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ORIGINAL CONTRIBUTION

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Structural observation of heterogeneous poly(*N*-isopropyl acrylamide-*co*-acrylic acid) hydrogels in highly hydrated states

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Abstract Heterogeneous hydrogels were prepared by γ-ray irradiation of aqueous solutions of *N*-isopropylacrylamide (NIPAAm) and acrylic acid (AAc) having various compositions above the lower critical solution temperature. The structures of the poly(*N*-isopropylacrylamide) (PNIPAAm) gel and poly(NIPAAm-*co*-AAc) gels in both their highly hydrated and their natural states were observed by environmental scanning electron microscopy. The heterogeneous structures of the homopolymer gel and the copolymer gels whose AAc contents were between 10–50% consisted of interconnected microspheres. In the copolymer gel with a high AAc content, the structure became a largely interconnected one which lacked micro-droplets. The hydrophobic interaction caused by hydrogen bonding between the unionized carboxylic acid groups of AAc and the amide groups of NIPAAm, the rates of polymerization, and the aggregation rates play important roles in the formation of interconnected microsphere gel structures.

Keywords Poly(*N*-isopropylacrylamide-co-acrylic acid) · Gels · Volume transition · Porous structure · Microsphere

Introduction

Polymer gels can undergo a reversible volume transition, depending on external physical and chemical factors such as temperature, solvent composition, ionic strength, pH, electric field, and light [1, 2]. This effect can be applied in a variety of fields, such as chemical engineering, medicine and pharmacy, life sciences, food, and agriculture. An important factor governing their application is the response time of their volume transitions. Tanaka and Fillmore reported that the swelling rate of a hydrogel is inversely proportional to the square of its linear size [3]. Hence, it is necessary to synthesize these gels in small sizes, such as microspheres, fine fibers and thin films, in order to improve their rates of change in volume; however, such small gels present some handling problems. In order to improve the response time without decreasing the gel size, the synthesis of microporous hydrogels by a phase separation of thermo-responsive polymers such as poly(*N*-isopropylacrylamide) (PNIPAAm) is very effective. PNIPAAm is well known as a thermo-responsive polymer, and its aqueous solution undergoes phase separation at the lower critical solution temperature (LCST) (31 °C) [4]. Several researchers have prepared porous and heterogeneous PNIPAAm gels by a radical copolymerization of *N*-isopropylacrylamide (NIPAAm) with a crosslinking monomer above the LCST [5, 6, 7]. Compared with homogeneous PNIPAAm hydrogels prepared below the LCST, these hydrogels have both a larger pore volume and an increased surface area; in addition, they show a rapid volume transition upon temperature alteration.

To prepare fast-response porous and heterogeneous hydrogels, γ-ray irradiation appears to be an effective and reproducible method [8, 9]. These porous PNIPAAm gels can be synthesized by radiation polymeri-

zation using an aqueous solution of NIPAAm without a crosslinking monomer [10]. Just after the irradiation begins, a radiation-induced polymerization produces an exothermic reaction, and during the course of the irradiation a radioactive heating takes place. These two factors keep the temperature of the monomer solution close to the radiation source (^{60}Co) above the LCST. The following phenomena occurred simultaneously under this irradiation: a radiation-induced polymerization of the monomers, an irradiation-produced phase separation of the polymers, and a crosslinking reaction of the phase-separated polymers. Thus, a heterogeneous and opaque gel with a phase separated, porous structure was formed. The advantages of this method are its easy reproducibility and adaptability to copolymerization with other water-soluble, functional monomers. Previously, we reported the preparation and properties of NIPAAm and acrylic acid (AAc) copolymer hydrogels with micro-porous structures [11]. The latter gels exhibited a rapid and reversible volume transition not only with changes in temperature but also with changes in pH.

The structural observation of these hydrogels is very important in clarifying the formation mechanism of the porous structures and the relationship between the structures and the responsiveness of the volume transition. Recently, the structures of the crosslinked dextrans (Sphadex) [12] and the biopolymer gel composites [13] were observed using an environmental scanning electron microscope (ESEM). Since the ESEM allows observation in the hydrated state, it may be ideal method to minimize the deformation or destruction of these soft, flexible, and highly hydrated polymer gels that might occur during the drying processes of sample preparations for SEM observations. Hence, ESEM studies would be helpful in investigating the detailed heterogeneous and porous structure of the γ-ray-prepared PNIPAAm homopolymer gel and the poly(NIPAAm-*co*-AAc) copolymer gels. In this work, we used ESEM to determine the heterogeneous structures of these polymer hydrogels ; it allowed us to control the humidity in the sample chamber and to obtain images under a high vapor pressure environment, even in the presence of liquid water.

Materials and methods

Preparation of the porous gels [11] The NIPAAm (Kohjin, Tokyo, Japan) was purified by the method reported by Gehrke et al [14]. The AAc (Wako Pure Chemical, Osaka, Japan) was distilled under reduced pressure before use. NIPAAm and AAc were dissolved in 16 ml of deionized water, and purged with nitrogen gas. The feed compositions of the NIPAAm and AAc were varied, but their sum was fixed to be 30 mmol (Table 1). These solutions were then transferred into several disposable cells (10×10×45 mm). The gels were prepared by γ-ray irradiation in the vicinity of a ^{60}Co source (110.5 TBq) with an intensity of 9.21 kGy h^{-1} and an exposure of 14 h; they were then cut into 10 mm slices and thoroughly washed several times with 5 °C water.

Measurements The fraction of AAc units in the copolymer gels was determined by an elemental analysis of the freeze-dried gels. The length of the gels in water was measured at various temperatures in order to calculate the degree of swelling. The degree of swelling in length, L/L_0, was calculated from the length (L) of the gel at various temperatures and the inner dimension of the disposable cells (L_0; 10 mm) used in their preparation. The weight percent of water [$(W–W_d)/W\times100$ (%)] was calculated from the weight of the gel at various temperatures (W) and the weight of the freeze-dried samples (W_d).

ESEM observation The ESEM images were obtained by the method reported by Kodaka et al [15]. The gels' swelling in deionized water at 2 °C was examined with the ESEM (Philips, XL30 ESEM-FEG) operated at 15 kV. The gels, which were prepared in the disposable cells described above, were cut into small pieces using a razor. We observed the sliced surface of these pieces. The pieces were set on the cooling stage of the ESEM adjusted to 2 °C and maintained above a saturated water pressure of 0.71 kPa (5.3 Torr). Under these conditions, the ESEM showed a water layer covering the sample surface. To prevent inordinate dehydration of the samples, the vapor pressure in the chamber was lowered gradually until the sample surface appeared.

Table 1 Monomer fractions for the preparation of microporous copolymer gels and in the resulting gels. *NIPAAm* *N*-Isopropylacrylamide, *AAc* acrylic acid, *PNIPAAm* poly(*N*-isopropylacrylamide)

[a]Determined by elemental analysis using freeze-dried samples

Sample code	Feed composition (mmol)		Monomer fraction (mol %)		Fraction in gel[a] (mol %)	
	NIPAAm	AAc	NIPAAm	AAc	NIPAAm	AAc
PGNA-0 (PNIPAAm gel)	30	0	100	0	100	0
PGNA-10	27	3	90	10	90	10
PGNA-30	21	9	70	30	70.2	29.8
PGNA-50	15	15	50	50	49.9	50.1
PGNA-70	9	21	30	70	30.1	69.9

The ESEM images were usually observed at a vapor pressure of 0.53–0.6 kPa (4–4.5 Torr). Since exposure to an electron beam over 5–7 min causes damage to the samples or local evaporation, we observed samples for 2–3 min, during which no structure destruction was observed in our samples.

Results and discussion

With all monomer compositions employed, opaque and heterogeneous poly(NIPAAm-*co*-AAc) gels were formed by γ-ray polymerization and crosslinking reactions at temperatures above the LCST of the copolymers. The NIPAAm and AAc fractions in the gels were calculated from the C/N ratio obtained from the results of elemental analysis of the freeze-dried samples (Table 1). The unit fraction in the gels was in good agreement with the monomer fraction for the synthesis of porous gels. Under γ-ray irradiation, random copolymerization between the NIPAAm and the AAc took place above the LSCT.

Table 2 shows the water contents by mass of the porous gels at various temperatures. The gels showed very large water contents, especially at 5 °C. The water content fell with increasing temperature, a phenomenon which resulted from a thermal shrinking of the gel. At 40 °C, the gels showed water contents of over 80% in spite of their shrinking states. In these shrinking states, a large amount of water was stored in the porous medium inside the gels.

The swelling and shrinking behaviors of the porous gels were studied in deionized water at various temperatures. The degree of swelling in length (L/L_0) was plotted against the temperature, as shown in Fig. 1. The heterogeneous homopolymer gel (PGNA-0) underwent a sharp thermal volume transition at around the LCST (31-33 °C), as did the homogeneous gel prepared by radical polymerization below the LCST [16]. Except for the PGNA-70, the porous copolymer gels also showed thermal volume transitions; however the LCST of the copolymer gels lowered with an increasing AAc content in the gels. On the other hand, the volume of the gel PGNA-70 decreased monotonically with a rise in the temperature of the swelling measurement from 5 to 40 °C.

These behaviors are explained by the interaction between the carboxylic acid groups of AAc and the amide groups of NIPAAm. It is well known that the thermal properties of the poly(NIPAAm-*co*-AAc) depend upon the pH of the solutions [17, 18, 19, 20]. The p K_a value of a poly(NIPAAm-*co*-AAc) was estimated to be 4.2 by Yoo et al [17]. At pH 7.4 (above the p K_a), the LCST of random copolymers increased rapidly with increasing AAc content and phase separation was not observed for copolymers without low AAc content, owing to electrostatic repulsion produced by the high ionization of carboxylic acid groups [18, 19]. On the other hand, at pH 2.2 (below the p K_a), the LCST of copolymers was lower than that of the PNIPAAm homopolymer (31–33 °C), and decreased with increasing AAc content [17]. The lowering of the LCST of copolymers in the pH range of 1.5–3.5 was also reported by Jones [20]. Under strongly acidic conditions below the p K_a, the carboxylic acid groups of the AAc unit changed to the undissociated and non-ionized state and formed inter- and intramolecular hydrogen bonds with the amide groups of NIPAAm [17, 20]. Jones considered that the formation of hydrogen bonding subsequently prevents the water-NIPAAm interactions and increases the hydrophobicity of the copolymer environment [20]. The dissociation constant of the carboxylic acid groups of the copolymers in pure water is estimated to be below 10^{-4} from the p K_a

Table 2 Weight percent water content ofmicroporous copolymer gels at various temperatures

Sample code	Weight percent water content (%)		
	5 °C	25 °C	40 °C
PGNA-0 (PNIPAAm gel)	97.0	95.0	80.8
PGNA-10	97.7	96.0	87.1
PGNA-30	97.4	93.8	85.4
PGNA-50	96.5	87.5	85.4
PGNA-70	94.3	90.9	88.6

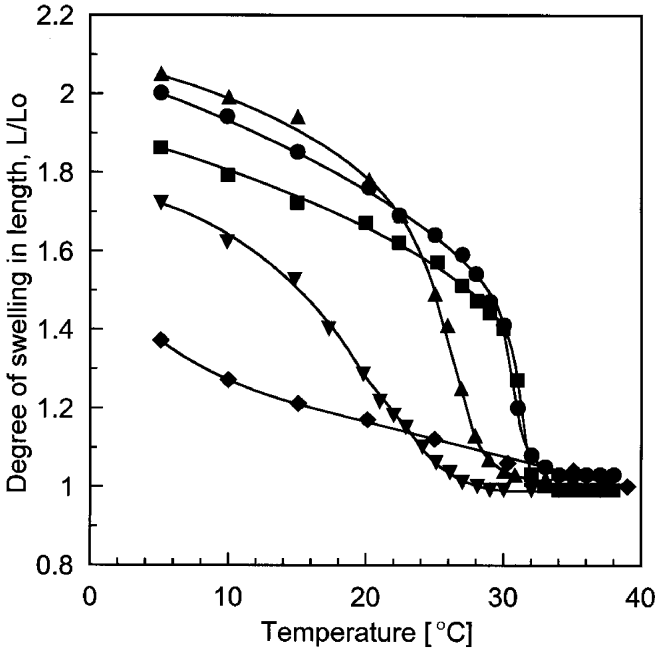

Fig. 1 Degree of swelling in length (L/L_0) of the gels plotted against the temperature. *Square* PGNA-0, *circle* PGNA-10, *up-triangle* PGNA-30, *down-triangle* PGNA-50, *diamond* PGNA-70. See Table 1 for explanation of codes

value. In pure water, most of the carboxylic acid groups of the copolymers might be undissociated, in which they form inter- and intra-molecular hydrogen bonds with amide groups in the NIPAAm units. Therefore, the reduced LCST of the gels in pure water (Fig. 1) seems to result from the increase in hydrophobicity caused by increasing hydrogen bonding between the AAc units and the NIPAAm units. In our previous report, an absorption band characteristic of a hydrogen-bonded AAc carbonyl group (1,619 cm^{-1}) was observed byTIR measurement in the freeze-dried poly(NIPAAm-*co*-AAc) gel [11]. The lowering of the LCST with increasing AAc units in pure water was also observed in another copolymer system consisting of *N, N*-diethylacrylamide and AAc [21].

Figure 2 shows the ESEM images of the homopolymer and copolymer gels. These structures are produced by the phase separation and crosslinking reaction of polymers formed by γ-ray polymerization, since the temperature during radiation is much higher than the LCSTs of the polymers. In Fig. 2a, a porous structure of interconnected microspheres approximately 0.7–0.9 μm in diameter was observed for homopolymer gel (PGNA-0). Previously, we reported a cryo-SEM observation of the homopolymer gel prepared in the same manner [10, 11]. The cryo-image of the PGNA-0 was that of a porous structure consisting of a fine fibrous network; the diameter of the fibers was < 2 μm and that of the particles approximately 3–5 μm. The highly hydrated structure obtained by ESEM (Fig. 2a) was different from the cryo-image. Since our heterogeneous porous gel has many cavities inside, it was in a soft, flexible, and highly hydrated state. Thus, its structure could be easily changed in the cryo-SEM observations in spite of the quick freezing and subsequent freeze-drying.

It is interesting to compare these interconnected microsphere structures with the hydrogel microspheres of PNIPAAm reported by Kawaguchi et al [22]. They synthesized independent and monodisperse microspheres using a dilute monomer solution, in which 5 g of monomers (4.9 g of NIPAAm and 0.1 g of *N, N'*-methylenebisacrylamide) were dissolved in 190 ml of water (monomer concentration: 2.56%). Since the monomer concentration was very low, the gel clusters could not grow to their full potential size, an inhibition which results in the formation of dispersed small gel droplets. On the other hand, the monomer concentration of our system was very high (monomer concentration: 17.5%), and the clusters were close to other clusters. It might be possible to grow larger interconnected structures instead of retaining discrete droplets. We thought that, unlike the phase separation structures in the usual polymer solutions, the crosslinking reaction of the gel preserved the droplet structure in the initial phase separation stage, which also obstructed the coarse graining processes that reduce the interfacial surfaces. This type of growth may occur when the crosslinking speed is considerably faster than that of the phase separation processes.

The ESEM images of the copolymer gels, PGNA-10, PGNA-30 and PGNA-50 are shown in Fig. 2b, c and d, respectively. The copolymer gels were also composed of interconnected microspheres, but their sizes were much larger than that of the homopolymer gel (PGNA-0). However, the size and shape of the microspheres of PGNA-10, 30 and 50 were almost the same, and independent of the AAc content of the gels.

The change in hydrophobicity resulting from hydrogen bond formation between carboxylic acid groups and amide groups may have affected the phase separation process of the polymers, which, in turn plays an important role in forming the structures of the gels. In the cases of the PGNA-10, 30 and 50, it seems that the radiation-induced phase separation of the copolymers starts at a temperature lower than that of the homopolymer, since the LCST of copolymers was lower than that of the PNIPAAm homopolymer. Therefore, the microspheres of PGNA-10, 30 and 50 might grow larger than those of the homopolymer system. In addition to the lowered LCST, the rates of copolymerization, the rates of crosslinking reactions, and the aggregation rates of the microsphere are considered important factors in the formation of gel structures. In fact, the morphology of the copolymer gel containing a large amount of AAc [PGNA-70 (Fig. 2e)] was completely different from the other copolymer or homopolymer gels. The structure of the high AAc copolymer gel was large, smoothly interconnected, and without micro-droplets. Apparently, during the gelation process, these factors interact in a complex fashion, resulting in the different structures and crosslinking densities formed by the γ-ray irradiation.

Conclusions

We prepared a heterogeneous PNIPAAm homopolymer gel and poly(NIPAAm-*co*-AAc) copolymer gels, and, in order to keep their original structure, observed their network morphologies by ESEM. Under γ-ray irradiation, the saturated monomer solution of NIPAAm produced heterogeneous structures. The ESEM observations revealed that these structures consisted of an interconnected microsphere network. The microsphere structures could be easily deformed and aggregated by quick-freezing and drying, changes which had caused difficulties in the previous observation by cryo-SEM. If the AAc contents were between 10 and 50%, the mixed solutions of NIPAAm and AAc also led to an interconnected microsphere structure. However, the microspheres of the copolymer gels became slightly larger than those of the homopolymer gel. Interestingly, the high AAc copolymer gels formed the usual intercon-

nected, large, and smoothly porous structures, in which microspheres were hardly to be found. The formation processes of these heterogeneous structures are affected by the hydrophobic interaction caused by hydrogen bonding between the unionized carboxylic acid groups of AAc and the amide groups of NIPAAm, the rates of polymerization, and the aggregation rates. To prevent drastic structural changes from freezing and drying, ESEM is considered to be a suitable method of structural observation of such highly hydrated samples as micro-porous hydrogels prepared by γ-ray irradiation with phase separation.

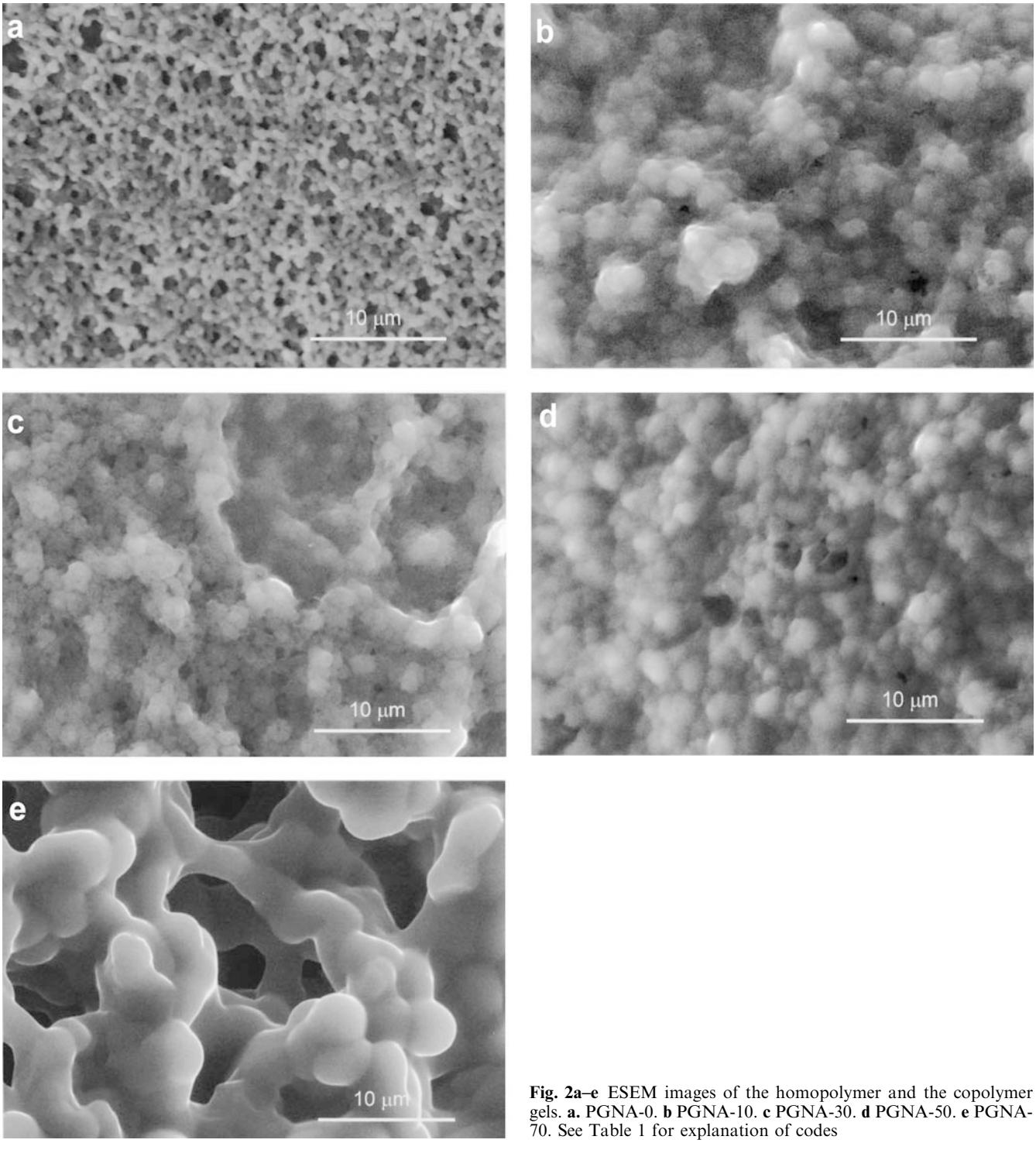

Fig. 2a–e ESEM images of the homopolymer and the copolymer gels. **a.** PGNA-0. **b** PGNA-10. **c** PGNA-30. **d** PGNA-50. **e** PGNA-70. See Table 1 for explanation of codes

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