Colloid Polym Sci (2004) 282: 693–698
DOI 10.1007/s00396-003-0999-6

ORIGINAL CONTRIBUTION

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Synthesis and characterization of thermo-responsive poly(*N*-isopropylacrylamide-co-*N*-hydroxymethylacrylamide-co-2-hydroxyethyl methacrylate) hydrogels

Received: 1 July 2003
Accepted: 22 August 2003

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Abstract Thermoresponsive hydrogels based on *N*-isopropylacrylamide, *N*-hydroxymethylacrylamide, and 2-hydroxyethyl methacrylate, poly(NIPAM–co-NHMAAm–co-HEMA), have been synthesized and their swelling—deswelling behavior studied as a function of NIPAM concentration, NIPAM/NHMAAm and NIPAM/HEMA mole ratio, and total monomer concentration. Copolymers varying in composition have been obtained by redox copolymerization of these three monomers. Temperature has been changed in the ranges from 4 to 70 °C at fixed pH and total ionic strength. Equilibrium swelling ratio, dynamic swelling ratio, and dynamic deswelling ratio were evaluated for all hydrogel systems. The equilibrium swelling ratios of the copolymeric gels decrease with increasing NHMAAm and HEMA content. The formation of the intermolecular hydrogen bonding between hydroxyl and amido groups decreases the hydrophilic group numbers of the gel and the affinity of the gel towards water decreases. The copolymer gels also showed rapid volume transitions with time. The time required for equilibrium shrinking increased with increasing NHMAAm and HEMA content in the gel.

Keywords *N*-Isopropylacrylamide · *N*-Hydroxymethylacrylamide · 2-Hydroxyethylmethacrylate · Thermoresponsive polymer · Swelling behavior

Introduction

Hydrogels, which are water-swollen polymer and copolymer networks, have been used in many fields of application, for example biotechnology, bioengineering, pharmacy, medicine, agriculture, and the food industry. According to the type of functional group the properties of hydrogels can be changed with temperature, pH, solvent composition, and salt concentration [1, 2, 3, 4]. Thermally reversible hydrogels have recently been of increasing interest in the biomedical field in biotechnology. Many scientific works in recent years have dealt with hydrogels incorporating *N*-isopropylacrylamide, (NIPAM), monomer. The reason is the lower critical solution temperature (LCST), around 32 °C, of poly(NIPAM) water solutions and hydrogels. The thermoresponsive behavior of poly(NIPAM) gels has been extensively investigated and modeled by different researchers [5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. Poly(NIPAM)-based hydrogels are being used as drug-delivery systems [10] because the critical temperature is close to the human body temperature. The copolymers of NIPAM have also been used as carrier matrices for immobilization of enzymes and cells and dewatering of protein solutions [15, 16, 17, 18, 19]. The other widely used hydrogel is water-swollen, crosslinked poly(2-hydroxyethyl methacrylate), poly(HEMA), which is inert to normal biological processes, is resistant to degradation, is not absorbed by the body, and can be prepared in a variety of shapes and forms. In the random copolymerization of NIPAM with HEMA no significant loss of thermoresponsivity was observed when the HEMA component of the copolymer was lower than 50 mol% [20]. In our previous study a random copolymer of NIPAM and *N*-hydroxymethylacrylamide [poly(NIPAM–co-NHMAAm)] with thermoresponsive properties was produced by redox copolymerization and had responsive behavior to temperature [21].

In this study, poly(NIPAM–co-NHMAAm–co-HEMA) copolymer gel was proposed as a new alternative thermoresponsive gel for the immobilization of biological agents. Hydrogels were obtained by changing the NIPAM/NHMAAm and NIPAM/HEMA initial mol ratio and the total monomer concentration. The hydrogels thus prepared were characterized in terms of the degree of swelling and deswelling and the swelling–deswelling kinetics.

The biomedical and biological applications of poly(NIPAM) gels may require derivatization of the gel without loss of thermoresponsivity. However, the derivatization of the poly(NIPAM) structure is usually difficult. In our previous study the influence of the initial monomer mole ratio, total monomer, PEG 4000, and crosslinker concentrations on the swelling properties and diffusional behavior of water were investigated [22]. In another previous study, the influence of NIPAM/NHMAAm mole ratio on the swelling properties was investigated [21]. In those study, we did not observe cracks on the gel surface. Therefore, HEMA and NHMAAm were good comonomers for the functionalization of NIPAM-based thermoresponsive gels. The monomer used for the preparation of hydrogels in this study are also hydrophilic monomers and can bind some dyes (e.g. an affinity dye, Cibacron blue F3GA containing Cl); therefore, some ligands which can interact with biological molecules may be incorporated more easily into the gel matrix. By introducing hydroxyl functional groups into a thermoresponsive copolymer gel the interaction ability of hydroxyl groups may be used together with the thermoresponsive behavior to control the interactions of various biological molecules with the derivatized gel structure.

Experimental

Materials

N-Isopropylacrylamide (NIPAM; Aldrich, USA) was recrystallized from hexane. 2-Hydroxyethyl methacrylate (HEMA) was supplied by BDH Chemicals (Poole, UK). HEMA stabilized with 0.12% (*w*/*v*) methoxyethylhydroquinone was vacuum distilled to remove the polymerization inhibitor. *N*-hydroxymethylacrylamide (NHMAAm) was obtained from Merck-Suchardt. The other chemicals used were *N*,*N*′-methylenebisacrylamide (MBAAm) from BDH, as a crosslinking agent, sodium persulfate (NaPS), as an initiator, and *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED) from Merck as an accelerator. Besides these, $Na_2HPO_4.2H_2O$ from Merck and KH_2PO_4 from BDH were used to prepare the phosphate buffer solution at pH 7.0.

Preparation of hydrogels

NIPAM/NHMAAm/HEMA hydrogels were prepared by radical redox copolymerization. A typical procedure for the copolymerization can be described as follows. NIPAM was dissolved in 0.4 mL distilled water. NHMAAm and HEMA were added to this solution. Crosslinking agent solution, MBAAm (0.05 g mL^{-1} water; 0.05 mL), 0.05 mL accelerator solution, 0.05 mL initiator solution, NaPS (0.05 g mL^{-1} water), and TEMED (0.1 mL/1.5 mL water) were included in the copolymerization medium. In general, a type of persulfate initiator and chemicals that contain diamine (ethylene diamine, tetramethyleneethylene diamine, etc.) are used to initiate the polymerization reaction at low temperature. NaPS and TEMED formed a redox pair for the purpose of radical polymerization. Polymerization was carried out in a glass tube 8 mm in internal diameter and 75 mm in length. After adding all the chemicals the tubes were left at 4 °C until polymerization was complete. The hydrogels were removed from the tubes by breaking the tubes. The copolymer blocks obtained in the form of short cylinders were collapsed twice at 70 °C for 2 h and were washed with distilled water at room temperature for 24 h to remove any unreacted monomers within the copolymer matrix. The conditions used for production of the hydrogels are summarized in Table 1. Under these conditions the conversion of monomers was checked by gravimetric determination. In most cases, nearly quantitative conversion values and complete incorporation of comonomers into the thermoresponsive gel matrix were achieved.

Determination of swelling ratio

To test the thermoresponsivity of the hydrogels produced, equilibrium and the dynamic swelling–deswelling studies were performed in phosphate buffer solution (pH 7.0, total ionic strength 0.1). These experiments were carried out by using a thermostatic water bath (Fryka, Kaltechnik KB 300, Germany).

To determine the variation of equilibrium swelling ratio of the gel with temperature cylindrical gel samples were incubated in phosphate buffer solution at 4 °C for 4 days. At the end of this period, the weight of the gel sample was recorded after removing excess surface water with a laboratory tissue. The temperature program was then applied. The hydrogels were left at 24, 26, 29, 35, 40, 45, 50, 60, and 70 °C in a thermostatically controlled water bath until they reached equilibrium in phosphate buffer solution at pH 7.0. The equilibrium swelling ratio was defined as $(W_e - W_d)/W_d$, where W_e is the weight of the gel after establishment of equilibrium in the buffer solution and W_d is the dry weight of the copolymer sample. The swelling behavior of hydrogels that were at equilibrium at 70 °C was determined in phosphate solution at 4 °C. During the period following the dynamic swelling behavior the gels were left in the buffer phosphate solution at 4 °C. The increase in the water content of the copolymer samples was followed by the determination of the gel weight against time. The dynamic swelling ratio is defined as:

$$\Phi = (W_t - W_d)/(W_{0(70\,^\circ\mathrm{C})} - W_d) \tag{1}$$

where Φ is the swelling ratio, $W_{0(70\,^\circ\mathrm{C})}$ is the gel weight at equilibrium at 70 °C, W_t is the weight of the gel at a particular time, and W_d is the dry weight of the copolymer sample. The deswelling kinetics of the copolymer samples was followed by applying a

Table 1 Production conditions for poly(NIPAM–co-NHMAAm–co-HEMA) hydrogels by radical polymerization (0.05 mL of the initiator solution, NaPS (50 mg mL^{-1} water), 0.05 mL accelerator solution, TEMED (0.1 mL/1.5 mL water), 0.05 mL crosslinking agent solution, MBAAm (50 mg mL^{-1} water), and 0.4 mL water were included in polymerization mediums of all the gels listed)

Monomer mol ratio	NIPAM (mg)	NHMAAm (μL)	HEMA (μL)
Effect of NHMAAm concentration			
60.22/17.44	50	30	20
51.28/29.70	50	50	20
39.50/45.84	50	100	20
Effect of HEMA concentration			
56.96/26.55	50	30	30
45.06/41.90	50	30	50
31.75/59.06	50	30	100
Effect of NIPAM concentration			
	50	30	20
	80	30	20
	100	30	20
Effect of total monomer concentration			
	25	15	10
	50	30	20
	100	60	40

temperature change in the opposite direction of the swelling behavior investigation. The copolymer samples equilibrated in the phosphate buffer solution at 4 °C were transferred into another phosphate buffer solution at 70 °C. The shrinking of the hydrogels was followed by determination of the decrease in the water content of the copolymer samples. The weight of the gels were recorded at particular times. The deswelling ratio is defined as:

$$\Theta = (W_t - W_d)/(W_{0(4\,^\circ\mathrm{C})} - W_d) \qquad (2)$$

where Θ is the deswelling ratio, $W_{0(4\,^\circ\mathrm{C})}$ the weight of the gel at equilibrium at 4 °C, W_t the weight of the gel at a particular time, and W_d is the dry weight of the copolymer sample. The experiments were performed on three samples for each data point on the graphs.

Results and discussion

In our previous studies poly(NIPAM) gel was produced with different concentrations of initiator, accelerator, and crosslinking agent. For the variation of the equilibrium swelling ratio by medium temperature, the thermoresponsive poly(NIPAM) gel exhibited a transition at 32 °C as was expected.

The effect of NIPAM/NHMAAm mole ratio on the thermosensitivities of the produced copolymers was studied by changing this ratio at a fixed temperature and fixed HEMA concentration. The temperature dependency of the equilibrium swelling ratio of these gels is given in Fig. 1. The data show that the samples with lower NHMAAm content had similar swelling behavior as a function of temperature, and the phase–transition temperatures or LCSTs of these hydrogels lay in the vicinity of 34–35 °C. Lower equilibrium swelling ratios were obtained at constant temperature by increasing the NHMAAm content of the gel below the transition temperature. The swelling of the gel with the higher NIPAM content was similar to that of the poly(NIPAM) gel and the lower plateau value in the equilibrium swelling curve was obtained at lower temperatures for the gel produced with higher NHMAAm content. For these gels significant loss of thermosensitivity was observed with increasing NHMAAm content and transition temperature shifted to higher temperatures by increasing the NHMAAm content of the gel. Incorporating more hydrophilic comonomers reduces the amount of the hydrophobic groups and increases the polymer hydrophilicity due to the strong interaction between water and the hydrophilic groups in the polymer. This leads to an increased LCST, since the hydrophobic interactions, which increase with the temperature, are compensated for up to a higher temperature by increased polymer–water interactions [16, 17, 23, 24, 25].

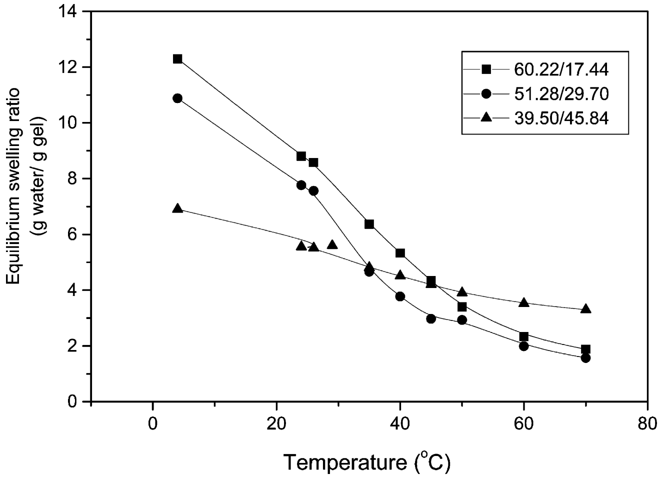

Fig. 1 Temperature-dependence of equilibrium swelling ratio for the gels produced with different NIPAM/NHMAAm mole ratios at fixed HEMA concentration

The swelling response of the copolymers produced with different NIPAM/NHMAAm mole ratios against the step input applied by decreasing the medium temperature from 70 to 4 °C is given in Fig. 2. In our previous study, poly(NIPAM) and poly(NHMAAm)

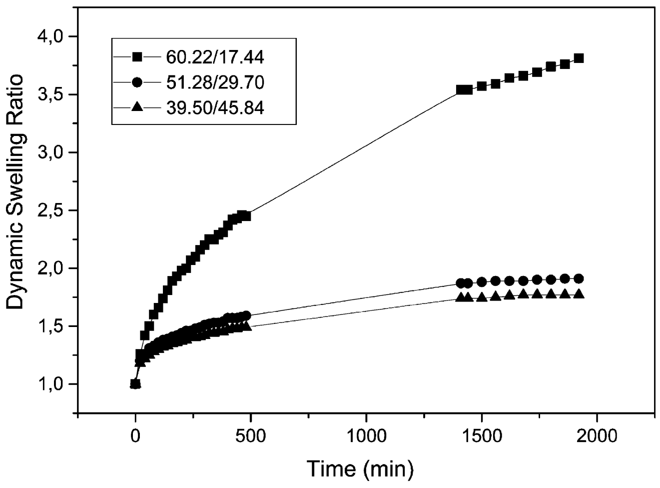

Fig. 2 Swelling kinetics of the gels produced with different NIPAM/NHMAAm mole ratios at fixed HEMA concentration. Magnitude of step input for medium temperature: 66 °C (from 70 to 4 °C)

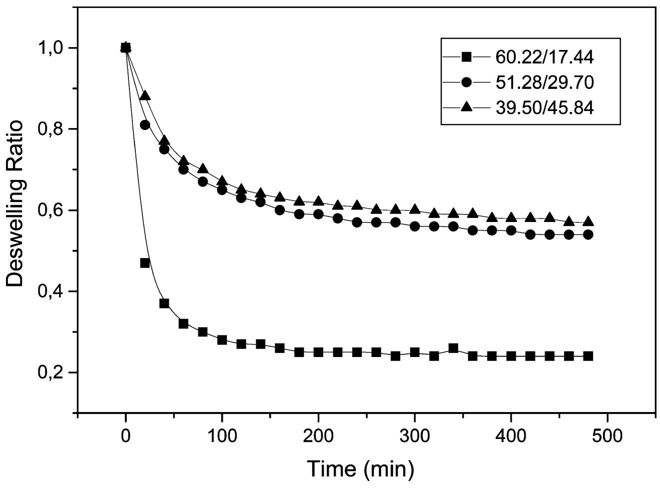

Fig. 3 Deswelling kinetics of the gel produced with different NIPAM/NHMAAm mole ratios at fixed HEMA concentration. Magnitude of step input for medium temperature: −66 °C (from 4 to 70 °C)

gels were produced with same concentrations of initiator, accelerator, and crosslinking agent [21]. The variation of the swelling rates (i.e. the variation swelling degree with the time) for poly(NIPAM) and poly(NHMAAm) gels was investigated in that study. Poly(NIPAM) gel exhibited the highest swelling ratio while poly(NHMAAm) had the lowest. The swelling rates of copolymers produced with different NIPAM/NHMAAm mole ratios were between these values. The swelling rates of the copolymers decreased and lower equilibrium swelling ratios were obtained at constant temperature by increasing the NHMAAm content of the copolymer. In other words, the lower plateau value in the equilibrium swelling curve was obtained at constant temperature and HEMA content. In the NHMAAm rich gels the equilibrium swelling ratios were possibly controlled by the NHMAAm part of the copolymeric gel. It is known that the dynamic equilibrium swelling ratio is related to ionic osmotic pressure, crosslinked density, and the hydrogel's affinity towards water. The total fixed charge was zero, and the crosslinked density was fixed in a series of NIPAM/NHMAAm/HEMA gels, so the influence of the dynamic swelling ratio of the gel is only affinity with water. The formation of intermolecular hydrogen bonding between amide and hydroxyl groups, decreases the hydrophilic group numbers of the gel and the affinity of the gel towards water decreases.

Fast responsive poly(NIPAM) gels were previously produced by applying special gelation procedures [26, 27]. The fast responsive type is usually achieved as a result of the macroporous structure of these gels created either by applying a vacuum during the gelation process (i.e. including a diluent in some cases) conducted at relatively higher temperatures [27] or by applying sudden temperature changes during the gelation [26]. To follow the shrinking kinetics of the copolymers the step input on the medium temperature was applied in the reverse direction. The dynamic shrinking behavior of the gels is given in Fig. 3. As seen here, the copolymer gel with a small NHMAAm content shrinks rapidly and this gel reached the equilibrium state first. The time required for equilibrium deswelling increased with increasing NHMAAm content in the copolymer structure. The gel deswelling magnitudes decrease with increasing NHMAAm content in the series of NIPAM-NHMAAm copolymer gels. Hydrophobic polymer aggregation forces in the phase transition are weakened because hydrated NHMAAm units disrupt regular aggregation of isopropylamide groups in polymer chains. As a result, water permeation from inside these gels increases with increasing NHMAAm content.

The effect of NIPAM/HEMA mole ratio on the thermosensitivities of the produced copolymers was studied by changing this ratio between 56.96/26.55 and 31.75/59.06. The concentration of NIPAM in the copolymerization medium was fixed at 100 mg mL^{-1}. The temperature dependency of the equilibrium swelling ratio of the gels produced with different NIPAM/HEMA mole ratios is given in Fig. 4. Lower equilibrium swelling ratios were obtained below the transition point by increasing the HEMA content of the copolymer structure. For copolymer gels, significant loss of thermosensitivity was observed with increasing HEMA content. The dynamic shrinking behavior of the HEMA-rich gels is given in Fig. 5. As seen here, the fastest shrinking was observed with the NIPAM-rich gel, which reached the equilibrium state first. The time required for equilibrium deswelling increased with increasing HEMA content in the gel matrix.

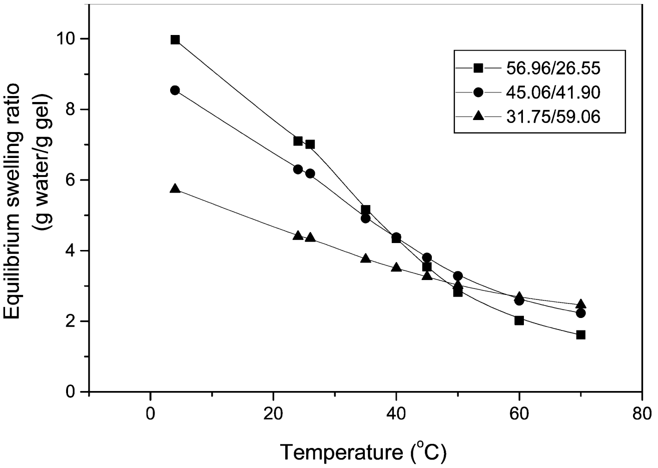

Fig. 4 Temperature-dependence of equilibrium swelling ratio for the gels produced with different NIPAM/HEMA mole ratios at fixed NHMAAm concentration

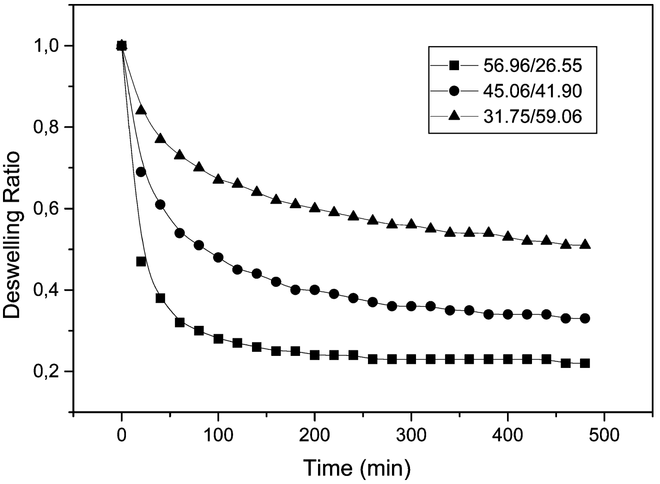

Fig. 5 Deswelling kinetics of the gel produced with different NIPAM/HEMA mole ratios at fixed NHMAAm concentration. Magnitude of step input for medium temperature: −66 °C (from 4 to 70 °C)

The variation of the dynamic swelling ratio of the gels produced with different NIPAM content at fixed HEMA and NHMAAm mole ratios was investigated. A decrease in the swelling rate and the fastest shrinking was observed with the NIPAM-rich gel (Figs. 6 and 7). The results show that the copolymeric gels exhibited deswelling behavior at higher temperature. This is because the amide group of NIPAM in the copolymer forms an intermolecular hydrogen bond with surrounding water at low temperature which would turn into an intramolecular hydrogen bond over its gel transition temperature. This phenomenon decreases the hydration capability of the gels and hydrophobicity of isopropyl groups of the NIPAM gel increases. These two

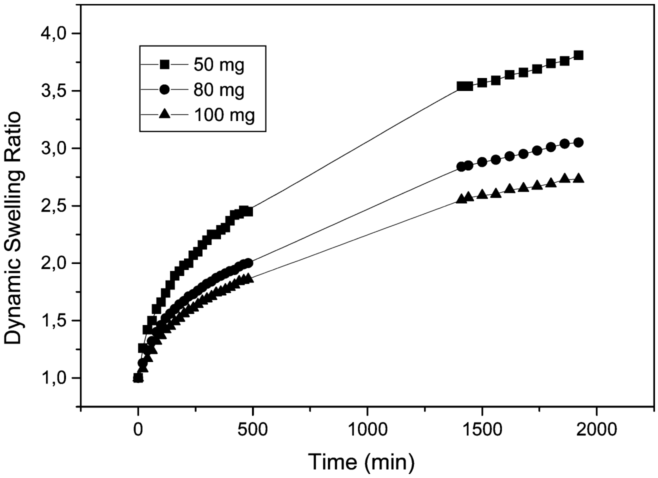

Fig. 6 Effect of the NIPAM concentration on the dynamic swelling behavior of the NIPAM–NHMAAm–HEMA gels. Magnitude of step input for medium temperature: 66 °C (from 70 to 4 °C)

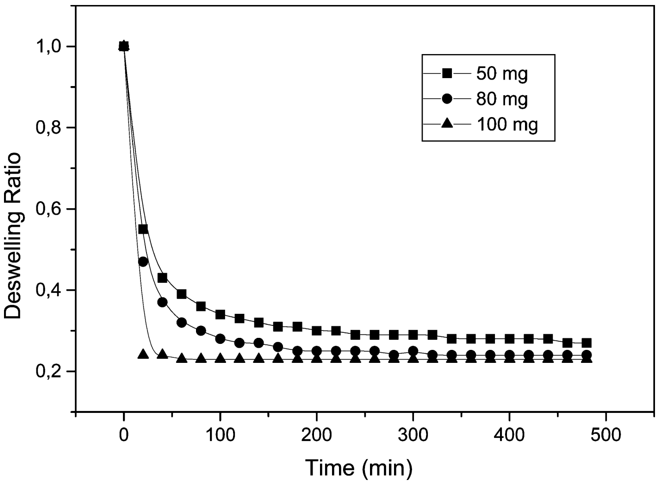

Fig. 7 Effect of the NIPAM concentration on the deswelling kinetics of the NIPAM–NHMAAm–HEMA gels. Magnitude of step input for medium temperature: −66 °C (from 4 to 70 °C)

effects make the state of the water molecule in the gel change from bound water to free water and makes the equilibrium swelling ratio of the gel decrease over its gel transition temperature. The time required for the gels to shrink to their equilibrium state was short and increased with decreasing NIPAM content in the copolymeric gel structure.

The total monomer concentration was changed between 50 and 200 mg/0.5 mL. The variation of the swelling ratio of the produced gels with temperature is given in Fig. 8. The equilibrium swelling ratio difference between 4 and 70 °C decreased and the temperature range where the volume change (the transition range) is shifted to higher temperatures is broadened for

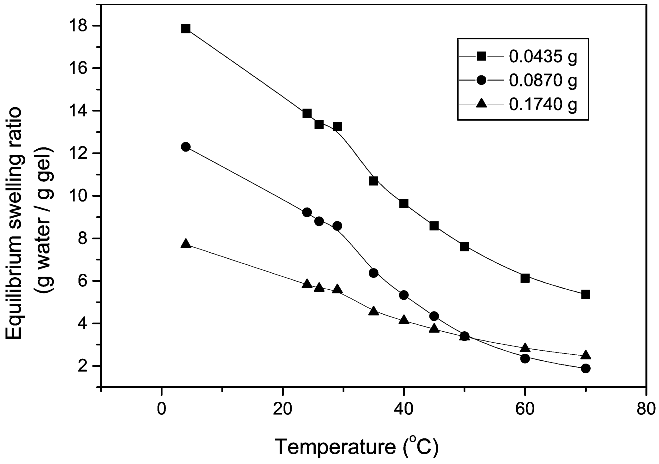

Fig. 8 Temperature-dependence of equilibrium swelling ratio for the NIPAM–NHMAAm–HEMA gels produced by different total monomer concentrations

poly(NIPAM–co-NHMAAm–co-HEMA) gel containing higher total monomer concentration. As seen Fig. 8, the equilibrium swelling ratio of the gels decreased with increasing total monomer concentration. The copolymer produced with total monomer concentration had higher thermosensitivity, because the equilibrium water content of the copolymer gel at 4 °C and the equilibrium swelling ratio difference between 4 and 70 °C increased with decreasing total monomer concentration. For constant volume the decrease in the total monomer concentration causes an increase in the microporosity of the gel. This case involves an increase in the equilibrium water content of the gel. Therefore, the observed increase in the thermosensitivity of the copolymeric gel may be explained by the increase in the microporosity of the structure with decreasing monomer concentration.

Conclusion

The polymerization of NIPAM with NHMAAm and HEMA yielded a thermoresponsive gel structure carrying hydroxyl functionality. The equilibrium swelling ratio of the copolymeric gels decreased with increasing NHMAAm and HEMA content. No undesired formations in the conventional poly(NIPAM) gel (e.g. the cracks on the gel surface) were observed with the copolymer gel structure produced. In addition, the copolymeric gels showed rapid volume transitions with time. The time required for equilibrium shrinking increased with the increasing NHMAAm and HEMA content in the gel matrix. The incorporation of hydroxyl groups into the poly(NIPAM) gels provided significant advantages for applications involving chemical derivatization of the thermoresponsive gel.

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