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Synthesis of thermoresponsive *N*-isopropylacrylamide–*N*-hydroxymethyl acrylamide hydrogels by redox polymerization

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Abstract

In this study, a random copolymer of *N*-isopropylacrylamide and *N*-hydroxymethyl acrylamide [poly(NIPAM-*co*-NHMAAm)], having a thermoresponsive character was prepared by a redox copolymerization method. Poly(ethylene glycol), PEG 4000, was used to increase the thermoresponsitivity of the resultant copolymeric structures. NIPAM-*co*-NHMAAm copolymers with different thermoresponsive properties were obtained by changing the initial NIPAM/NHMAAm mole ratio, total monomer, PEG 4000, and *N*,*N*′-methylenebisacrylamide, (MBAAm), concentrations. © 2000 Published by Elsevier Science Ltd.

Keywords: *N*-Isopropylacrylamide; *N*-Hydroxymethyl acrylamide; Thermoresponsive polymer

1. Introduction

Gels undergo reversible and discontinuous volume changes in response to the changes in environmental conditions such as solvent composition, temperature, salt concentration, pH [1–4]. Thermally reversible hydrogels have recently been of increasing interest in the biomedical field and in biotechnology. Poly(*N*-isopropylacrylamide), [Poly(NIPAM)], hydrogel is also a thermally reversible hydrogel and exhibits a lower critical solution temperature (LCST) at 32°C in aqueous solutions. The thermoresponsive behavior of poly(NIPAM) gels was extensively investigated and modeled by different researches [5–14].

Poly(NIPAM) gels have recently been of increasing interest in the fields of controlled drug delivery, immobilization of enzymes and cells, and dewatering of protein solutions [15–20].

Thermosensitive copolymeric structures are produced by the copolymerization of a temperature-sensitive monomer with an acrylate-based one. The behavior of thermosensitive poly(acrylamide-*co*-acrylic acid) copolymer gels was explained by hydrogen-bond formation [21]. Amphoteric copolymer gels with different thermoresponsive properties were synthesized by the copolymerization of NIPAM with *N*-3-dimethylaminopropylacrylamide, sodium 2-acrylamide-2-methylpropylsulfonate and betaine [22].

The form of copolymerization determines the phase transition behavior of the resultant copolymeric structure against temperature. Chen and Hoffman produced copolymers of NIPAM and acrylic acid (AA) by grafting temperature-sensitive oligo-(NIPAM) chains onto a pH-sensitive polymer backbone [i.e. poly(AAc)], and exhibited responsive behavior against either temperature or pH changes [23]. In the random copolymerization, a significant loss of thermosensitivity was observed when the AAc component of the copolymer was higher than 10 mol% [24]. In the copolymerization of a thermoresponsive monomer with a nonresponsive one, the critical point is the reflection of thermoresponsive monomer properties into the resultant copolymeric structure. When the nonresponsive comonomer content is high, the random copolymerization of NIPAM with an acrylate-based comonomer usually results in copolymeric structures having relatively weak thermosensitivity.

The primary purpose of the present study is to develop a thermoresponsive hydrogel, in which biocatalysts will be immobilized. The monomer used for the preparation of hydrogels in this study is *N*-hydroxymethyl acrylamide (NHMAAm), which is one of the hydrophilic monomers. NHMAAm can bind some dyes [i.e. an affinity dye, Cibacron Blue F3GA containing Cl], therefore some ligands having interaction abilities with biological molecules may be incorporated more easily into the gel matrix. By introducing an NHMAAm-based structure into a copolymer gel which

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is thermoresponsive in a proper temperature range, the binding ability of NHMAAm may be used together with the thermoresponsive behavior to control the interactions of various biological molecules with the derivated gel matrix. In this respect a series of temperature-sensitive copolymeric hydrogels was prepared by the redox copolymerization of *N*-isopropylacrylamide and *N*-hydroxymethyl acrylamide. Poly(NIPAM-*co*-NHMAAm) copolymers were obtained by changing the initial NIPAM/NHMAAm mole ratio, the total monomer, and PEG 4000 concentrations. The hydrogels thus prepared were characterized in terms of swelling–deswelling degree and swelling–deswelling kinetics. The gels will later be evaluated for potential enzyme immobilization systems.

2. Experimental

2.1. Chemicals

N-isopropylacrylamide (NIPAM) and *N*-(hydroxymethyl) acrylamide (NHMAAm) were used as monomers. The former was obtained from Aldrich Chemical Company, Inc., and the latter from Merck–Schuchardt. Poly(ethylene glycol) 4000 (PEG 4000) is added as a diluent. The other chemicals which were used are *N*,*N*′-methylenebisacrylamide (MBAAm) from BDH as a crosslinking agent, potassium persulphate (KPS) as an initiator and *N*,*N*,*N*′,*N*′-tetramethylethylen-diamin (TEMED) from Merck as an accelerator. Besides these, $HNa_2O_4P{\cdot}2H_2O$ from Merck and KH_2PO_4 from BDH were used to prepare the phosphate buffer solution at pH 7. The experiments were carried out in a thermostatic water bath (Fryka, Kaltechnik KB 300, Germany).

2.2. Preparation of hydrogels

N-isopropylacrylamide and *N*-(hydroxymethyl) acrylamide random copolymers were prepared by radical polymerization. NIPAM was dissolved in 0.4 ml of distilled water, NHMAAm was added to this solution and PEG 4000 should be dissolved if the hydrogel would be with PEG. After the addition of the two monomers, water and PEG 4000; 0.05 ml of the crosslinking agent solution, MBAAm (0.05 g/ml water), 0.05 ml of the initiator solution, KPS (0.05 g/ml water), 0.05 ml of the accelerator solution, TEMED (0.1 ml/1.5 ml water), were included in the polymerization medium. In general, a type of persulphate initiators, chemicals that contain diamin (ethylen-diamin, tetramethylethylen-diamin, etc.) are used in order to initiate the polymerization reaction at low temperature. KPS and TEMED are formed a pair of redox 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 completed. The hydrogels were removed from the tubes by breaking the tubes. After synthesis at room temperature the gels were short cylinders of 8 mm in diameter and 10–12 mm in length. 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 to remove the unreacted monomers and physically entrapped PEG 4000 within the gels. The production conditions of NIPAM/NHMAAm copolymers are given in Table 1.

2.3. Determination of swelling ratio

The cylindrical hydrogels were incubated in phosphate buffer solution at pH 7.0 and 0.1 M ionic strength, and at 4°C for 4 days. At the end of this period, the weight of the gel sample was measured after removing the excess surface water with a laboratory tissue. Then, the temperature program was applied. The hydrogels were left at 21, 30, 40, 45, 50, 60 and 70°C in the thermostatic water bath until they reach equilibrium in the 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 the establishment of equilibrium in the buffer solution and W_d the dry weight of the copolymer sample. The swelling behavior of the hydrogels was investigated by transferring the gels that were at equilibrium at 70°C into the phosphate solution at 4°C. During the period following the dynamic swelling behavior, the gels are 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:

$$\Phi = (W_t - W_d)/(W_{o(70°C)} - W_d)$$

where Φ is the swelling ratio, $W_{o(70°C)}$ the gel weight at equilibrium at 70°C, W_t the weight of the gel at a particular time and W_d the dry weight of the copolymer sample. The deswelling kinetics of the copolymer samples was followed by applying a 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 the determination of the decrease in the water content of the copolymer samples. The weight of the gels was recorded at particular times. The deswelling ratio is defined as:

$$\Theta = (W_t - W_d)/(W_{o(+4°C)} - W_d)$$

where Θ is the deswelling ratio, $W_{o(+4°C)}$ the weight of the gel at equilibrium at +4°C, W_t the weight of the gel at a particular time and W_d the dry weight of the copolymer sample.

Table 1
Production conditions of poly(NIPAM-*co*-NHMAAm) copolymers by radical polymerization (0.05 ml of the initiator solution, KPS (0.05 g/ml water), 0.05 ml of the accelerator solution, TEMED (0.1 ml/1.5 ml water), and 0.4 ml water were the included polymerization mediums of all the gels listed)

NIPAM/NHMAAm mole ratio	NIPAM (mg)	NHMAAm (μl)	MBAAm (μl)	PEG 4000 (mg)
Effect of NIPAM/NHMAAm mole ratio				
100:0	50	–	50	–
77.54:22.46	50	25	50	–
63.32:36.68	50	50	50	–
46.28:53.72	50	100	50	–
0:100	–	50	50	–
Effect of total monomer concentration				
63.32:36.68	25	25	50	–
63.32:36.68	50	50	50	–
63.32:36.68	75	75	50	–
Effect of PEG 4000 concentration				
77.54:22.46	50	25	50	50
63.32:36.68	50	50	50	50
53.45:46.55	50	75	50	50
46.28:53.72	50	100	50	50
36.50:63.50	50	150	50	50
53.45:46.55	50	75	50	0
53.45:46.55	50	75	50	50
53.45:46.55	50	75	50	100
63.32:36.68	25	25	50	50
63.32:36.68	50	50	50	50
63.32:36.68	75	75	50	50
Effect of MBAAm concentration				
63.32:36.68	50	50	0	0
63.32:36.68	50	50	50	0
63.32:36.68	50	50	200	0

3. Results and discussion

3.1. Swelling behavior of poly(NIPAM) gel and effect of NIPAM/NHMAAm mole ratio

Poly(NIPAM) gel was prepared by radical polymerization. The crosslinking was achieved by MBAAm and the monomer concentration was fixed at 50 mg/ml. The effect of temperature on the swelling behavior of the produced gel was studied in phosphate buffer medium (pH 7.0, total ionic strength 0.1 M). For the variation of the equilibrium-swelling ratio by medium temperature, the thermoresponsive poly(NIPAM) gel exhibited a sharp transition at 32°C as was expected. Note that the poly(NIPAM) gel produced by redox polymerization exhibited a very similar response against the temperature to that of poly(NIPAM) gels produced by other redox polymerization procedures [25].

The effect of NIPAM/NHMAAm mole ratio on the thermoresponsitivities of the produced copolymers was studied by changing this ratio between 77.54:22.46 and 46.28:53.72. The concentration of NIPAM in the polymerization medium was fixed at 50 mg/ml. The temperature dependency of the equilibrium-swelling ratio of the gels produced with different NIPAM/NHMAAm mole ratios is given in Fig. 1. Lower equilibrium-swelling ratios were obtained at constant temperature by increasing the NHMAAm content of the gel structure. As was expected, the swelling behavior of the gel obtained with 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 with the gels produced with higher NHMAAm content. For these gels, significant loss of thermosensitivity was observed with increasing NHMAAm content and transition temperature shifted to the right by increasing the NHMAAm content of the gel. Incorporating more hydrophilic or hydropobic monomers in the gel composition can control the phase transition behavior. Incorporation of hydrophilic comonomers reduces the amount of hydrophobic groups and increases the polymer hydrophility 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 temperature, are compensated for up to a higher temperature by the increased polymer–water interactions [17,18,23,24,26].

The dynamic swelling ratios of the copolymers produced with different NIPAM/NHMAAm mole ratios are given in Fig. 2. The dynamic swelling behaviors of poly(NIPAM) and poly(NHMAAm) gels are also included in this figure. As seen in Fig. 2, the poly(NIPAM) gel exhibited the highest swelling rate while the poly(NHMAAm) gel had the

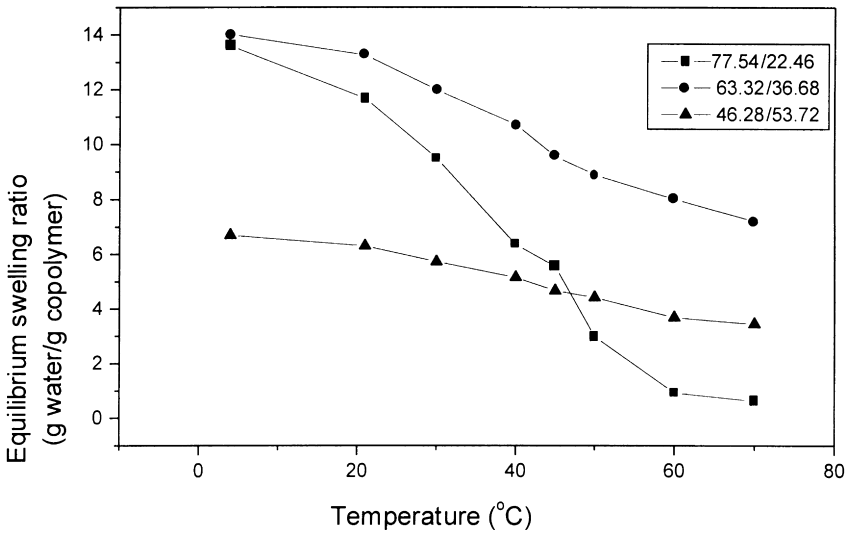

Fig. 1. Temperature dependence of equilibrium-swelling ratio for the NIPAM–NHMAAm copolymers produced by different NIPAM/NHMAAm mole ratios; NIPAM concentration, 50 mg/ml.

lowest. The dynamic swelling behaviors of poly(NIPAM-*co*-NHMAAm) gels were between them. 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 higher plateau value in the equilibrium-swelling curve was obtained at constant temperature with the gel produced with higher NIPAM content. The swelling behaviors of the copolymers obtained with higher NIPAM concentrations (i.e. 77.54 mol%) were similar to that of the poly(NIPAM) gel.

To follow the shrinking kinetics of the copolymers, the step input on the medium temperature was applied in the reverse direction. The dynamic shrinking behaviors of the gels are given in Fig. 3. As seen here, the fastest shrinking was observed with the poly(NIPAM) gel, which reached the equilibrium state first. The time required for equilibrium deswelling increased with the increase of NHMAAm content in the copolymer.

3.2. Effect of total monomer concentration

The total monomer concentration was changed between 67 and 193 mg/ml by fixing the initial NIPAM/NHMAAm mole ratio to 63.32:36.68. The variation of the swelling ratio of the produced gels with the temperature is given in Fig. 4. The equilibrium-swelling ratio difference between 4 and 70°C decreased and the temperature range where the volume change (the transition region) is shifted to higher temperatures is broadened with all poly(NIPAM–NHMAAm) gels

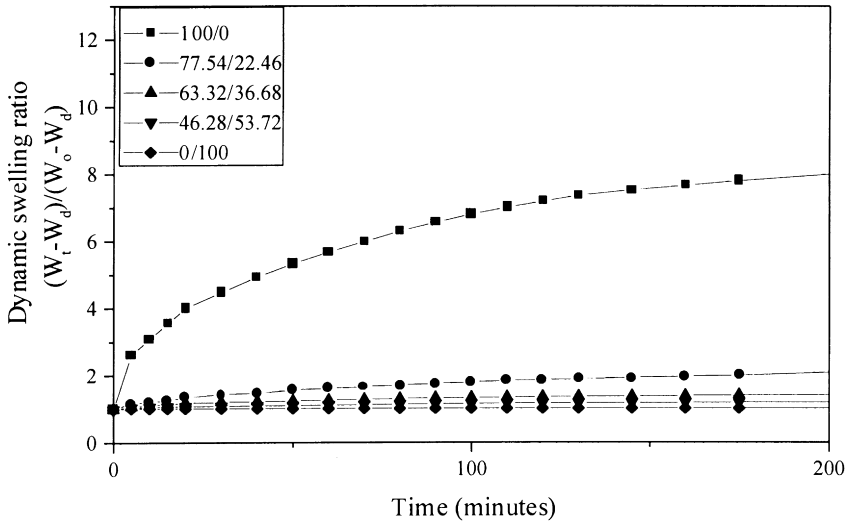

Fig. 2. Swelling kinetics of NIPAM–NHMAAm random copolymers produced with different NIPAM/NHMAAm mole ratios. Magnitude of step input for medium temperature, 66°C (from 70 to 4°C).

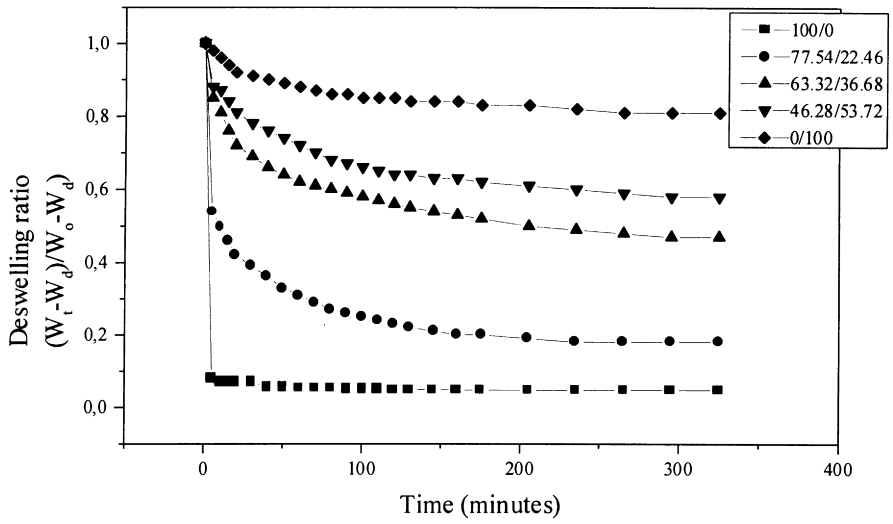

Fig. 3. Deswelling kinetics of NIPAM–NHMAAm random copolymers produced with different NIPAM/NHMAAm mole ratios. Magnitude of step input for medium temperature, −66°C (from 4 to 70°C).

relative to the poly(NIPAM) gel. As seen here, the equilibrium-swelling ratio of the gels decreased with increasing total monomer concentration. The copolymers produced with lower total monomer concentrations exhibited higher thermosensitivity since 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 the constant gel 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 copolymer gel may be explained by the increase in the microporosity of the gel with decreasing total monomer concentration.

3.3. Effect of MBAAm concentration

In the copolymerization experiments, MBAAm concentration was changed by fixing the initial NIPAM/NHMAAm mole ratio to 63.32:36.68. The variation of the swelling ratio of the produced gels with the medium temperature is given in Fig. 5. As seen here, the equilibrium-swelling ratio of the

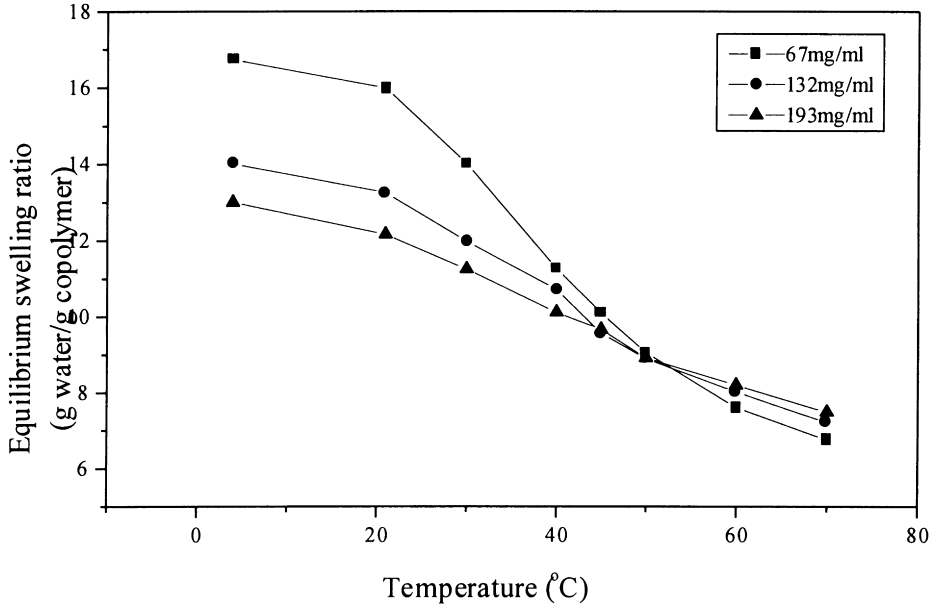

Fig. 4. Temperature dependence of equilibrium-swelling ratio for the NIPAM–NHMAAm copolymers produced by different total monomer concentrations. NIPAM/NHMAAm mole ratio, 63.32:36.68.

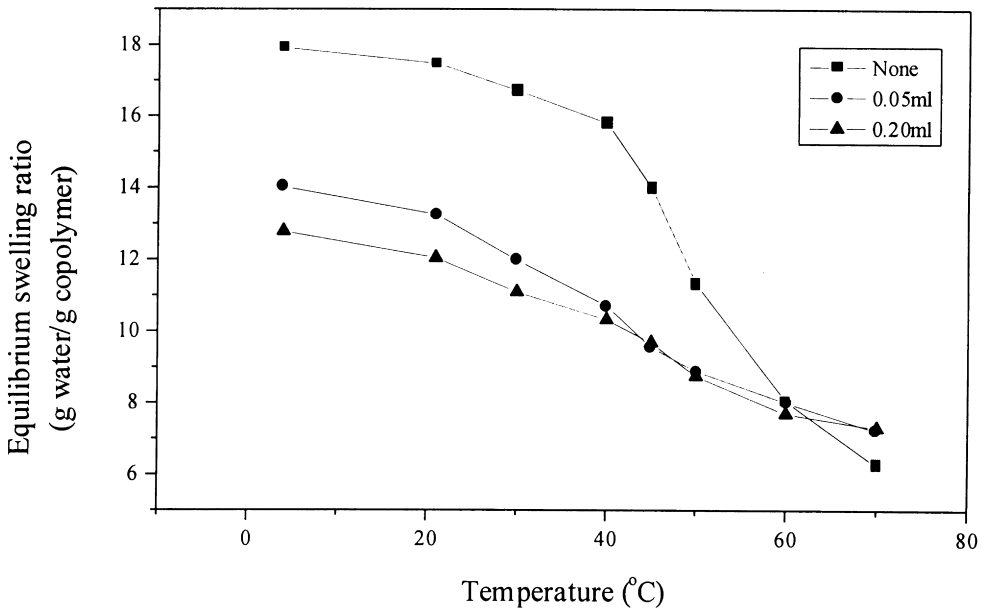

Fig. 5. Effect of MBAAm concentration on the temperature dependence of equilibrium-swelling ratio for the NIPAM–NHMAAm copolymers produced by 63.32:36.68 mole ratio.

copolymers decreased with increasing MBAAm concentration, as was expected. The swelling behavior of the gel that was obtained without any crosslinker is also included in the same figure. This gel may be obtained via complex formation between NIPAM and NHMAAm involving carboxylic acid groups. These hydrogen-bonded structures were observed between the carboxyl and amide groups in interpenetrating networks of the PAAc and acrylamide copolymer, and the PNIPAM and PAAc graft copolymers [23,27,28].

3.4. Effect of PEG 4000 concentration

To increase the thermoresponsitivity of the produced gels by creating additional microporosity within the gel, PEG 4000 was tried since it was soluble in the initial copolymerization medium and its low molecular weight enabled its removal from the gel after copolymerization by a diffusion process induced by the shrinking of the gel. Hydroxypropyl cellulose (HPC) was used as a pore forming agent in the literature [29].

The effect of PEG 4000 on the swelling behavior of the poly(NIPAM–NHMAAm) gel at a constant total monomer concentration and at a constant monomer concentration was studied by changing the PEG concentration in the gelation medium between 0 and 100 mg/ml. The total monomer concentration and NIPAM/NHMAAm mole ratio were fixed at 132 mg/ml and 53.45:46.55, respectively. The variation of the equilibrium-swelling ratio of the gels produced with different PEG 4000 concentrations is given in Fig. 6. The use of PEG 4000 caused a significant decrease in the thermosensitivity of the copolymer. The equilibrium-swelling ratios at lower temperatures and the difference between the upper and lower plateau regions of the equilibrium-swelling curve decreased with decreasing PEG 4000 concentration.

The effect of total monomer concentration on the swelling behavior of the gels produced in the presence of PEG 4000 is given in Fig. 7. In these experiments, the PEG 4000 concentration was fixed at 50 mg/ml and the total monomer concentration was changed between 67 and 193 mg/ml. When the swelling behaviors given in Fig. 7 are compared with the results in Fig. 4, it is clearly seen that the equilibrium-swelling ratios at lower temperatures and the difference between the upper and lower plateau regions of the equilibrium-swelling curve increased in the presence of PEG 4000.

The variation of the equilibrium-swelling ratio with temperature is given in Fig. 8 for the gels produced by changing the NIPAM/NHMAAm mole ratio in the presence of PEG 4000. Here, the NIPAM and PEG 4000 concentrations were fixed at 50 mg/ml. The equilibrium-swelling ratio difference between the upper and lower plateau regions of the curves slightly increased with all NIPAM/NHMAAm ratios in the presence of PEG 4000 relative to those given in Fig. 1 sketched for the gels produced in the absence of PEG 4000.

To observe the effect of PEG 4000 on the dynamic swelling behavior, poly(NIPAM-*co*-NHMAAm) gels prepared with different NIPAM/NHMAAm mole ratios were utilized. The dynamic swelling ratio with time is given in Fig. 9 for the gels produced in the presence of PEG 4000. When the swelling behaviors in Fig. 9 are compared with those obtained for the gels produced in the absence of PEG 4000 (Fig. 2), it is seen that the use of PEG 4000 caused a slight increase in the swelling of the gel produced with

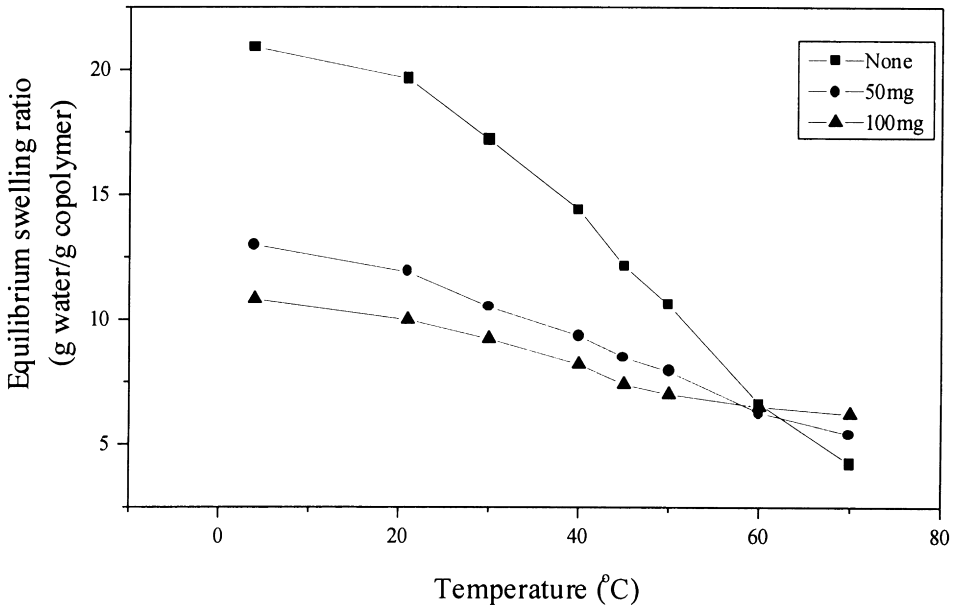

Fig. 6. Temperature dependence of equilibrium-swelling ratio for the NIPAM–NHMAAm copolymers produced by different PEG 4000 concentrations. Total monomer concentration, 132 mg/ml; NIPAM/NHMAAm mole ratio, 53.45:46.55.

NIPAM/NHMAAm having a mole ratio of 77.54:22.46. But no change was observed in the swelling rate of the gel with an NIPAM/NHMAAm mole ratio of 46.28:53.72. Therefore, the effect of PEG 4000 on the swelling of the gel was strongly related to the gel composition. The increase in the swelling rate of the NIPAM-rich gel may be explained by the formation of additional microporosity by the introduction PEG 4000, which accelerated the water diffusion into the gel.

The variation of the deswelling ratio with time is given in Fig. 10 for the gels produced in the presence of PEG 4000. In the production of these gels, the PEG 4000 concentration was fixed at 50 mg/ml. The comparison of shrinking behaviors in Fig. 10 with those observed for the gels having the same composition and produced without using PEG 4000 (Fig. 3) indicated that the shrinking rate of the gel was not significantly affected by the introduction of PEG 4000 in the studied gel compositions. The type of porosity is one of the most important factors for controlling the rate of volume

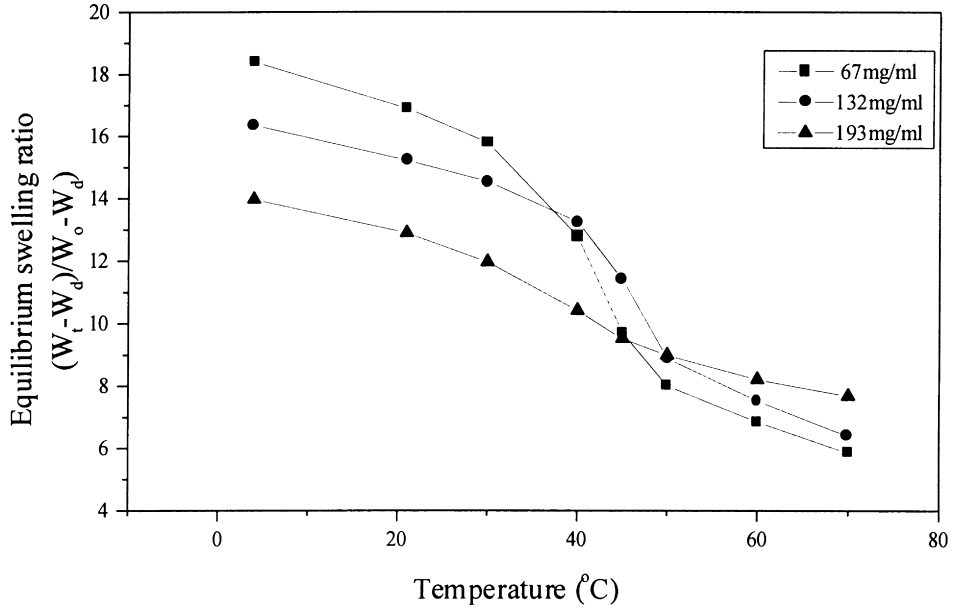

Fig. 7. Temperature dependence of equilibrium-swelling ratio for the NIPAM–NHMAAm copolymers produced by different total monomer concentrations in the presence of PEG 4000 as a diluent. NIPAM/NHMAAm mole ratio, 63.32:36.68.

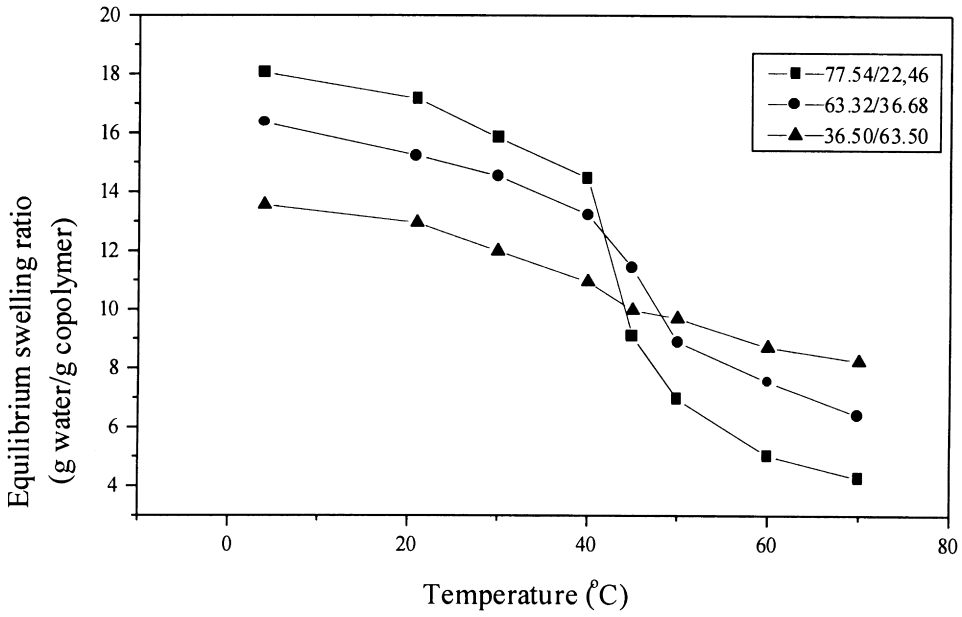

Fig. 8. Variation of the swelling ratio with the medium temperature for the copolymers produced by changing the NIPAM/NHMAAm mole ratio and in the presence of PEG 4000. PEG 4000 concentration, 50 mg/ml.

change in thermally reversible gels. The macroporous poly(NIPAM) gels shrunk more rapidly relative to the conventional microporous one and the shrinking rate was higher than that observed with the poly(NIPAM) gels prepared with the conventional recipes, since the existence of large pores prevents the skin formation and the large pores cannot close up completely even in the shrunken state [29]. In our results, the shrinking rates of gels produced in the absence and presence of PEG 4000 were very close for constant NHMAAm. In the presence of a microporous structure, the skin formation on the gel surface cannot be prevented, which determines the swelling rate in the shrinking process [29]. So, the increase in the microporosity may not be effective on the shrinking rate due to the dominant effect of skin formation. Therefore, the equality of deswelling rates obtained in the absence and in the presence of PEG 4000 also indicated that no significant macropore formation occurred within the gel by introducing the diluent into the copolymerization recipe.

4. Conclusions

A new series of temperature sensitive hydrogels was

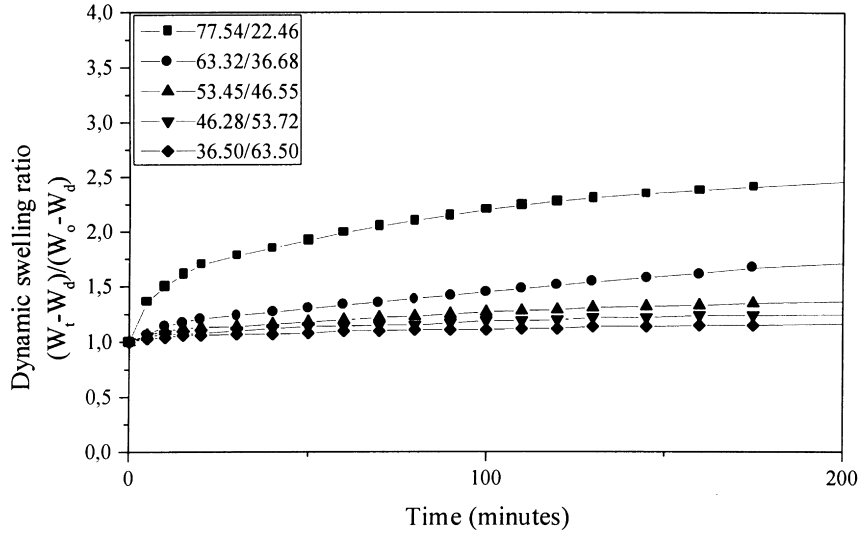

Fig. 9. Swelling kinetics of NIPAM/NHMAAm random copolymers produced with different NIPAM/NHMAAm mole ratios in the presence of PEG 4000 as a diluent. Magnitude of step input for medium temperature, 66°C (from 70 to 4°C).

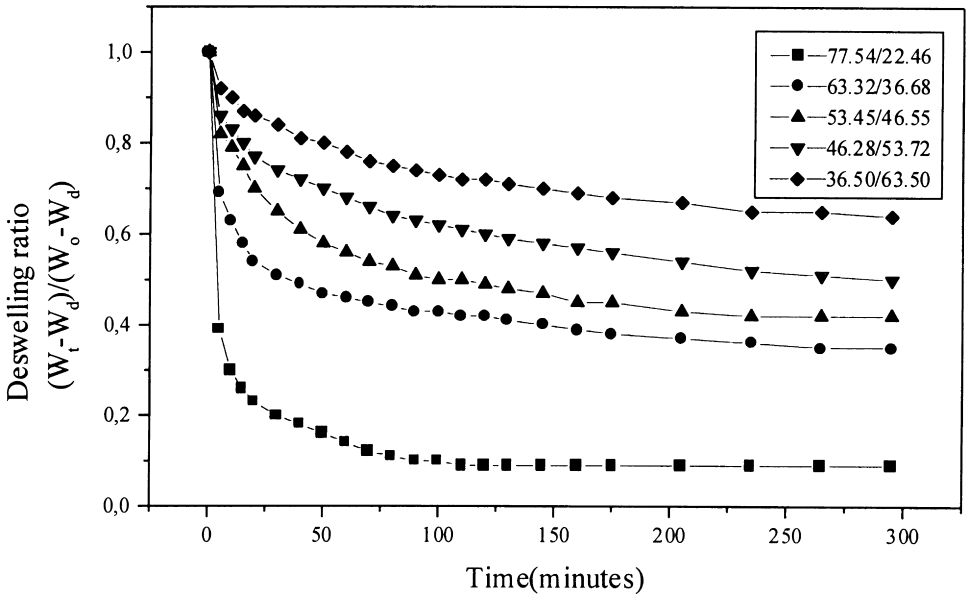

Fig. 10. Deswelling kinetics of the NIPAM–NHMAAm copolymers produced with different NIPAM/NHMAAm mole ratios in the presence of PEG 4000 as a diluent. Magnitude of step input for medium temperature, 66°C (from 4 to 70°C).

synthesized in aqueous solution and characterized in terms of swelling–deswelling degree and swelling–deswelling kinetics. The swelling, deswelling behaviors and temperature dependencies of the gels dependent on the NIPAM/NHMAAm mole ratio, total monomer, MBAAm concentration and the use of PEG 4000 as a diluent caused an increase in the equilibrium-swelling ratio.

As a result, these gels can be used in applications such as immobilization of enzymes and size-selective separation processes.

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