Preparation of Alginate/Poly(*N*-isopropylacrylamide) Semi-Interpenetrating and Fully Interpenetrating Polymer Network Hydrogels with γ-Ray Irradiation and Their Swelling Behaviors

Sang Bong Lee,[1]* Eun Kyoung Park,[1] Youn Mook Lim,[1] Seong Kwan Cho,[1] So Yeon Kim,[1] Young Moo Lee,[1] Young Chang Nho[2]

[1]*School of Chemical Engineering, College of Engineering, Hanyang University, Seoul 133-791, South Korea*
[2]*Radiation Application Research Division, Korea Atomic Energy Research Institute, Daejeon 305-353, South Korea*

ABSTRACT: Semi-interpenetrating polymer network (semi-IPN) and fully interpenetrating polymer network (full-IPN) hydrogels composed of alginate and poly(*N*-isopropylacrylamide) were prepared with γ-ray irradiation. The semi-IPN hydrogels were prepared through the irradiation of a mixed solution composed of alginate and *N*-isopropylacrylamide (NIPAAm) monomer to simultaneously achieve the polymerization and self-crosslinking of NIPAAm. The full-IPN hydrogels were formed through the immersion of the semi-IPN film in a calcium-ion solution. The results for the swelling and deswelling behaviors showed that the swelling ratio of semi-IPN hydrogels was higher than that of full-IPN hydrogels. A semi-IPN hydrogel containing more alginate exhibited relatively rapid swelling and deswelling rates, whereas a full-IPN hydrogel showed an adverse tendency. All the hydrogels with NIPAAm exhibited a change in the swelling ratio around 30–40°C, and full-IPN hydrogels showed more sensitive and reversible behavior than semi-IPN hydrogels under a stepwise stimulus. In addition, the swelling ratio of the hydrogels continuously increased with the pH values, and the swelling processes were proven to be repeatable with pH changes. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 4439–4446, 2006

Key words: interpenetrating networks (IPN); hydrogels; radiation

INTRODUCTION

γ-ray irradiation is a valuable technique for the modification of the chemical and physical properties of polymeric materials. The usage of γ-ray-irradiation methods has advantages, such as relatively simple manipulation and the uselessness of any extra agents for polymerization and crosslinking,[1–3] whereas the method of thermal activation requires radical initiators and crosslinkers such as *N,N*-methylenebis(acrylamide) for the formation of poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels.[3–5] In particular, irradiative polymerization and crosslinking with γ rays concurrently take place during the preparation of hydrogels.[4] In the meantime, the degree of crosslinking, which strongly determines the extent of swelling in hydrogels, can be easily controlled by the variation of the dose rates. For these reasons, the γ-ray-irradiation method is very useful for preparing hydrogels for medical applications, for which even a small contamination is undesirable, and is often used to sterilize biomedical devices for medical and veterinary applications.[6–10]

In this study, alginate and PNIPAAm were used to prepare pH- and temperature-sensitive interpenetrating polymer network (IPN) hydrogels. Alginate is derived from marine algae, and its molecular structure is a linear copolymer of 1,4-linked β-D-mannuronate and α-guluronate residues.[11,12] It has been much used in medical applications, including dental impressions, wound dressings, drug delivery vehicles, and cell transplantation matrices, because of its biocompatibility and gentle gelling behavior in the presence of divalent cations such as calcium ions to form hydrogels.[12–17] In addition, temperature-sensitive PNIPAAm hydrogels have been used as another component consisting of an IPN because PNIPAAm chains are hydrated to form an expanded structure when the temperature of water is kept below its lower critical solu-

TABLE I
Composition and Designation of the Hydrogels

| Type[a] | Sample code | Weight ratio (wt %)[b] | |
		Alginate	NIPAAm
Hydrogel	Alginate[c]	100	0
	PNIPAAm	0	100
Semi-IPN hydrogel	SAN37	30	70
	SAN55	50	50
	SAN73	70	30
Full-IPN hydrogel[c]	FAN37	30	70
	FAN55	50	50
	FAN73	70	30

[a] γ-ray irradiation = 30 kGy.
[b] Concentrations of alginate aqueous solution and NIPAAm monomer aqueous solution = 5 wt %.
[c] Crosslinking of alginate alone and full-IPN hydrogels: 1 h in 1 wt % $CaCl_2$ aqueous solution.

tion temperature (LCST) around 32°C.[11,18–20] PNIPAAm hydrogels are versatilely applied to biomedicine and biotechnology as well as drug delivery systems.[21–23]

Thus, the main purpose of this study was to prepare semi-interpenetrating polymer network (semi-IPN) and fully interpenetrating polymer network (full-IPN) hydrogels with various compositions of alginate and N-isopropylacrylamide (NIPAAm) with γ-ray irradiation and calcium ions and to investigate their swelling behaviors in the equilibrium state and the pulsatile pattern with respect to various external stimuli such as pH and temperature.

EXPERIMENTAL

Materials

NIPAAm (Aldrich Chemical Co., Milwaukee, WI) was purified by recrystallization from n-hexane/toluene (Duksan Pure Chemical, Seoul, Korea). Sodium alginate (mannuronate/gluronate ratio for alginate = 1.56) was purchased from Aldrich Chemical. The molecular weight of the alginate was determined with gel permeation chromatography (GPC; model 510 HPLC pump, Waters, Billerica, MA) in water with the Millennium software program. The number-average and weight-average molecular weights were 339,000 and 1,073,000, respectively. Calcium chloride (Sigma Chemicals, St. Louis, MO) and methanol (Duksan Pure Chemical) were used as received. Water was first treated with a reverse osmosis system (Sambo Glove Co., Ansan, Korea) and was further purified with a Milli-Q Plus system (Waters). All other chemicals were reagent grades and were used as purchased without further purification.

Preparation of the alginate/NIPAAm semi-IPN and full-IPN hydrogels

An aqueous solution of alginate and NIPAAm monomer at a concentration of 5 wt % were mixed in

various ratios (0/100, 30/70, 50/50, 70/30 and 100/0), as listed in Table I. The mixed solutions were then irradiated by γ rays from a Co-60 source (ACEL type C-1882, Korea Atomic Energy Research Institute, Daejeon, Korea) with 30 kGy under a nitrogen atmosphere at room temperature.

The semi-IPN hydrogel was prepared via the drying of the irradiated solution in a vacuum oven at 40°C to a constant weight. The NIPAAm homopolymer that formed during γ-ray irradiation was removed by a Soxhlet extraction apparatus with methanol for 48 h, and then the resulting product was dried *in vacuo* at 40°C. To prepare full-IPN hydrogels, the dried semi-IPN hydrogel (10×10 cm^2) was immersed in 200 mL of a 1 wt % $CaCl_2$ aqueous solution for 1 h at room temperature. The samples were washed with deionized water and were dried in a vacuum oven at 40°C.

Figure 1 schematically describes the procedure for preparing the semi-IPN and full-IPN hydrogels with γ-ray irradiation. The changes in the chemical structure in the IPN hydrogels were investigated with Fourier transform infrared (FTIR) spectroscopy (model Magna IR 550, Nicolet, Madison, WI).

Measurement of the swelling ratio

Swelling studies were conducted on alginate alone and semi- and full-IPN hydrogels composed of alginate and PNIPAAm as functions of the temperature and pH in the swelling medium. To measure the swelling ratio, preweighed dry samples were immersed in deionized water (pH 5.4) or buffer solutions (pH 2–6). When the samples reached the equilibrium state, the weight of the swollen samples was measured after the excessive water on the surface was removed
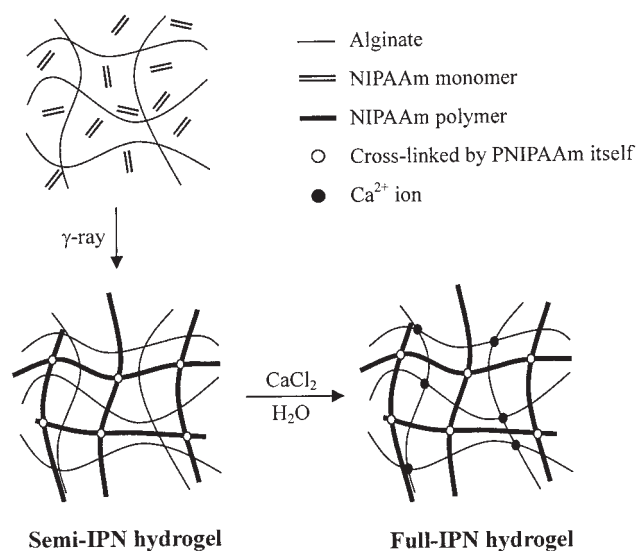
Figure 1 Schematic structures for preparing semi-IPN and full-IPN hydrogels.

with filter paper. The swelling ratio is defined as follows:

$$\text{Swelling ratio} = [(W_s - W_d)/W_d] \quad (1)$$

where W_s and W_d are the weights of the IPN sample at the sufficiently swollen and dried states, respectively.

The deswelling kinetics were measured after the samples swollen at 25°C were immersed in hot water at 40°C. During the deswelling process, the weight change of the hydrogels was recorded at each given time. In addition, the pulsatile swelling behavior was observed in deionized water at alternate temperatures of 25 and 40°C and in buffer solutions with alternate pH values between 2 and 6. The swelling ratio was measured in a pulsatile pattern depending on external stimuli such as the pH and temperature.

RESULTS AND DISCUSSION

Preparation of the semi-IPN and full-IPN hydrogels

Figure 1 shows the schematic structure of the semi-IPN and full-IPN hydrogels with various compositions of alginate and NIPAAm, which were irradiated by γ rays and crosslinked with calcium ions. After the solutions were irradiated by γ rays from Co-60, a water-insoluble fraction was generated by the formation of crosslinked PNIPAAm. This means that the NIPAAm monomers were concurrently polymerized and crosslinked without initiators and crosslinkers during the γ-ray irradiation. The crosslinking mechanism of irradiated PNIPAAm revealed that two possible ways existed during the irradiation: the formation of mid-chain radicals during polymerization by an intramolecular H-shift and the formation of radicals by hydrogen abstraction from the growing polymer.[4] It was reported that PNIPAAm gels obtained over a dose of 20 kGy of γ irradiation were dimensionally stable and easy to handle.[24]

However, it is thought that H · and · OH radicals formed by radiolysis during the irradiation of water accelerate the molecular chain scission of alginate[25] because polysaccharides are typical degradable materials under ionizing irradiation according to the reduction of the molecular weight.[26] A reaction between the aforementioned free radicals and alginate molecules leads to rapid degradation of alginate in an aqueous solution. The decrease in the molecular weight of alginate is influenced by the irradiation dose and the concentration of alginate in the aqueous solution. Nagasawa et al.[25] studied the change in the molecular weight of alginate according to the dose of irradiation and conditions of alginate such as the blowing gas and concentration of alginate.[25] The molecular weight of alginate in an aqueous solution exponentially decreased with the irradiation dose, and this indicated that the chain scission of alginate due to the suppression of the recombination of the main chain was caused by the high mobility of alginate chains in an aqueous solution.

The number of chain breakages per single molecule (G_d) can be calculated from the change in the molecular weight, as previously reported:[27]

$$G_d = (M_n - M_i)/M_i \quad (2)$$

where M_i is the number-average molecular weight of irradiated alginate and M_n is the number-average molecular weight of nonirradiated alginate. If eq. (2) is applied to this study (30 kGy and 5 wt %), the value of G_d is 4.6 because M_i has been measured to be 61,200 g/mol by GPC. However, although the degradation caused by irradiation has a detrimental effect on the mechanical properties of hydrogels, the proper degradation of alginate improves cell viability and facilitates excretion from the body because of the low molecular weight.[27]

In this study, IPN hydrogels were prepared through irradiation with a γ-ray dose of 30 kGy, which was simultaneously considered with the gelation of PNIPAAm and the degradation of alginate. The backbone of PNIPAAm in semi-IPN hydrogels was sufficiently crosslinked by itself to be handled in a swollen state. The full-IPN hydrogels were additionally crosslinked with $CaCl_2$ solutions to overcome the weakened mechanical property caused by a decrease in the molecular weight of alginate. Its gelling properties arose from the cooperative binding of divalent or trivalent cations (usually Ca^{2+}) between the homopolymeric sequences of gluronate residues in alginate.

FTIR spectra

To confirm the changes in the chemical structure of the alginate–PNIPAAm blend, FTIR spectroscopic measurements were carried out. Figure 2 shows the FTIR spectra for alginate, full-IPN hydrogels, semi-IPN hydrogels, PNIPAAm, and NIPAAm monomer.

The FTIR spectrum of alginate [Fig. 2(a)] indicated that peaks appearing at 3450 and 1615 cm^{-1} could be assigned to a hydroxyl group and carbonyl stretching vibration, respectively. Figure 2(h), obtained from the NIPAAm homopolymer, shows significant peaks at 1650, 1550, and 1387 and 1367 cm^{-1} that can be attributed to the characteristic peaks of the amide I, amide II, and methyl groups in —CH(CH$_3$)$_2$, respectively. Also, the characteristic peaks at 1620 (C=C) and 1413 cm^{-1} (CH$_2$=) and C—H vinyl out-of-plane bending vibrations, observed in the spectrum of the monomer [Fig. 2(i)], disappeared.
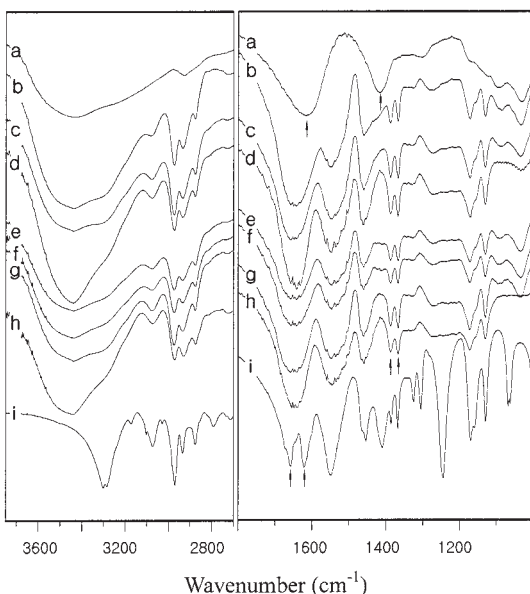
Figure 2 FTIR spectra for (a) alginate, (b) FAN37, (c) FAN55, (d) FAN73, (e) SAN37, (f) SAN55, (g) SAN73, (h) PNIPAAm, and (i) NIPAAm monomer.

In the IPN hydrogels, an increase in the characteristic peaks of amide I and II compared with alginate itself was observed, and the peaks at 1387 and 1367 cm^{-1} significantly increased because of the methyl group in —$CH(CH_3)_2$. This result clearly indicated that PNIPAAm chains were woven between the alginate chains. Unfortunately, the distinctive peaks caused by calcium ions between semi-IPN and full-IPN hydrogels were not shown in the FTIR spectra.

Swelling–deswelling behavior of the hydrogels

Figure 3(a,b) shows the swelling kinetics of IPN hydrogels composed of alginate and PNIPAAm at 25°C in distilled water (pH 5.4).

The alginate hydrogel reached an equilibrium swelling state within 2 h, whereas the PNIPAAm hydrogel showed slow swelling kinetics. In the semi-IPN hydrogels, the swelling ratio increased with the alginate content in the hydrogels. On the other hand, the swelling ratio of the full-IPN hydrogels decreased with increasing alginate content in the hydrogels. This was due to the additional crosslinking of the carboxylic groups in the alginate backbone by the calcium ions. Thus, hydrogels containing the weight ratio 7:3 (alginate/NIPAAm) showed relatively small swelling ratios in full-IPN hydrogels but high swelling ratios in semi-IPN hydrogels.

In particular, the swelling ratios of SAN73 and SAN55 slowly decreased after their equilibrium state was reached because alginate chains without crosslinking were extracted from the swollen hydrogels. However, the SAN37 hydrogel did not show a decrease in the swelling ratio because PNIPAAm in the hydrogel was abundant and thus prevented the alginate chains from being removed.

Figure 4(a,b) shows the deswelling kinetics of hydrogels pre-equilibrated at 25°C by the elevation of the temperature to 40°C. The PNIPAAm gel was quickly shrunk during the deswelling process, whereas the swelling ratio of alginate did not change according to the increasing temperature. In all the sample compositions, the deswelling rate of semi-IPN hydrogels was slower than that of full-IPN hydrogels. In full-IPN hydrogels, the FAN73 and FAN55 hydrogels showed a relatively fast deswelling rate in comparison with the FAN37 hydrogel. Relatively rapid assembly in the
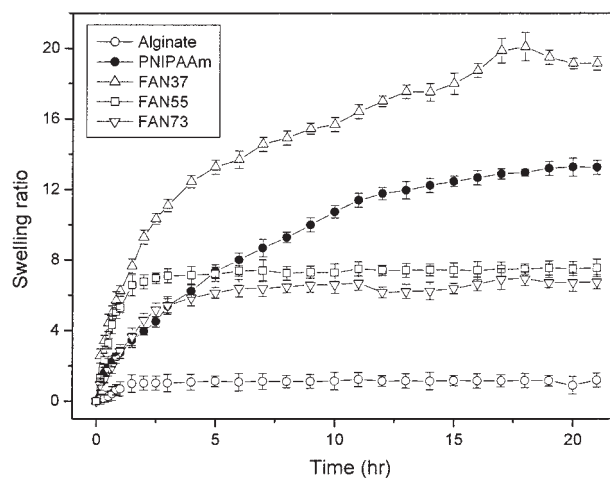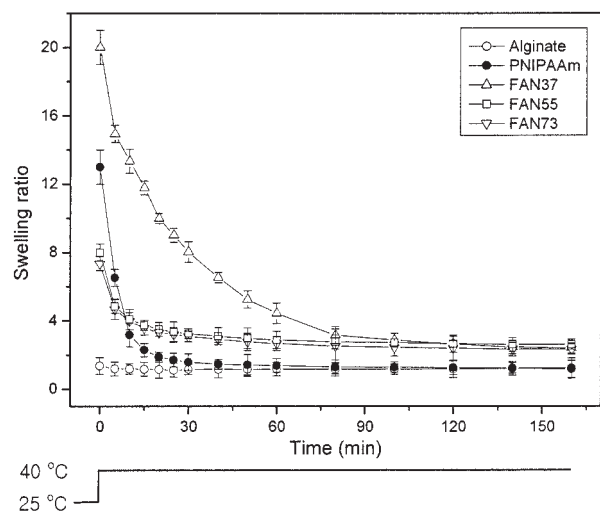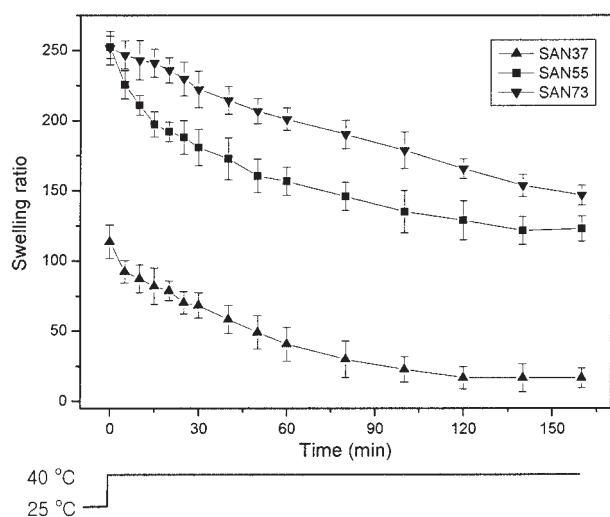

(a)
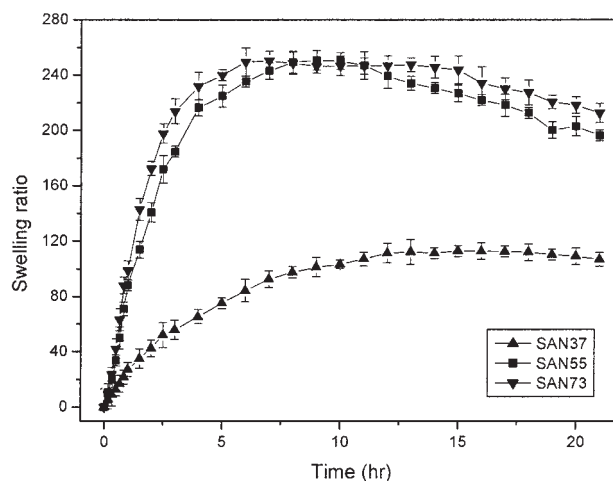
(b)

Figure 3 Swelling kinetics of alginate, PNIPAAm, and IPN hydrogels in deionized water (pH 5.4) at 25°C: (a) alginate, PNIPAAm, and full-IPN hydrogels and (b) semi-IPN hydrogels.

(a)

(b)

Figure 4 Deswelling kinetics of alginate, PNIPAAm, and IPN hydrogels in deionized water (pH 5.4) at 40°C from the equilibrium swelling state at 25°C: (a) alginate, PNIPAAm, and full-IPN hydrogels and (b) semi-IPN hydrogels.

main chains of alginate was due to the relatively small amount of PNIPAAm in the hydrogel.

Temperature-sensitive swelling behaviors

Temperature-dependent equilibrium swelling of semi-IPN and full-IPN hydrogels is shown in Figure 5(a,b). All the hydrogels except the alginate alone had significant changes in the swelling ratios over the temperature range of 30–40°C because PNIPAAm in water exhibited a reversible phase transition in response to small temperature changes around 32°C.[28] It could be concluded that the temperature sensitivity of

PNIPAAm was due to the dissociation of ordered water molecules surrounding hydrophobic *N*-isopropyl groups in PNIPAAm.

In full-IPN hydrogels, FAN37 containing more NIPAAm showed a drastic decrease in the swelling ratios, whereas FAN55 and FAN73 exhibited gentle slopes in the swelling ratio between 30 and 40°C. Semi-IPN hydrogels composed of alginate and PNIPAAm also underwent a volume phase transition in water around the LCST of PNIPAAm. The change in the swelling ratio of semi-IPN hydrogels was higher than that of full-IPN hydrogels with increasing temperature.

Figure 6 shows stepwise swelling behaviors in water with the temperature alternating between 25 and
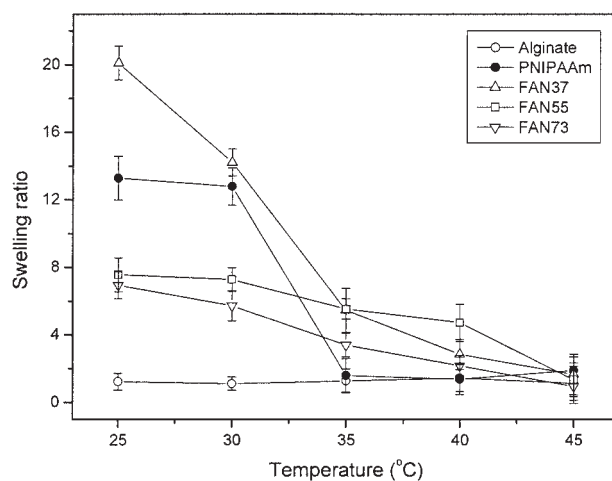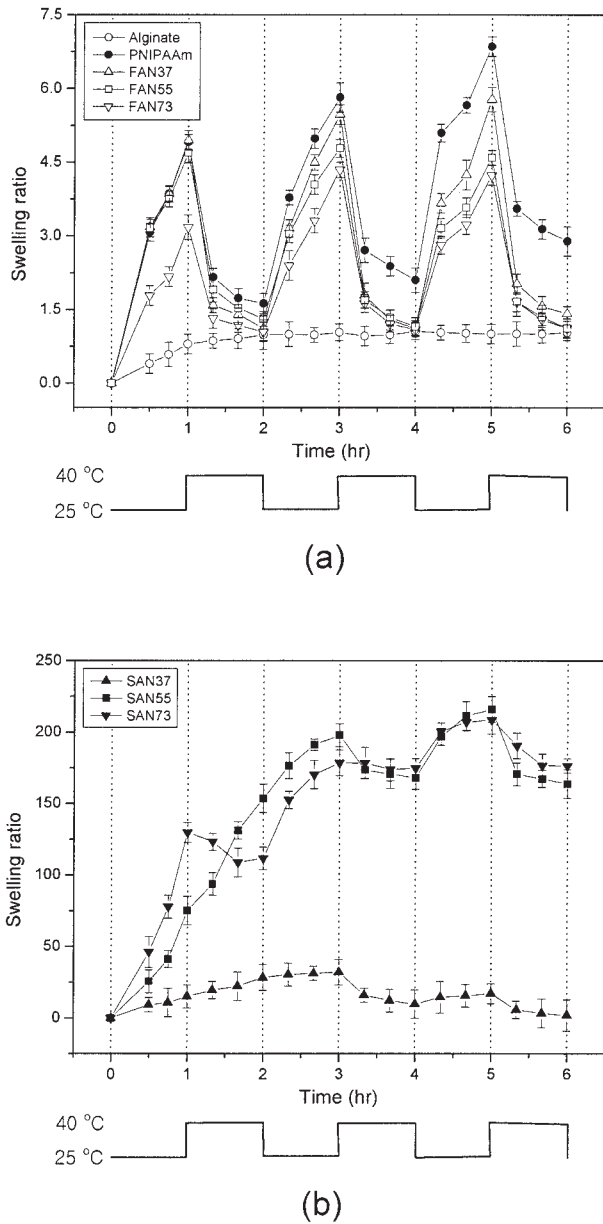

(a)
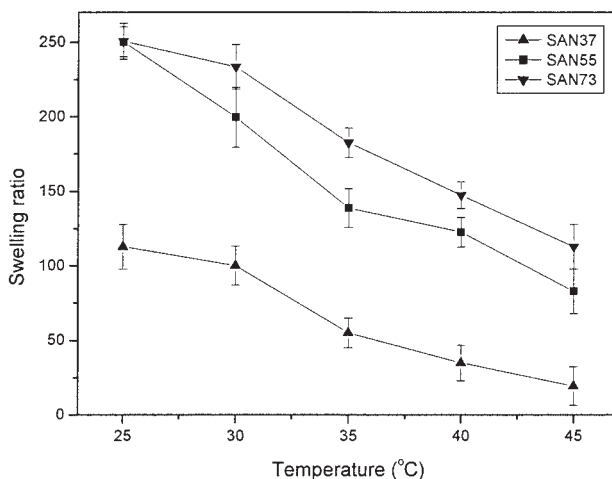
(b)

Figure 5 Swelling ratio of alginate, PNIPAAm, and IPN hydrogels as a function of temperature in deionized water (pH 5.4): (a) alginate, PNIPAAm, and full-IPN hydrogels and (b) semi-IPN hydrogels.
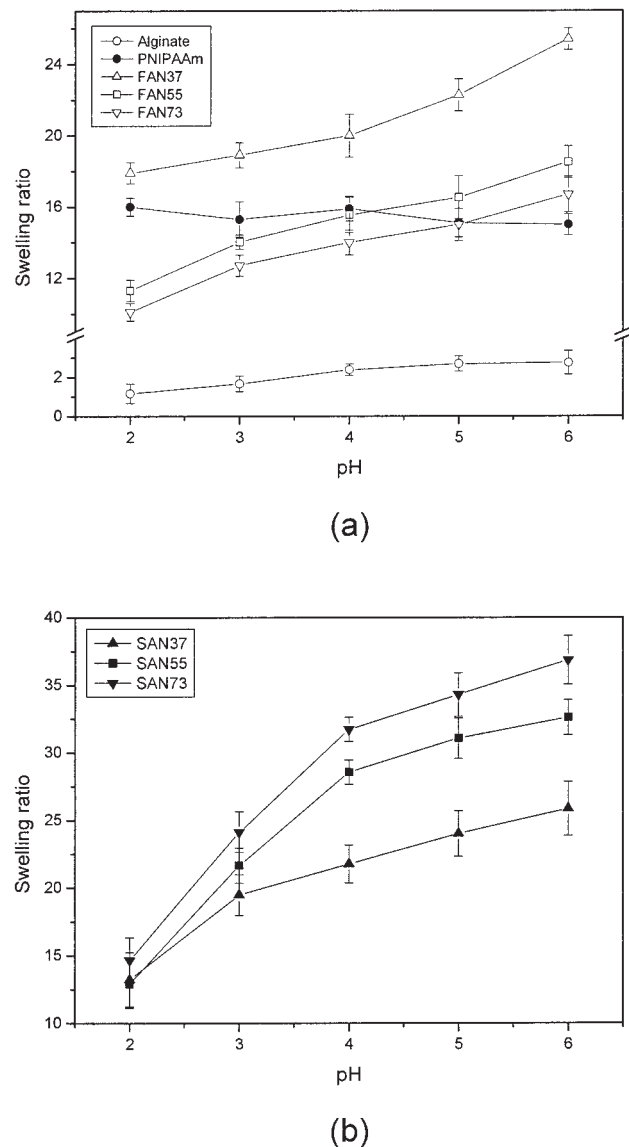
(a)

(b)

Figure 6 Pulsatile temperature-dependent swelling behaviors of alginate, PNIPAAm, and IPN hydrogels in distilled water (pH 5.4): (a) alginate, PNIPAAm, and full-IPN hydrogels and (b) semi-IPN hydrogels.

40°C. The swelling ratio of the IPN hydrogels was measured every hour. Swelling processes in full-IPN hydrogels were proved to be repeatable with temperature changes. Full-IPN hydrogels responded to temperature changes more rapidly and orderly than semi-IPN hydrogels, whereas semi-IPN hydrogels showed a little disordered swelling ratio.

pH-sensitive swelling behavior of the hydrogels

pH-dependent swelling behaviors were observed at 25°C under various pH conditions, as shown in Figure

7. The swelling ratio of alginate hydrogels continuously increased with the pH, whereas that of the PNIPAAm hydrogel was sustained or a little decreased. The pH sensitivity of alginate was due to pK_a of alginate, which was about 3.2 and 4 for guluronic and mannuronic acids, respectively.[12] At a low pH region, most carboxylic acid groups in alginate were in the form of COOH. As the pH of the solution increased, COOH groups became ionized, and the resulting electrostatic repulsion caused the hydrogels to swell. In addition, the slope of the swelling ratios in either semi-IPN or full-IPN hydrogels versus pH increased with the alginate content of the hydrogel because ionic groups of alginate were affected by pH changes.

(a)

(b)

Figure 7 pH-dependent swelling behaviors of alginate, PNIPAAm, and IPN hydrogels at 25°C: (a) alginate, PNIPAAm, and full-IPN hydrogels and (b) semi-IPN hydrogels.
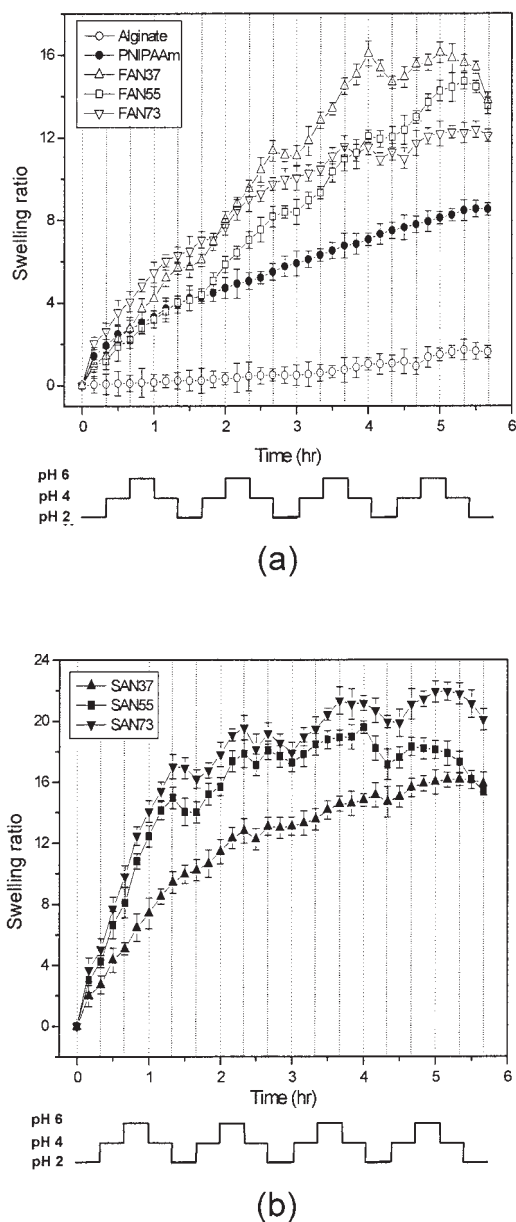
Figure 8 Pulsatile pH-dependent swelling behaviors of alginate, PNIPAAm, and IPN hydrogels at 25°C: (a) alginate, PNIPAAm, and full-IPN hydrogels and (b) semi-IPN hydrogels.

Figure 8 shows oscillatory swelling behaviors of IPN hydrogels at 25°C with pH alternating between 2, 4, and 6. The swelling ratio was measured every 10 min, and the pH was switched every 20 min. After 1 h, pH-dependent pulsatile swelling behaviors were observed. Swelling processes were proven to be repeatable with pH changes. However, the pH-sensitive behaviors of the full-IPN hydrogels occurred more slowly or were less dramatic than the temperature-sensitive response because alginate networks were anchored at several points on account of the crosslinking with calcium ions on each chain restricting the local

mobility. On the other hand, the semi-IPN hydrogels such as SAN73 and SAN55 showed the most drastic changes in the swelling ratio because of the large amount of alginate.

CONCLUSIONS

We designed semi-IPN and full-IPN hydrogels composed of alginate and PNIPAAm. Semi-IPN hydrogels were prepared after γ-ray irradiation of alginate and NIPAAm at 30 kGy. During the irradiation, the polymerization and self-crosslinking of NIPAAm concurrently occurred without any initiators and crosslinking agents. Full-IPN hydrogels were formed by the immersion of the semi-IPN film in a calcium-ion solution, and this resulted in the formation of ionic bonds between the carboxylic groups of alginate. The swelling ratios of the full-IPN hydrogels were much smaller than those of the semi-IPN hydrogels, and the deswelling rate of the semi-IPN hydrogels was slower than that of the full-IPN hydrogels. All the hydrogels with NIPAAm exhibited a change in the swelling ratio around 30–40°C, and the full-IPN hydrogels showed more sensitive and reversible behavior than the semi-IPN hydrogels under the oscillatory stimulus of temperature. Under various pH conditions, the swelling ratio of the hydrogels continuously increased with the pH values (2–6), and the swelling processes were proven to be repeatable with pH changes.

References

1. Maolin, Z.; Ning, L.; Jun, L.; Min, Y.; Jiuqiang, L.; Hongfei, H. Radiat Phys Chem 2000, 57, 481.
2. Xuequan, L.; Maolin, Z.; Jiuqing, L.; Hongfei, H. Radiat Phys Chem 2000, 57, 477.
3. Maolin, Z.; Hongfei, H.; Jilan, W. Radiat Phys Chem 1993, 42, 919.
4. Strauss, P.; Knolle, W.; Naumov, S. Macromol Chem Phys 1998, 199, 2229.
5. Okano, T.; Yamada, N.; Okuhara, M.; Sakai, H.; Sakurai, Y. Biomaterials 1995, 16, 297.
6. Bhattacharya, A. Prog Polym Sci 2000, 25, 371.
7. Rosiak, J. M.; Ulanski, P.; Pajewski, L. A.; Yoshii, F.; Makuuchi, K. Radiat Phys Chem 1995, 46, 161.
8. Rosiak, J. M.; Yoshii, F. Nucl Instrum Methods Phys Res Sect B 1999, 141, 56.
9. Kaetsu, I. Radiat Phys Chem 1996, 47, 419.
10. Benson, R. S. Nucl Instrum Methods Phys Res Sect B 2002, 191, 752.
11. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. Polymer 2001, 42, 6851.
12. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. J Appl Polym Sci 2002, 83, 1128.
13. Kikuchi, A.; Kawabuchi, M.; Watanabe, A.; Sugihara, M.; Sakurai, Y.; Okano, T. J Controlled Release 1999, 58, 21.
14. Choi, Y. S.; Hong, S. R.; Lee, Y. M.; Song, K. W.; Park, M. H.; Nam, Y. S. Biomaterials 1999, 20, 409.
15. Choi, Y. S.; Lee, S. B.; Hong, S. R.; Lee, Y. M.; Song, K. W.; Park, M. H. J Mater Sci: Mater Med 2001, 12, 67.
16. Gacesa, P. Carbohydr Polym 1988, 8, 161.

17. Kim, J. H.; Lee, S. B.; Kim, S. J.; Lee, Y. M. Polymer 2002, 43, 7549.

18. Kim, S. Y.; Cho, S. M.; Lee, Y. M.; Kim, S. J. J Appl Polym Sci 2000, 78, 1381.

19. Lee, J. W.; Lee, D. S.; Kim, S. W. Macromol Res 2003, 11, 189.

20. Nam, I.; Bae, J. W.; Jee, K. S.; Lee, J. W.; Park, K. D.; Yuk, S. H. Macromol Res 2002, 10, 115.

21. Kaneko, Y.; Nakamura, S.; Sakai, K.; Aoyagi, T.; Kikuchi, A.; Sakurai, Y.; Okano, T. Macromolecules 1998, 31, 6099.

22. Kaneko, Y.; Sakai, K.; Kikuchi, A.; Yoshida, R.; Skurai, Y.; Okano, T. Macromolecules 1995, 28, 7717.

23. Wu, W. S.; Hoffman, A. S.; Yager, P. J Polym Sci Part A: Polym Chem 1992, 30, 2121.

24. Panda, A.; Manohar, S. B.; Sabharwal, S.; Bhardwaj, Y. K.; Majali, A. B. Radiat Phys Chem 2000, 58, 101.

25. Nagasawa, N.; Mitomo, H.; Yoshii, F.; Kume, T. Polym Degrad Stab 2000, 69, 279.

26. Leonhardt, J.; Arnold, G.; Baer, M.; Langguth, H.; Gey, M.; Hubert, S. Radiat Phys Chem 1985, 25, 887.

27. Kong, H. J.; Smith, M. K.; Mooney, D. J. Biomaterials 2003, 24, 4023.

28. Schild, H. G. Prog Polym Sci 1992, 17, 163.