

Swelling and Network Parameters of pH-Sensitive Poly(acrylamide-co-acrylic acid) Hydrogels

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ABSTRACT: Poly(acrylamide-co-acrylic acid) [P(AAm-co-AAc)] hydrogels were synthesized by the free-radical crosslinking copolymerization of acrylamide (AAM) monomer at fixed amount, but at changing amount of acrylic acid (AAc) as the ionic comonomer in water. The swelling behavior of the hydrogels thus obtained was analyzed in buffer solutions at various pHs. The polymer-solvent interaction parameter (χ) and the average molecular mass between crosslinks (\bar{M}_c) of the hydrogels were calculated from swelling studies in buffer solutions at various pHs and were related to the AAc content. The results indicated that the swelling behavior of the hydrogels at different pHs agreed with the modified Flory-Rehner equation based on the affine network model and the ideal Donnan theory. In addition, from differential curves of dO_v/dpH versus pH, the volume

phase transition pH (VPT-pH) was found to be 4.33 ± 0.05 , independent of the AAc content in the hydrogel. Scanning electron microscope (SEM) was used to identify the morphological changes in the hydrogel as the concentration of AAc increased. In water at 22°C, an increase of the AAc content further increased the response rate of the hydrogels because of simultaneous increase of both the porosity and hydrophilicity of the network. In buffer solution with a pH increase from 9.0 to 2.0 at 22°C, the hydrogels with more AAc also exhibited a more rapid deswelling rate than that of the hydrogels with less AAc. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 106: 2000–2007, 2007

Key words: poly(acrylamide-co-acrylic acid); pH-sensitive hydrogels; network structure

INTRODUCTION

Hydrogels of poly(acrylamide) (PAAm) have a broad applications, such as electrophoretic media for the separation or purification of biomolecules, model drug delivery systems due to having random morphologies with a wide distribution of pore size,^{1,2} matrix for enzymes and living cells immobilization owing to its controllable pore size and no need for enzyme reactive groups for the attachment of an insoluble support, high residual activity, etc.³ Therefore, investigation of the swelling behavior of acrylamide (AAM)-based hydrogels have received considerable attention in the last four decades. These hydrogels are mostly prepared by the free-radical crosslinking copolymerization of AAM and *N,N'*-methylenebis(acrylamide) (MBAAM). To increase their swelling capacity, an ionic comonomer is also included into the reaction mixture. The hydrogels carrying ionic moieties respond to pH changes because of the presence of ionizable groups like carboxyl groups or amino groups. One of the frequently studied pH-sensitive hydrogels is poly(acrylic acid) (PAAc),

which extends and shrinks at pH value above and below the pK_a of PAAc (about pH 4.75), respectively.^{4–6} On the basis of their dramatic swelling and deswelling behaviors, pH-sensitive hydrogels are being utilized for new potential applications in numerous fields including chemical transducer,⁷ chemical separation,^{8,9} drug delivery,^{10,11} and artificial organ.¹² pH-sensitive hydrogels can be prepared by combining PAAm with a pH-sensitive polymeric component, such as acrylic acid (AAc),^{13–17} and copolymerization is a common method used to make such compounds.

In contrast to the extensive amount of literatures on the synthesis and swelling properties of AAM-based hydrogels, the systematic investigations of the effect of the AAc concentration on the network structure and responsive dynamics of the poly(acrylamide-co-acrylic acid) [P(AAm-co-AAc)] hydrogels, have not been reported before in the literature.

In this study, we focus on how the initial ionic comonomer concentration affects the responsive dynamics, morphology, and network parameters such as the average molecular mass between crosslinks (\bar{M}_c) and the polymer-solvent interaction parameter (χ) of the AAM-based hydrogels by influencing the chain charge density of the first formed polymers in the reaction mixture. For this purpose, we synthesized the poly(acrylamide-co-acrylic acid) [P(AAm-co-AAc)] hydrogels with four different AAc concentration and

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measured their swelling ratios (SRs) in buffer solutions at various pHs. The measured swelling data of the hydrogels were compared with those predicted by the modified Flory–Rehner approach.^{18,19} The simple equation correctly predicted the swelling behavior of the hydrogels.

EXPERIMENTAL

Materials

AAm (Aldrich), AAc (Aldrich), MBAAm, (Aldrich), ammonium persulfate (APS, Aldrich), *N,N,N',N'*-tetramethylethylenediamine (TEMED, Aldrich) were used as received. Glacial acetic acid, phosphoric acid (85%), boric acid, and standardized sodium hydroxide were used to prepare Britton–Robinson (B-R) buffers. A B-R buffer solution was prepared in such a way that 2.5 mL of glacial acetic acid, 2.7 mL of phosphoric acid, and 2.47 g of boric acid were dissolved by dilution deionized water to 1.0 L; 50-mL portions of this solution were taken, and the pH was adjusted between 2.0 and 9.0 by the addition of an appropriate amount of 2.0M sodium hydroxide solution.

Synthesis of hydrogels

Hydrogels were prepared by free-radical crosslinking copolymerization of AAm and AAc with a small amount of MBAAm as the crosslinker. APS (0.06M) and TEMED (0.064M) were used as the redox initiator system. The hydrogels were prepared as two set; in first set, the initial monomer concentration was fixed at 3.02M, while the amount of the crosslinker MBAAm was varied between at 0.6 and 2.9 mol %; in second set, the crosslinker amount was fixed at 1.3 mol %, while the amount of AAc was changed from 7.3 to 29.1 mol %. The synthesis parameters used in the preparation of the hydrogels are represented in Table I.

To illustrate the synthesis procedure, we give details for the preparation of the hydrogels with various AAc content.

The solutions containing AAm (1.0 g), MBAAm (0.035 g), APS (1.0 mL), TEMED (0.25 mL), and AAc (0.05, 0.10, 0.15, and 0.20 mL) were prepared in 4.0 mL of deionized water. After nitrogen bubbling for 15 min, these solutions were placed in poly(vinylchloride) straws 4 mm in diameters and about 20 cm long. The poly(vinylchloride) straws were sealed and immersed in a thermostated water bath at 22°C, and the polymerization was conducted for 24 h. After the reaction, the hydrogels were cut into specimens, approximately 10 mm long, dried in a vacuum oven at 50°C to constant weight, and subjected to Soxhlet extraction with water to remove the uncross-linked polymer and unreacted AAc from the copolymers.

TABLE I
Characteristic Parameters Used in Preparation of P(AAm-co-AAc) Hydrogels

f_i	$C_0 \times 10^3$ (mol mL ⁻¹)	\bar{V}_r (mL mol ⁻¹)
0.082	2.8	52.71
0.151	2.9	52.76
0.197	3.0	52.76
0.237	3.1	52.82

The extracted gels were dried again in vacuum oven at 50°C to a constant weight. When the aqueous extract was titrated with NaOH (0.05M), end-point detection was not observed because there was no unreacted AAc. The volume fraction of polymer network after preparation (v_{2r}) was calculated as follows:

$$v_{2r} = \left[1 + \frac{\left(\frac{m_r}{m_d} - 1\right)\rho_2}{\rho_1} \right]^{-1} \quad (1)$$

where m_r and m_d are the masses of the hydrogel after the preparation and after drying and ρ_2 and ρ_1 are densities of the polymer network and solvent, respectively. The ρ_2 and ρ_1 values were 1.35 and 1.0 g/mL, respectively. The density of the hydrogel was determined by a pycnometer with acetone as a nonsolvent. The density value of the hydrogels did not change so much with the AAc content. On the other hand, v_{2r} could also be calculated from the initial monomer concentration (C_0 mol mL⁻¹) as follows:²⁰

$$v_{2r} = C_0 \bar{V}_r \quad (2)$$

where \bar{V}_r is the average molar volume of the polymer repeat units. \bar{V}_r can be calculated as follows:

$$\bar{V}_r = \frac{M_{AAm}f_{AAm} + M_{AAc}f_{AAc}}{\rho_2} \quad (3)$$

where M_{AAm} is the molecular mass of AAm, f_{AAm} is the mole fraction of AAm, M_{AAc} is the molecular mass of AAc and f_{AAc} is the mole fraction of AAc in the gel system.

The theoretical values of v_{2r} calculated with eq. (2) are shown in Figure 1 as open symbols together with the experimentally determined v_{2r} values (filled symbols). The experimental v_{2r} values are larger than the theoretical ones because of the presence of bound water in the hydrogel. Hence, the theoretical v_{2r} values were used for further calculations.

Scanning electron microscopy observation

The hydrogels were first equilibrated in deionized water at 22°C, then quickly frozen in liquid nitrogen, and further freeze-dried in a Virtis freeze drier (Lob-conco) *in vacuo* at -50°C for at least 24 h until all the

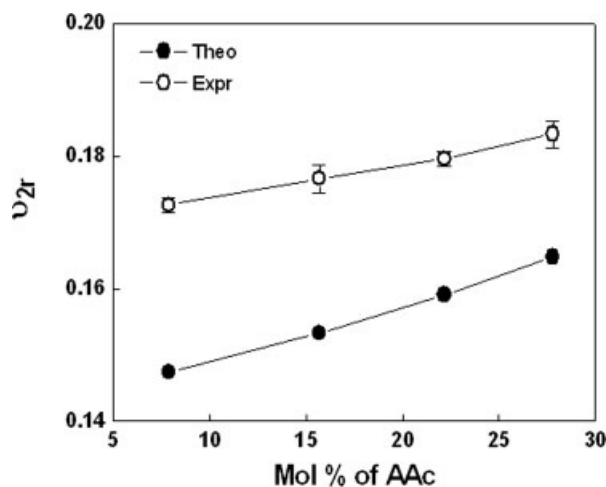


Figure 1 Variation of v_{2r} with the mole percentage of AAc. v_{2r} values experimentally and theoretically determined are indicated in the figure.

solvent was sublimed. Then, the freeze-dried hydrogels were fractured, and their cross section or interior morphologies were determined with SEM (JEOL JSM-6360 LV SEM instrument) after being fixed on aluminum stubs and coated with gold for 40 s.

Measurement of the equilibrium swelling ratio

To measure the equilibrium SR, preweighed dry samples were immersed in the pH buffer solutions. After the excessive water was wiped off the sample surface with moistened filter paper, the mass of the swollen samples was measured in the pH range of 2–9. The equilibrium SR of hydrogels (Q_v) was determined as follows:

$$Q_v = 1 + \frac{\left(\frac{m_s}{m_d} - 1\right)\rho_2}{\rho_1} \quad (4)$$

where m_s is the mass of the hydrogel after equilibrium swelling in the pH solution. The volume fraction of polymer network (v_{2m}) was calculated as follows:

$$v_{2m} = 1/Q_v \quad (5)$$

Measurement of the swelling and deswelling kinetics

For the swelling kinetics measurements, the hydrogels were immersed in deionized water. At a prescribed time interval, the hydrogels were taken out from the water and weighed after wiped off the surface of hydrogel. The SR of the hydrogels was defined as follows:

$$SR = \frac{m_t - m_d}{m_d} \quad (6)$$

where m_t is the mass of the wet hydrogel at each time.

The kinetics of deswelling of the hydrogels was also followed gravimetrically, in pH 2.0 buffer solution. The swollen hydrogels equilibrated first in pH 9.0 buffer solution were transferred into pH 2.0 buffer solution, then after a certain time, the hydrogels were surface dried with filter paper. The weight changes of the hydrogels were recorded during the course of deswelling at these time intervals. The normalized deswelling ratio (NDR) of the hydrogels was calculated as follows:

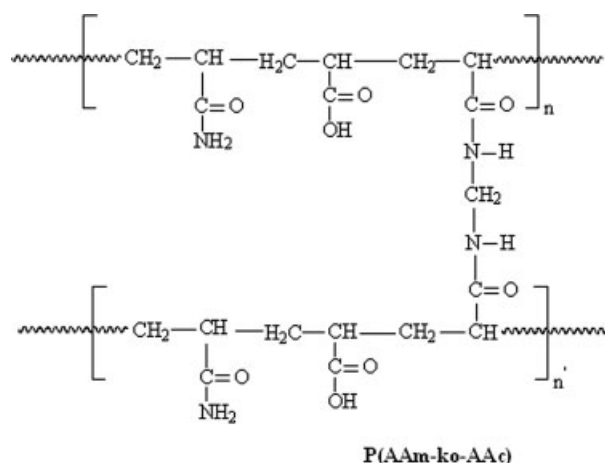
$$NDR = \frac{m_t - m_d}{m_e - m_d} \quad (7)$$

where m_e is the mass of the swollen hydrogel at the equilibrium. All the reported swelling and deswelling values are an average of at least three separate measurements.

RESULTS AND DISCUSSION

Preparation of P(AAm-co-AAc) hydrogels

P(AAm-AAc) hydrogels were prepared by solution polymerization in aqueous medium with the existence of AAm, AAc as monomers, MBAAm as crosslinker, and redox initiators, as shown in Scheme 1. In our work, we employed deionized water as the polymerization solvent, and the pH value of polymerization system measured by pH meter was in the range from 6.8 to 7.0. Because the pH value is remarkably higher than pK_a of AAc (nominal $pK_a = 4.75$). AAc was a negatively charged in the polymerization system. The strong electrostatic repulsions among AAc carboxylate anions ($-\text{COO}^-$) could have resulted in expanded network of the hydrogel, which might have had an extremely high water uptake. Because the hydrogel network is reported to retain memory of its



Scheme 1 Chemical structure of P(AAm-co-AAc) hydrogels.

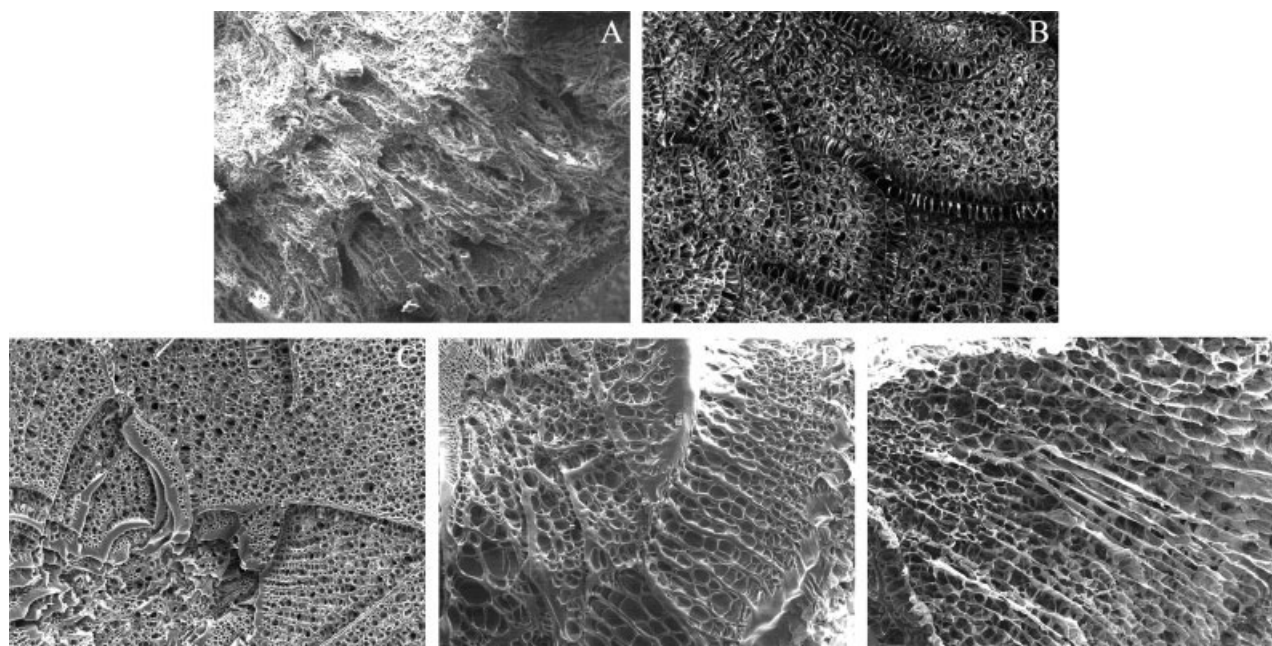


Figure 2 The cross-sectional SEM micrographs of PAAm (A), mol % 7.3 AAc (B), mol % 14.9 AAc (C), mol % 21.9 AAc (D), mol % 29.1 AAc (E) (magnification $\times 100$).

formation history and molecular conformation,^{21–23} an expanded network structure with a special conformation would remain even after the hydrogel had been transferred into acidic medium after the synthesis and after $-\text{COO}^-$ had changed to $-\text{COOH}$. The expanded structure was confirmed by the porous structure observed by SEM after the swollen hydrogel samples were freeze-dried and fractured, as illustrated below.

Interior morphology of the hydrogels

Figure 2 shows the SEM micrographs of the internal structure of the freeze-dried hydrogels, from which we can see that the PAAm hydrogel has a relatively dense structure, whereas the P(AAm-co-AAc) hydrogels show a porous network structure in character. Their pore sizes increase with increasing AAc content in the hydrogel. These results support our above analysis that a highly expanded network can be generated by electrostatic repulsions among carboxylate anions ($-\text{COO}^-$) of AAc during the polymerization process. With increasing AAc content, expansion of the hydrogel matrices enhanced, resulting in the increase in the pore size. During the shrinking or deswelling processes, the water molecules are easy to diffuse out as a result of numerous small pores in the hydrogel. Hence, the response rate could greatly be enhanced by the incorporation AAc into the PAAm network during the deswelling process.

pH-dependent swelling behavior

It is well known that while PAAm does not respond to changes in pH buffer solutions, PAAc is a typical pH-sensitive polymeric material that can deprotonate its carboxyl moieties in alkaline solution and protonate them in acidic solution. The dissociation degree of carboxyl groups is closely related to the pH value of the medium. To investigate the influence of pH value of the medium on the equilibrium SRs for the P(AAm-co-AAc) hydrogels, thus, the pH range is selected from 2.0 to 9.0 in this study. As shown in Figure 3(A), SRs of the hydrogels with various AAc contents are low in the pH value range from 2.0 to 3.0 at 22°C. It is due to the formation of hydrogen bond between $-\text{COOH}$ of the AAc and $-\text{CONH}_2$ of the AAm units in the hydrogel and, thus, leading to polymer-polymer interactions predominating over the polymer-water interactions, as a result, the SRs of the hydrogels decreases. In the pH range from 3.0 to 6.0, the SRs of the hydrogels drastically increase with increasing pH values. This mainly attributed to the carboxyl group of AAc in the hydrogel, in which the $\text{p}K_a$ of AAc is about 4.75. On the other hand, by presenting the equilibrium swelling data in a differential form, i.e., $dQ_v/d\text{pH}$ versus pH, in Figure 3(B), the volume phase transition pH (VPT-pH) can be located more easily. The maximum for each hydrogel occurs at $\text{pH} = 4.33 \pm 0.05$. This value is also very close to the $\text{p}K_a$ value of AAc units in the hydrogel. Above the VPT-pH value ($\text{pH} > 5$), an electrostatic repulsive force operating between the charged carboxyl groups

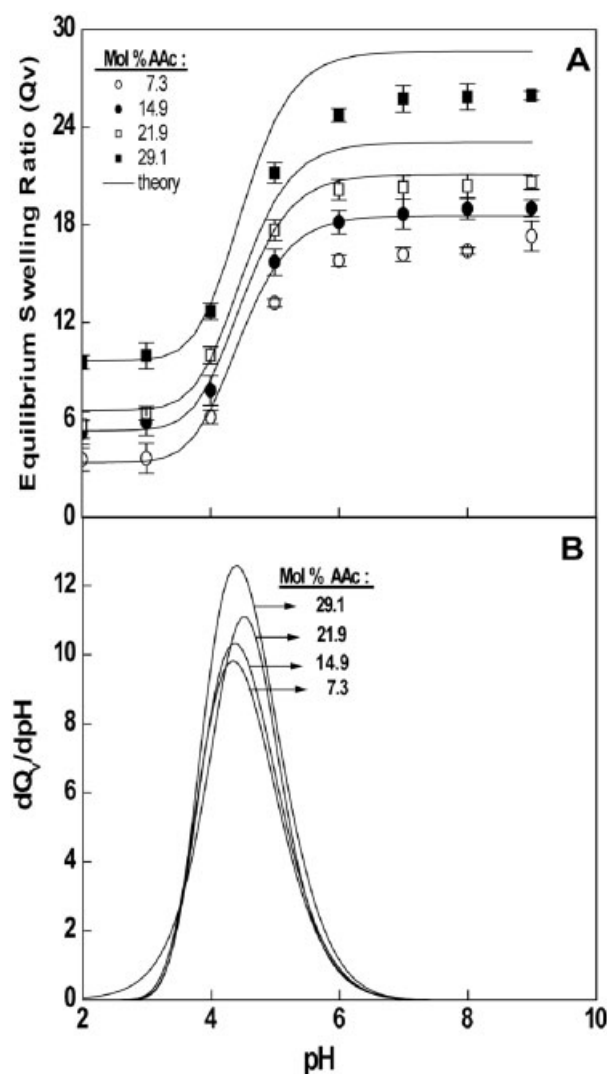


Figure 3 (A) Variation of equilibrium SR of P(AAm-co-AAc) hydrogels with different AAc content as function of pH. Experimental data are shown as symbols. The solid curves were calculated using eq. (8). (B) Data for hydrogels in (A) plotted in the differential form of dQ_v/dpH versus pH.

of AAc increased the hydration of the hydrogels, causing swelling. On the contrary, the hydration of the hydrogels decreased below the VPT-pH value because the electrostatic repulsive force was vanished between the uncharged carboxyl groups. In addition, the pH sensitivity was also proportional to the AAc content of the hydrogels. An increase of AAc concentration in the hydrogel produces an equilibrium SR, which increase dramatically at all pH values, especially for hydrogels with 21.9 and 29.1 mol % AAc, for which the number of ionic units is relatively high. Also notable is the fact that the addition of only a minute amount of AAc (14.9 mol %) radically changes the swelling behavior of these hydrogels. This is due to the fact that as the AAc content in the hydrogel

increases, the charges on the polymeric chains also increase. As a result, this leads to the expansion of the hydrogels because of the high electrostatic repulsion, so the swelling capacities of the hydrogels increase.

On the other hand, the effect of the amount of the crosslinker on the SRs was also investigated and the results were given in Figure 4(A). The data in Figure 4(A) clearly show that the SRs of these hydrogels decreased 48% in pH 2 and 31% in pH 9 buffer solutions with increasing amount of MBAAm from 0.6 to 2.9%. It is believed that an increase in the level of crosslinking agent would reduce the free volume within hydrogel network structure in which water would reside during swelling and would also lead to the reduction of pore size of the corresponding

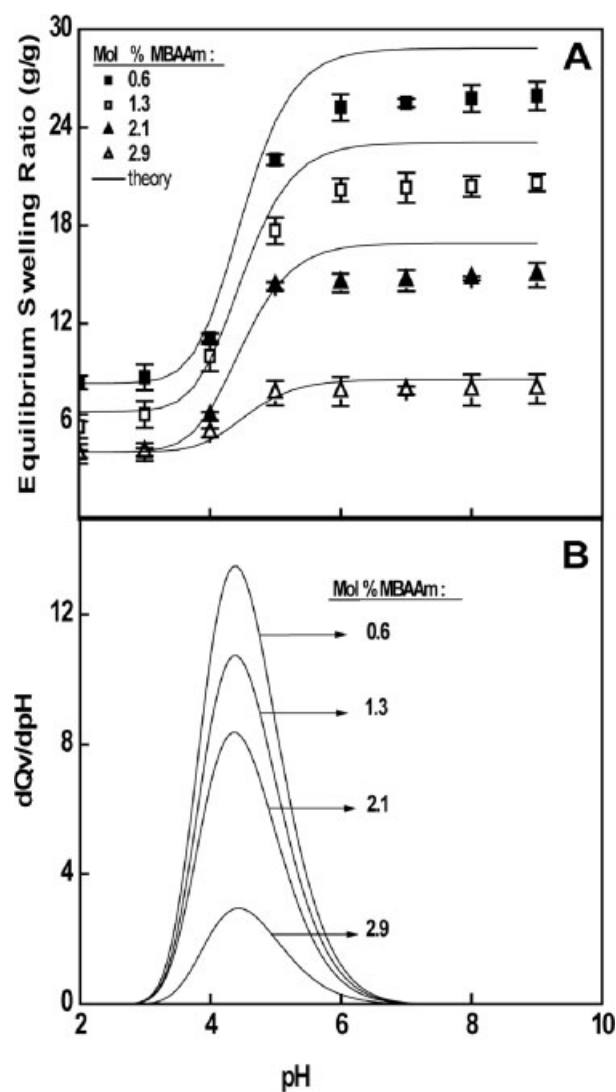


Figure 4 (A) Variation of equilibrium SR of P(AAm-co-AAc) hydrogels with different MBAAm content as function of pH. Experimental data are shown as symbols. The solid curves were calculated using eq. (8). (B) Data for hydrogels in (A) plotted in the differential form of dQ_v/dpH versus pH.

TABLE II
Network Parameters of the P(AAm-co-AAc) Hydrogels

MBAAm (mol %)	AAc (mol %)	χ	\overline{M}_{cE} (g/mol)	\overline{M}_{cT} (g/mol)
1.3	7.3	0.497 ± 0.001	8700	2400
1.3	14.9	0.480 ± 0.003	2900	2500
1.3	21.9	0.449 ± 0.006	1600	2700
1.3	29.1	0.386 ± 0.013	1400	2800
0.6	21.9	0.448 ± 0.004	2400	5900
1.3	21.9	0.449 ± 0.006	1600	2700
2.1	21.9	0.462 ± 0.002	1000	1700
2.9	21.9	0.348 ± 0.003	300	1200

hydrogels, which in turn reduced the water holding capacity because of the decreased pore volume. The VPT-pH value of the hydrogels was also found as 4.38 ± 0.06 [Fig. 4(B)]. In this case, it is said that an increase in the level of crosslinking agent had no effect on the VPT-pH value of the hydrogels.

Network parameters

The average molecular mass between crosslinks (\overline{M}_c) and the polymer-solvent interaction parameter (χ) were determined from the swelling data of the hydrogels in the pH buffer solutions using the following the modified Flory–Rehner equation based on the affine network model and the ideal Donnan theory:^{18,19}

$$\left[\frac{K_a}{10^{-\text{pH}} + K_a} \right]^2 \frac{V_1 f_i^2 v_{2m}^2}{4I\overline{V}_r^2} = \ln(1 - v_{2m}) + v_{2m} + \chi v_{2m}^2 + F \frac{V_1 \rho_2 v_{2r}^{2/3} v_{2m}^{1/3}}{\overline{M}_c} \quad (8)$$

In this equation, the relevant experimental parameters for the hydrogels are as follows: the dissociation constant of AAc, ($K_a = 5.6 \times 10^{-5}$), molar volume of the solvent ($V_1 = 18 \text{ mL mol}^{-1}$), the ionic strength of the swelling medium ($I = 1.0 \times 10^{-4} \text{ mol mL}^{-1}$), and the front factor for the affine network ($F = 1$). Details about the eq. (8) have been reported elsewhere.^{18,19}

The change in the equilibrium SR of the hydrogels in pH buffer solutions was used to evaluate \overline{M}_c and χ . For this purpose, by expansion of the logarithmic series, eq. (8) was rearranged for the determination of \overline{M}_c and χ , eq. (9) was obtained:

$$\left[\frac{K_a}{10^{-\text{pH}} + K_a} \right]^2 \frac{V_1 f_i^2}{4I\overline{V}_r^2} + 0.5 = \chi + F \frac{V_1 \rho_2 v_{2r}^{2/3} v_{2m}^{-5/3}}{\overline{M}_c} \quad (9)$$

With the experimentally measured polymer volume fraction (v_{2m}) of the gels in their equilibrium-swollen state and the aforementioned data, a plot of the left-hand side of the eq. (9) versus $FV_1 \rho_2 v_{2r}^{2/3} v_{2m}^{-5/3}$ gives a straight line, with $1/\overline{M}_c$ and χ as the slope

and intercept of the horizontal axis, respectively. The \overline{M}_c and χ values determined via linear regression analysis are listed in Table II.

The \overline{M}_c values of the hydrogels decreased from 8700 to 1400 g/mol with the increasing of AAc content. This behavior can be interpreted as AAc being effective in the crosslinking process. In addition, the reduction of the \overline{M}_c values with increasing AAc content can be connected to the condensation of counterions to ion pairs.^{20,24} Ion pairs attract each other because of dipole–dipole interactions and thus act as physical crosslinks in the hydrogel. However, the χ parameter of the hydrogels is slightly decreased by the incorporation of AAc into the polymer chain. It is known that this parameter depends on the temperature and, for many systems, also on the composition. The use of eq. (9) for calculating the parameter neglects both the composition and temperature dependence. In this case, one possible explanation for the variation of χ with the AAc content is related to the change in the chemical structure of the network chains, which can alter the value of the χ parameter.

On the other hand, assuming that all crosslinker molecules used in the hydrogel synthesis participate in forming effective crosslinks, theoretical \overline{M}_c values of the hydrogels were calculated by the following equation:²⁰

$$\overline{M}_{cT} = \frac{\rho_2 \overline{V}_r}{2X} \quad (10)$$

where X is the crosslinker ratio (mole ratio of MBAAm to AAm+AAc). The relevant experimental and theoretical parameters of the hydrogels were collected in Table II. As can be seen from Table II, the experimental \overline{M}_c values of the hydrogels decreased from 5900 to 1200 g/mol with increasing amount of MBAAm, ranging from 0.6 to 2.9%. The experimentally found \overline{M}_c value of the hydrogel with 0.6% MBAAm is 2.5 times higher than its theoretically calculated counterpart. This value changed 1.7 and 4.0 when 2.1% and 2.9% MBAAm was used in the hydrogel preparation, respectively. The difference between theoretical and experimental \overline{M}_c values of the hydrogels indicates that a significant fraction of the cross-

linker MBAAM is wasted during the crosslinking copolymerization, probably because of the cyclization and multiple crosslinking reactions.²⁰ The high degree of dilution during the hydrogel preparation as well as the higher crosslinker reactivity is mainly responsible for these reactions. In other words, at high AAc concentrations, theoretically calculated \bar{M}_c values of the hydrogels are higher than their experimentally found counterparts. In this case, one possible explanation for the difference between the experimental and theoretical \bar{M}_c values of the hydrogels is related to the anhydride formation in the AAc units of the hydrogel via decarboxylation occurring simultaneously with water elimination during the crosslinking process, which may decrease the value of the \bar{M}_c .

On the other hand, to control the modified Flory–Rehner equation and validity of \bar{M}_c and χ of the hydrogels, the theoretical SRs depending on pH were calculated by using eq. (8). The solid curves in Figures 3(A) and 4(A) represent the theoretical SR of the hydrogels as a function of pH. The prediction of the modified Flory–Rehner equation based on the affine network model and the ideal Donnan theory agrees reasonably well with the experimental swelling data of the hydrogels in buffer solutions at various pHs. We can thus conclude that the modified Flory–Rehner equation based on the affine network model and the ideal Donnan theory can be used to predict the swelling behavior of an ionic hydrogels in buffer solutions at various pHs.

Swelling and deswelling kinetics of hydrogels

The swelling kinetic curves of the hydrogels in deionized water are shown in Figure 5. As usual, the SRs of these hydrogels increase at short times and level off to maxima at longer time. The data shows that the

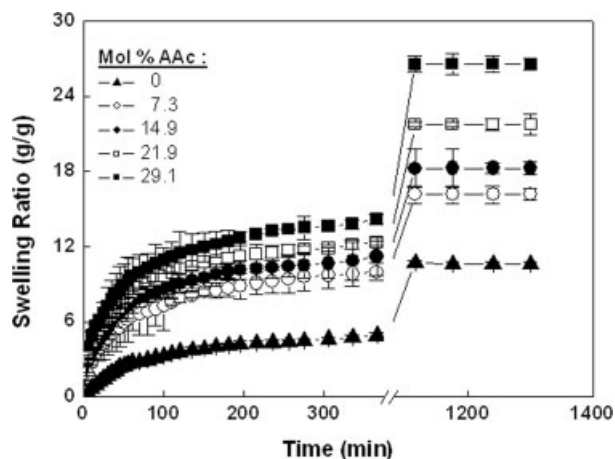


Figure 5 Swelling kinetics of P(AAm-co-AAc) hydrogels with different AAc content in deionized water at 22°C. Experimental data are shown as symbols.

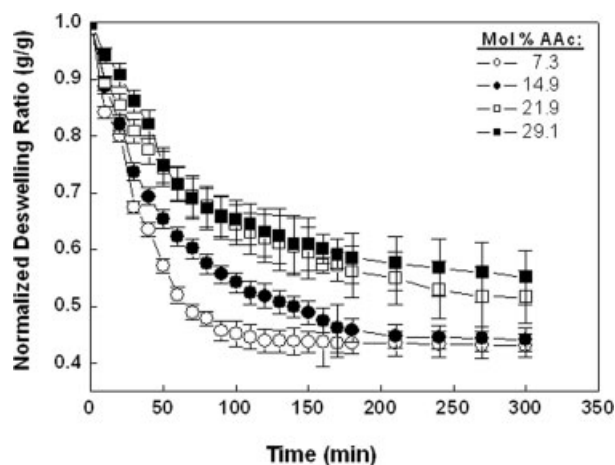


Figure 6 Deswelling kinetics of P(AAm-co-AAc) hydrogels with different AAc content in pH 2 buffer solution at 22°C. Experimental data are shown as symbols.

swelling rate increased with increasing AAc content in the hydrogel. In addition, the SRs of the P(AAm-co-AAc) hydrogels are also higher than those of the pure PAAm hydrogels in deionized water.

The deswelling kinetics of the P(AAm-co-AAc) hydrogels from the swollen state in the buffer solution of pH 9.0 to the shrunken state in the buffer solution of pH 2.0 were also measured and are shown in Figure 6. The data illustrate that the hydrogel with 29.1 mol % AAc has the lowest SR, whereas the hydrogel with 7.3 mol % AAc has the highest SR. As a result, the hydrogels with more AAc content showed rapid swelling in deionized water and rapid shrinking in the buffer solution of pH 2.0. In deionized water, the AAc unit of the hydrogel was a negatively charged polyelectrolyte because of the pH value is remarkably higher than pK_a of AAc and the electrostatic repulsion between $-\text{COO}^-$ groups causes the hydrogel to swell. In the buffer solution of pH 2.0, the hydrogels shrank and aggregated with each other because of the disappearance of electrostatic repulsion and the generation of hydrophobic interactions owing to hydrogen bond formation between $-\text{COOH}$ and $-\text{CONH}_2$ groups.

References

- Ding-Gan, L. *Anal Biochem* 2005, 339, 351.
- Christopher, L. L.; Shannon, M.; Smith, C.; Allan, G. *Macromolecules* 2001, 34, 8587.
- Yankov, D. *Enzyme Microbial Technol* 2004, 34, 603.
- Kawasaki, H.; Sasaki, S.; Maeda, H. *J Phys Chem B* 1997, 101, 5089.
- Lui, Y.; Velada, J. L.; Huglin, M. B. *Polymer* 1999, 40, 4299.
- Wang, Y.; Lui, Z. M.; Han, B. X.; Dong, Z. X.; Wang, J. Q.; Sun, D. H.; Huang, Y.; Chen, G. W. *Polymer* 2004, 45, 855.
- Kown, L. C.; Bae, Y. H.; Kim, S. W. *Nature* 1991, 354, 291.
- Kawaguchi, H.; Fujimoto, K. *Bioseparation* 1998, 7, 253.
- Kasgoz, H.; Orbay, M. *Polymer* 2003, 44, 1785.

10. Sauzedde, F.; Pichot, C. *Colloid Polym Sci* 1999, 277, 846.
11. Hoffman, A. S. *Adv Drug Deliv Rev* 2002, 54, 3.
12. Shiino, D.; Murata, Y.; Kataoka, K.; Koyama, Y.; Yokoyama, M.; Okano, T.; Sakurai, Y. *Biomaterials* 1994, 15, 121.
13. Park, T. G.; Hoffman, A. S. *J Appl Polym Sci* 1992, 46, 659.
14. Serizawa, T.; Wakita, K.; Akashi, M. *Macromolecules* 2002, 35, 10.
15. Ju, H. K.; Kim, S. T.; Kim, S. T.; Lee, Y. M. *J Appl Polym Sci* 2002, 83, 1128.
16. Chui, H. C.; Yang, C. H. *Polymer J* 2000, 32, 574.
17. Shibayama, M.; Fujikawa, Y.; Nomura, S. *Macromolecules* 1996, 29, 6535.
18. Caykara, T.; Akcakaya, I. *Eur Polym J* 2006, 42, 1437.
19. Özyurek, C.; Çaykara, T.; Kantoğlu, Ö. *J Polym Sci Part B: Polym Phys* 2000, 38, 3309.
20. Okay, O.; Durmaz, S. *Polymer* 2002, 43, 1215.
21. Zhang, X. Z.; Yang, Y.; Wang, F. J.; Chung, T. S. *Langmuir* 2002, 2013, 18.
22. Nakamoto, C.; Motonaga, T.; Shibayama, M. *Macromolecules* 2001, 34, 911.
23. Alvarez, L. G.; Guney, O.; Oya, T.; Salaj, Y.; Kobayashi, M.; Tanaka, K.; Masamune, G.; Tanaka, T. *Macromolecules* 2000, 33, 8693.
24. Ilavsky, M.; Sedlakova, Z.; Bouchal, K.; Plestil, J. *Macromolecules* 1995, 28, 6835.