = X - n_0(1 - X)$$

The average molar enthalpy of water in hydrogels (ΔH_{mol}) was calculated as follows:

$$\Delta H_{\text{mol}} = \Delta H_1[X - n_0(1 - X)] = \Delta H_1[(1 + n_0)X - n_0]$$

$$X_0 \leq X < X_1$$

where ΔH_1 is the molar enthalpy of freezing bound water, ΔH_2 is the molar enthalpy of free water. The molar of nonfreezing bound water in polymer unit of each molar was n_0.

TABLE I
The Content of Different Kind of Water in the GPNA Hydrogels

Total water content (w%)	ΔH (J/mol)	Peak (°C)	Onset (°C)	End (°C)	Total freezing bound water content (w%)	No freezing bound water content (w%)
99.5	5018.16	0.717	−2.120	2.587	95.9	3.6
99.0	4909.75	0.092	−2.900	2.354	94.8	4.2
98.5	4751.84	0.0024	−3.211	2.587	91.7	6.8
98.0	4750.80	−0.135	−3.511	3.054	91.0	7.0
97.0	4569.59	−0.723	−3.991	2.087	88.2	8.8
96.0	4443.29	−0.798	−4.704	2.354	86.1	9.9
94.0	4294.20	−1.227	−5.725	1.172	81.5	12.5
H_2O	5178.45	1.283	−0.595	3.054		

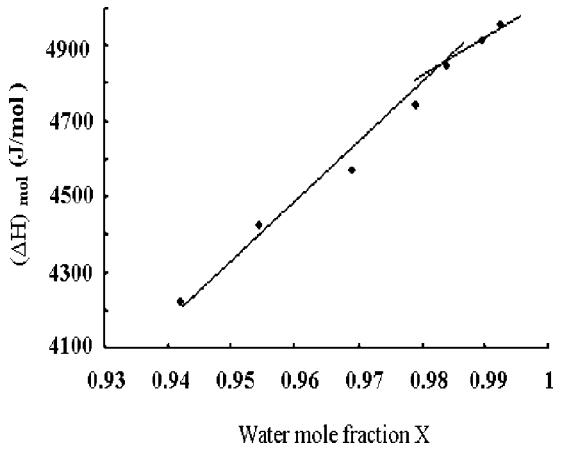

Figure 4 Dependence of ΔH_{mol} on the water content of GPNA hydrogels.

The water in hydrogels comprised freezing bound water, free water, and nonfreezing bound water when the water content was more than X_1. An average molar enthalpy (ΔH_{mol}) equaled approximately to the enthalpy of freezing bound water and free water. The total molar of freezing bound water was calculated as follows:

$$n_1(1-x) = n_1(1-X)$$

where n_1 is the molar of freezing bound water in polymer unit of each molar.

The molar of free water was calculated as follows:

$$x - (n_0 + n_1)(1-x) = X - (n_0 + n_1)(1-X).$$

The average molar enthalpy (ΔH_{mol}) was calculated as follows[13]:

$$\begin{aligned}\Delta H_{mol} &= \Delta H_1 n_1(1-X) + \Delta H_2[X - (n_0+n_1)(1-X)] \\ &= [n_1\Delta H_1 - (n_0+n_1)\Delta H_2] + [\Delta H_2(1+n_0+n_1) \\ &\qquad - n_1\Delta H_1]X \\ X_1 &\le X < 1\end{aligned}$$

ΔH_{mol} versus a total molar of water in hydrogels is shown in Figure 4.

The two linear-fit equations of intersection bilateral were shown in eqs. (1) and (2) as follows:

$$\Delta H_{mol,1} = 13912X - 8898.7 \qquad R = 0.9959 \qquad (1)$$

$$\Delta H_{mol,2} = 13189X - 8187.2 \qquad R = 0.9985 \qquad (2)$$

The slope rates of the curves (k_1 and k_2) and intercept were calculated as follows:

$$\begin{aligned}k_1 &= \Delta H_1(1+n_0) \\ k_2 &= \Delta H_2(1+n_0+n_1) - n_1\Delta H_1 \\ X_0 &= n_0/(1+n_0) \\ X_1 &= (n_0+n_1)/(1+n_0+n_1)\end{aligned}$$

where X_0 was an intersection of eq (1) and X-axis, X_1 was a value on X-axis corresponding to the intersection of two curves.

The result is shown in Table II. As the water content in hydrogels achieves 98.45%, a bump appears in the line (see Fig. 4), which indicate the appearance of another kind of water. The result is consistent with the result of theoretical analysis.

ΔH_1 is larger than ΔH_2, but the difference is not obvious (see Table II), so it is difficult to distinguish them quantitatively. n_1 is larger than n_0, indicating the content of freezing bound water was higher than that of nonfreezing water, which is in accordance with the previous report.[14]

Shrinking capacity

The GPNA hydrogels were elastic gel. Generally, elastic gel can absorb liquid not only by wetting but also by imbibition; the volume of elastic gel reduces with water loss; elastic gel can be extended.[15] The aggregates of the polymer chains resulted in phase-transition phenomenon. During the phase-transition, polymer changed from extended chain conformation to shrinking state. The shrinkage includes three steps as follows:[15] first, the shrinkage initiated on the surface of gel, and shrinking surface inhibited the exudation of solvent. The shrinkage would stop once the wizened surface layer formed. Second, pressure accumulating inside the gel resulted in tympanic bulla on the gel surface. Third, the pressure was powerful enough to make the solvent inside outflow, and the shrinkage went on.

The shrinking process was obviously different at the temperature above or below LCST (see Fig. 5). At 35°C, amount of water loss raised proportionately to the square root of time. At 40°C, the hydrophobic surface layer resulting from initial gel shrinking could inhibit solvent to exudation. At the same time, since part of the energy coming from the outside

TABLE II
The Results of Linear Fit of GPNA Hydrogels

Sample	k_1	k_2	n_0	n_1	X_0	X_1	ΔH_1	ΔH_2
GPNA	13,912	13,189	1.774	60.118	0.6396	0.9841	5013.88	5002.38

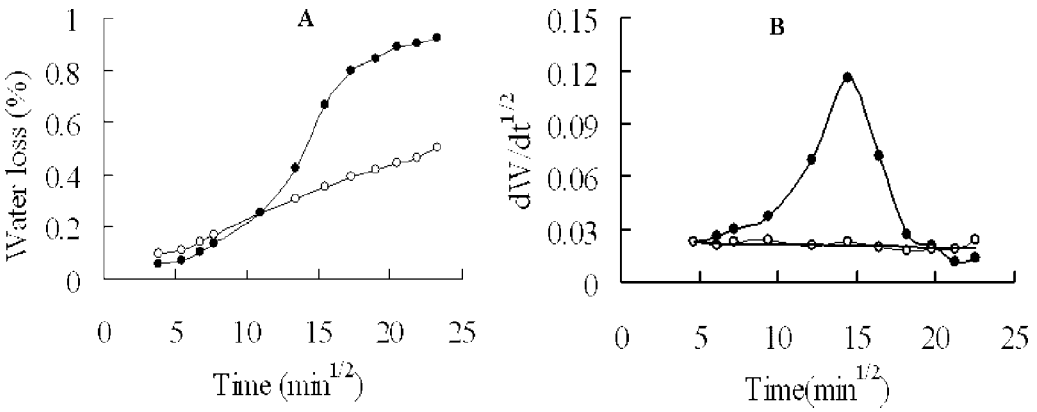

Figure 5 Effect of temperature on the mass and the water loss rate of hydrogel disks with a wet thickness of 1.0 cm and diameter of 1.5 cm (○) 35°C, (●) 40°C (n = 3).

was used to change the conformation of GPNA, the energy attributing to losing water was reduced. This was another reason for the decreasing of water loss rate. When the conformation change was complete, the pressure and energy inside gel increased, which accelerated the exudation of solvent. Under the powerful stress, more and more cracks formed on the surface, the structure of gel was destroyed, and the rate of losing water accelerated further.

With an increase in crosslinker, the rate of water loss increased (see Fig. 6). The main reason was that polymer structure became more compact with more crosslinker, mutual action between the polymer chain, and the water weakened, and water loss became easy.

In vitro drug release

SH-HCl release from hydrogels showed significant changes with temperature (see Fig. 7). At 35°C (below the LCST) SH-HCl released more rapidly, the behavior of drug release followed pseudozero-order or first-order kinetics. By contrast, at 40°C (above the LCST), it was difficult for SH-HCl to diffuse out of the hydrogels, so the drug released slowly. When the temperature increased, the shrinking surface of the hydrogels extruded the drug near the surface, resulting in an initial burst effect. However, the dense surface structure would then limit or prevent more drugs from being released. At low temperatures, the hydrogels swelled and allowed the drug to be released. Okano et al. found that the release of indomethacin from IPNS of PNIPAAm and polytetramethylene ether glycol could be correlated with the observed swelling properties. Indomethacin release was similar to SH-HCl from PNIPAAM-GG gel, pseudozero-order or first-order release kinetics at low temperatures but no release at elevated temperatures.[7]

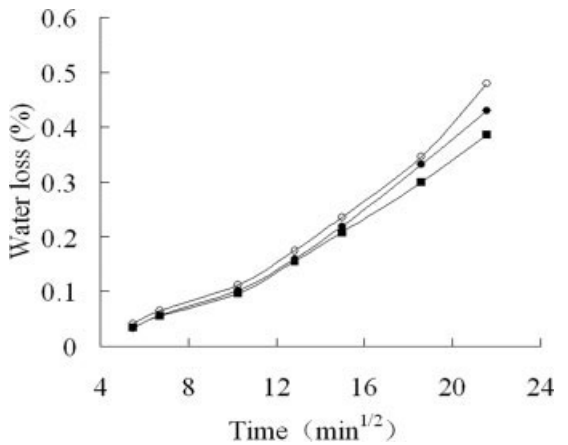

Figure 6 Effect of GA content on the shrink of gel disks with a wet thickness of 1.0 cm and diameter of 1.5 mm at 30°C (n = 3). GA (v/w): (○) 25%, (●) 15%, (■) 10%.

CONCLUSIONS

The GPNA hydrogels were synthesized successfully and some of the physicochemical properties were measured. The structure changes of GPNA led to the

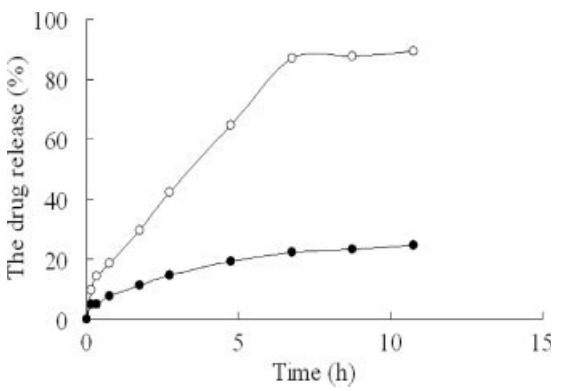

Figure 7 The drug release from hydrogels with 20% GA at temperature (●) 40°C and (○) 35°C (n = 3).

change of physicochemical properties. For example, the hydrogels had thermosensitivity with LCST at 37.5°C, the hydrogels were pseudoplastic fluid below LCST, but were dilatant fluid above LCST. Crosslinking can also change GPNA hydrogels from dilatant fluid to pseudoplastic fluid. Above and below LCST of hydrogels, the water loss rate and procedure were different. The drug was released slowly from the hydrogels above LCST and more rapidly below LCST. Based on the obvious thermosensitivity, GPNA hydrogels can be used as the carriers of drug delivery.

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