

Preparation and Characterization of Micrometer-Sized Poly(*N*-isopropylacrylamide) Hydrogel Particles

By Hidenobu SHIMIZU,^{1,*} Risei WADA,¹ and Masaru OKABE¹

The present study deals with preparation and swelling properties of micrometer-sized poly(*N*-isopropylacrylamide) (PNIPAM) hydrogel particles. Aqueous precipitation polymerization of NIPAM with *N,N'*-methylenebisacrylamide (MBAAm) was performed with varying electrolyte concentrations in the polymerization media. Sodium chloride was used as an electrolyte. According to the particle size analysis by transmission electron microscope, the particle size increased in the range of 0.55 to 1.60 μm with an increase in the electrolyte concentration up to 1.6 wt %. The concentration is based on the NIPAM monomer weight. Dynamic light scattering data showed that swelling ratio of PNIPAM hydrogel particles in water varied from 10 to 40 as the electrolyte concentration at particle preparation increased from 0 to 1.6 wt %. Time-course analysis of particle size showed that an increase in particle size would be caused by aggregation of smaller particles. In conclusion, micrometer-sized PNIPAM particles with high swelling capacity can be obtained by the addition of an electrolyte in polymerization media.

KEY WORDS: Poly(*N*-isopropylacrylamide) / Thermosensitive Particle / Precipitation Polymerization / Hydrogel / Volume Phase Transition /

Thermosensitive hydrogel particles containing poly(*N*-isopropylacrylamide) (PNIPAM) moieties have attracted much attention because they exhibit sharp and reversible volume phase transition at the lower critical solution temperature (LCST).^{1,2} PNIPAM particles also show the dramatic change of their colloidal properties such as hydrophobicity,³ particle size,⁴ electrophoretic mobility,^{5,6} colloidal stability,^{7,8} rheology^{9,10} etc. in response to temperature changes across the LCST. These unique features make PNIPAM particles desirable for numerous applications, including controlled drug delivery,^{11,12} control of enzymatic activity,^{13,14} tunable optics,^{15,16} and bioseparation.¹⁷

Colloidally stable PNIPAM particles were first prepared by precipitation polymerization of NIPAM with *N,N'*-methylenebisacrylamide (MBAAm) as a cross-linking agent in an aqueous medium at 60 to 70 °C.¹⁸ The resulting particles showed a volume phase transition at the LCST around 32 °C. In precipitation polymerization, the initial state of the reaction mixture is a homogeneous solution. When a water-soluble initiator is added to the mixture, initiation and propagation take place largely in the homogeneous medium, followed by phase separation of growing PNIPAM chains because PNIPAM is insoluble in the medium at the reaction temperature. This leads to continuous nucleation and coagulation of the resulting nuclei to form larger particles. Since the first report, various methods have been studied so far for preparation of PNIPAM hydrogel particles. Pelton and co-workers reported that addition of surfactant gave smaller and more uniform PNIPAM particles than those obtained without surfactant.¹⁹ Yi *et al.* reported that monodisperse thermosensitive poly(styrene-*co*-NIPAM) particles with diameters in the range of 100 to 130 nm could be

prepared by emulsifier-free emulsion polymerization with microwave irradiation.²⁰ In addition, many researchers have focused on studying synthesis and characterization of PNIPAM particles with core-shell morphologies.^{4,21,22} However, the sizes of such particles obtained so far are limited in the submicron range and there are only few reports about preparation of micrometer-sized PNIPAM particles.²³

We recently reported a simple and convenient method to prepare micrometer-sized PNIPAM hydrogel particles. Such larger particles can be obtained by precipitation polymerization at higher initiator concentrations.^{24,25} Increase in initiator concentrations provided the increased number of precipitated PNIPAM chains, resulting in the production of larger particles. In view of our results, it would be a key process for precipitated chains to efficiently coagulate during polymerization for production of larger particles.

We report here the effect of an electrolyte concentration on the particle size. It is expected that the addition of an electrolyte in aqueous medium causes solubility of growing polymer chains to lower and aggregation reaction between growing polymer chains is prone to take place. Sodium chloride was used as an electrolyte. The morphology, size, and size distribution of the obtained PNIPAM particles were studied with transmission electron microscopy (TEM) and dynamic light scattering (DLS).

EXPERIMENTAL

Materials

N-Isopropylacrylamide (NIPAM, Wako Pure Chemical Industries, Ltd.) was purified by recrystallization from a

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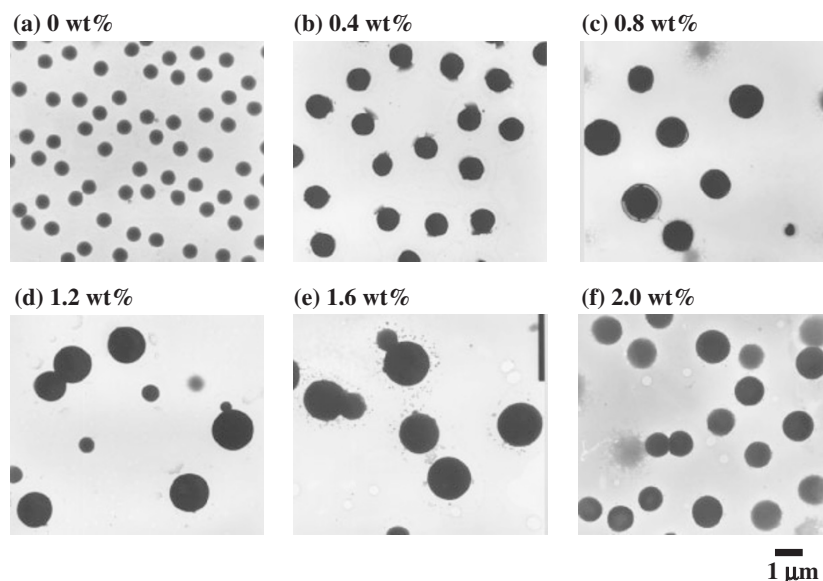


Figure 1. TEM images of PNIPAM hydrogel particles prepared by precipitation polymerization as a function of electrolyte concentrations.

mixture of hexane and toluene (v/v: 1/1). Potassium persulfate (KPS, Kanto Chemical Co., Inc.) was recrystallized from distilled water and used as an initiator. *N,N'*-methylenebisacrylamide (MBAAm) and sodium chloride were purchased from Kanto Chemical Co., Inc. and used as received. Distilled water with a conductivity of *ca.* 0.10 mS/m was used as a polymerization medium.

Synthesis of PNIPAM Hydrogel Particles

PNIPAM hydrogel particles were prepared by using precipitation polymerization. The NIPAM monomer (2.5 g), MBAAm cross-linker (0.025 g), and various amounts of sodium chloride were dissolved in 45 g of distilled water and then the mixture was added in a four-necked round bottom flask equipped with a reflux condenser, a Teflon paddle stirrer, and a nitrogen gas inlet. The reaction mixture was heated up to 70 °C under a stream of nitrogen. To the mixture, 2.5 g of distilled water containing 0.01 g of KPS was added to initiate polymerization. The polymerization was carried out at 70 °C for 1 h under a nitrogen atmosphere. All particles were purified by repetitive centrifugation and redispersion in distilled water several times, followed by dialysis to remove free electrolytes.

Characterization

The morphology and size of the PNIPAM hydrogel particles were characterized by using transmission electron microscopy (TEM, JEOL 2000EX). The obtained dispersion was diluted with distilled water. A drop of the diluted dispersion was placed on a collodion-coated copper grid, and the grid was then dried at 40 °C. The polydispersity index was evaluated by TEM observations. The index was defined by the uniformity ratio U :

$$U = D_w/D_n$$

where D_w is the weight average diameter and D_n is the number average diameter.

The average hydrodynamic diameters of the PNIPAM hydrogel particles were measured by using a homemade light scattering apparatus equipped with an ALV 5000/60X0 Tau digital correlator and a 20 mW He-Ne laser ($\lambda = 632.8$ nm). The dispersed particles in water were allowed to equilibrate thermally before measurements were taken at given temperature. Scattering data were collected at 90° and the time correlation functions were obtained. The average hydrodynamic diameters at given temperature were calculated from the translational diffusion coefficients by using the Stokes-Einstein equation.

RESULTS

Particle Size and Morphology

Aqueous precipitation polymerization of NIPAM with MBAAm was performed as a function of electrolyte concentrations. The feed molar ratio of NIPAM with MBAAm held constant at 136. Sodium chloride was used as an electrolyte. Figure 1 shows the TEM micrographs of the resulting particles as a function of electrolyte concentrations. The concentration was based on NIPAM monomer weight. Particle size was then determined from the TEM images and particle size distribution are shown in Figure 2.

According to the TEM images, all particles were almost spherical and the surface of PNIPAM particles seems to give fuzzy images especially at higher electrolyte concentrations. In addition, the polydispersity index U was below 1.01 when the electrolyte concentrations were at 0 and 0.4 wt %, indicating that monodisperse PNIPAM particles were obtained. On the other hand, electrolyte concentrations of 0.8 wt % or above made the particles polydisperse (U was above 1.10); particularly bimodal distribution seemed to be observed at the concentration of 1.2 and 1.6 wt %. The smaller particles had about a half size of the larger particles. These results may be

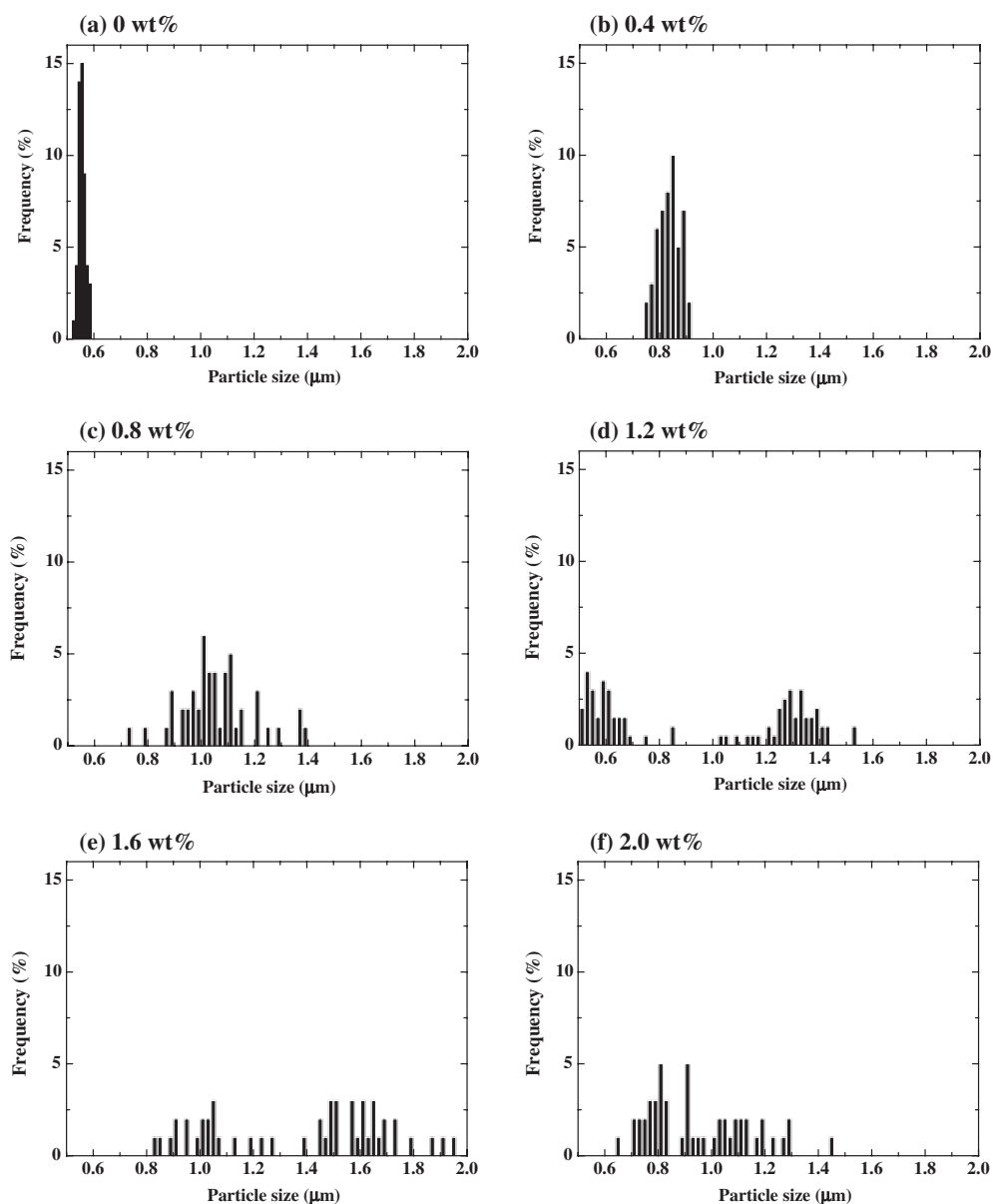


Figure 2. Particle size distribution for precipitation polymerization of NIPAM with MBAAm as a function of electrolyte concentrations.

concerned about the mechanism for production of micrometer-sized PNIPAM particles. We will discuss it later.

Figure 3 summarized the influence of the electrolyte concentration on the particle size. In bimodal distribution, the larger size with a polydispersity index of about 1.02 was plotted as the average size. In addition, the average size of the particles prepared at higher electrolyte concentrations had a somewhat wide range even if the experimental conditions were the same. The size increased from 0.55 to 1.60 μm as the electrolyte concentration was increased from 0 to 1.6 wt %. At higher concentrations, however, the size decreased to about 1.1 μm. Higher electrolyte concentrations provided many macroscopic precipitates (above 90 wt % in the feed), leading to the decrease in NIPAM monomer concentration in aqueous media. As a result, smaller particles were obtained at higher

electrolyte concentrations. These results indicate that the size of PNIPAM hydrogel particles can be controlled by varying an electrolyte concentration in the range of 0.55 to 1.60 μm.

Volume Phase Transition Behavior

The hydrodynamic diameters of PNIPAM particles were measured by using DLS at different temperatures. The results are shown in Figure 4. The size distribution was unimodal and very narrow. Higher electrolyte concentrations at particle preparation resulted in larger hydrodynamic diameters both in the swollen and collapsed state. These results were in agreement with the TEM results. Dose-dependence of the particle size by DLS, however, was a little different from that by the TEM images. Hydrogel particles may have a tendency to flatten and spread on the TEM grid during the sample

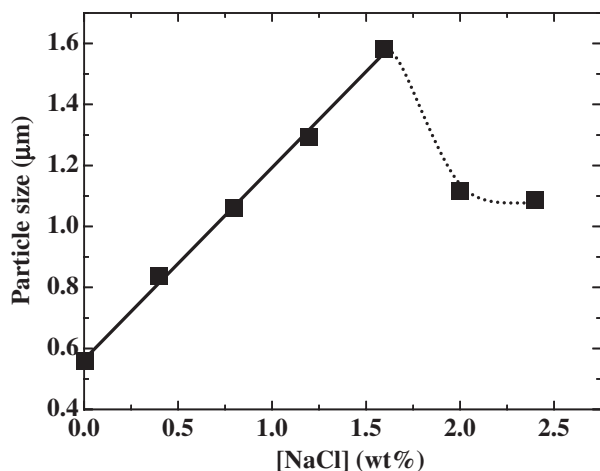


Figure 3. Particle size determined by TEM as a function of electrolyte concentrations.

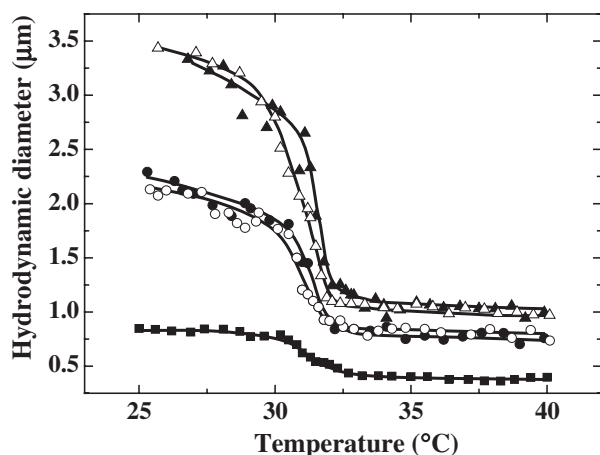


Figure 4. Temperature dependence of hydrodynamic diameter of PNIPAM particles prepared with electrolyte concentration of (■) 0 wt %, (●) 0.4 wt %, (○) 0.8 wt %, (▲) 1.2 wt %, and (△) 1.6 wt %.

preparation. Softness of the PNIPAM particles therefore may be involved in the particle size by TEM. In addition, the data show that all of the hydrogel particles shrank as the temperature was increased and underwent a remarkable deswelling around 32 °C. This phase transition temperature corresponds with that of linear PNIPAM chains in pure water^{26,27} and little effect of MBAAm incorporation on the LCST was observed because the MBAAm content was very low. The diameters of larger PNIPAM particles seem to have a large change in response to the variation in temperature. We then calculated the swelling ratio from these results.

Swelling/Deswelling

The swelling-deswelling process can be described quantitatively in terms of the amount of solvent filled in hydrogel particles. The swelling was evaluated by the $(d/d_0)^3$ ratio, where d is the hydrodynamic diameter at given temperature and d_0 is that at 40 °C. The results are shown in Figure 5. The data indicate that the swelling ratio of these hydrogel particles

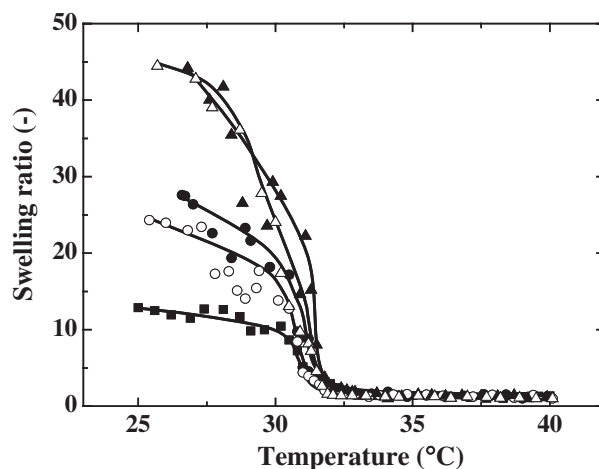


Figure 5. Temperature dependence of swelling ratio of PNIPAM particles prepared with electrolyte concentration of (■) 0 wt %, (●) 0.4 wt %, (○) 0.8 wt %, (▲) 1.2 wt %, and (△) 1.6 wt %.

increased as the temperature was decreased. Moreover, the ratio was strongly affected by the electrolyte concentrations at particle preparation in spite of the same cross-linker concentration. The volume of the conventional PNIPAM particles, which were prepared in pure water, increased approximately 10 times when temperature was decreased from 40 °C to room temperature. On the other hand, micrometer-sized thermosensitive particles swelled about 40 times with a decrease in temperature. These high swelling ratios of the larger particles may be due to the difference in internal particle structure.

Kinetic Study

In order to clarify particle formation and growth process of larger PNIPAM particles, we investigated how particle size varies with polymerization time. Polymerization of NIPAM was performed as previously described in the EXPERIMENTAL and the samples were withdrawn from the reaction mixture at various time intervals. Figure 6 shows the TEM images of PNIPAM particles as a function of polymerization time. NaCl was added at the concentration of 1.6 wt % to produce as large particles as possible. Figure 7 also shows particle size distribution obtained by the TEM images. At the early stage of polymerization (Figure 7a), monodispersed particles with a diameter of 0.33 μm were produced. As polymerization proceeds, particle size distribution seems to be broad and then the average particle size appears to increase not continuously but stepwise. In fact, the average particle size increased to 0.68 μm as the polymerization proceeded to 10 min, indicating that the volume of the particles were 8 times larger. Monomer conversion at 5 min was about 50% and thus the particles at 5 min could not grow larger without a change in the particle number. As a result, particle number would decrease during polymerization. These results indicate that an increase in particle size would be caused by aggregation of smaller particles. Finally, the average size led to 1.24 μm, suggesting that the final particles would consist of approximately 50 primary particles if they were the particles at 5 min.

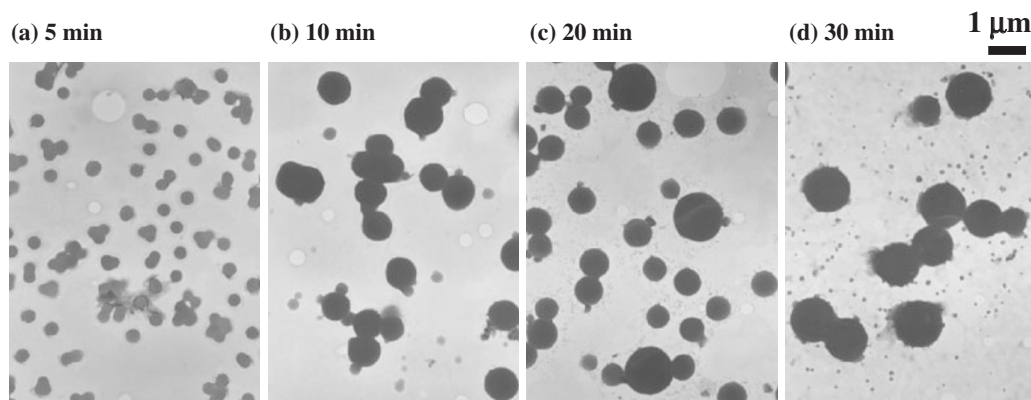


Figure 6. TEM images of PNIPAM hydrogel particles prepared by precipitation polymerization as a function of polymerization time.

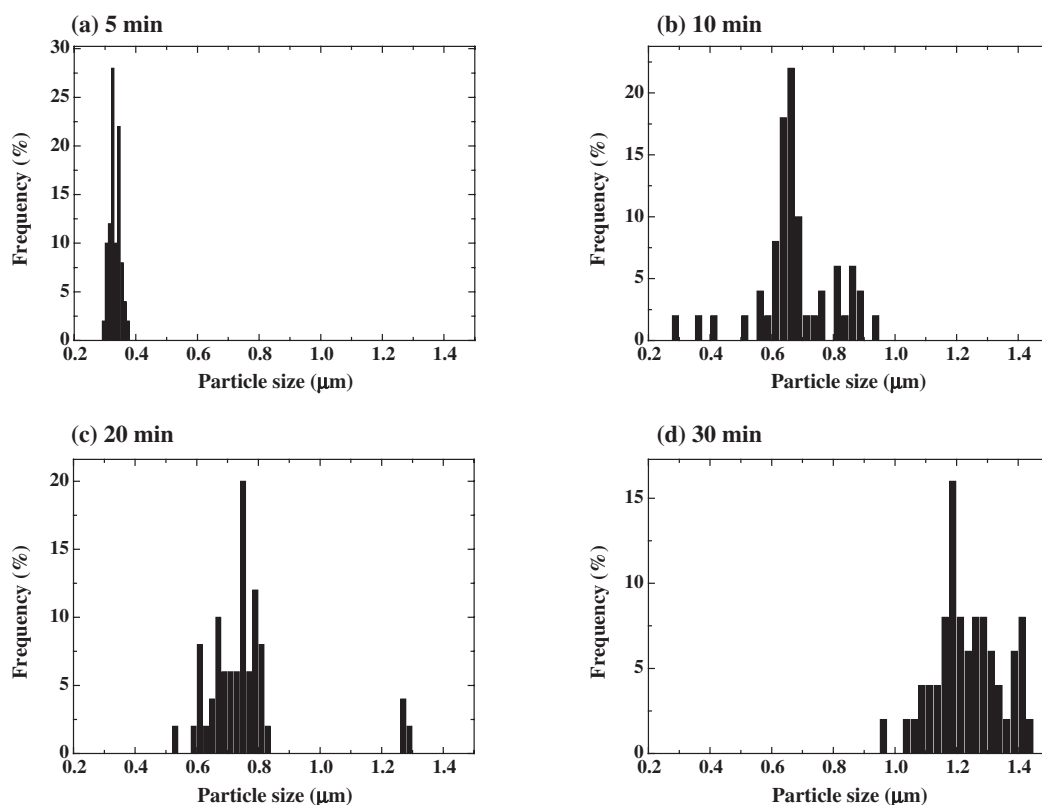


Figure 7. Particle size distribution for precipitation polymerization of NIPAM with MBAAm in the presence of 1.6 wt % NaCl concentration as a function of polymerization time.

This is probably due to reduced colloidal stability at higher ionic strength. These particle-particle interactions may be relatively weak because larger PNIPAM particles have a high swelling capacity as shown in Figure 5.

Effect of Injection Stage

In order to investigate the effect of an electrolyte on particle formation and growth, PNIPAM hydrogel particles were prepared by varying the conversion at NaCl injection. Figure 8 shows the TEM images of PNIPAM particles prepared by an injection of the electrolyte at different conversions. NaCl

concentration was kept constant at 1.6 wt %. In the above, the electrolyte was in the polymerization media before starting polymerization, namely conversion was 0%. Size distribution of the particles obtained was relatively narrow in spite of the injection conversion. At lower injection conversions (< 35%), micrometer-sized PNIPAM particles were obtained. But, particle size decreased as the injection conversion was higher. These results indicate that an electrolyte should be injected at the early stage of polymerization to prepare micrometer-sized PNIPAM particles. In other words, an electrolyte cannot make mature particles larger.

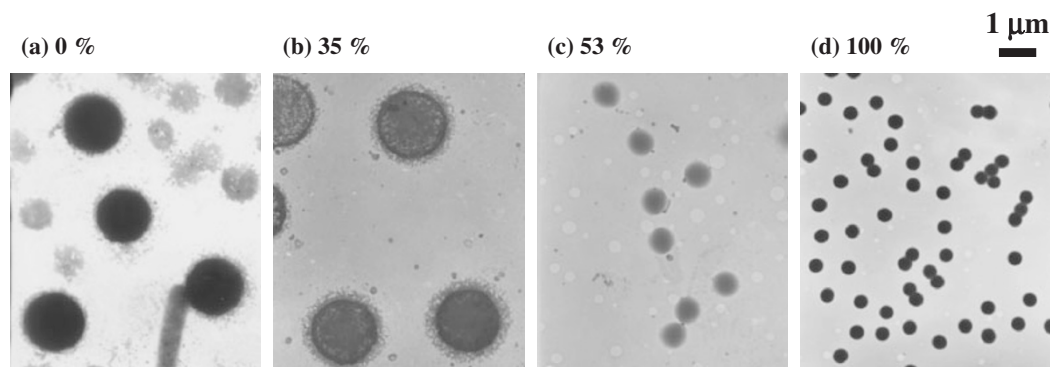


Figure 8. TEM images of PNIPAM hydrogel particles prepared by varying the conversion at NaCl injection.

DISCUSSION

Particle Formation Mechanism

Micrometer-sized PNIPAM hydrogel particles have been successfully prepared by aqueous precipitation polymerization of NIPAM with MBAAm in the presence of an electrolyte. The particle size can be controlled in the range of 0.55 to 1.60 μm by altering the electrolyte concentrations. It has already been reported that the average particle size increased with an increase in the concentration of salt in emulsion polymerization.^{28–31} Tanrisever *et al.* proposed that both rates of nucleation and aggregation are involved in determining the final particle size. This mechanism is probably applicable to precipitation polymerization. We will then discuss the mechanism for particle formation and growth in aqueous precipitation polymerization of NIPAM at different electrolyte concentrations.

NIPAM monomer is initially dissolved in distilled water to form a homogeneous solution. Polymerization reaction is started by the addition of a water-soluble initiator (KPS in the present study) and then the polymer chains propagate. The polymer chains keep growing until they reach a critical length. We consider that an electrolyte little affects the polymer chain growth process although an electrolyte may reduce the water solubility of NIPAM. When the growing chains reach their critical length, the chains precipitate and agglomerate each other to form primary particles. These particles are electrostatically stabilized by sulfonic acid groups. Accordingly, the increased electrolyte concentrations caused the decreased electrostatic stabilization of primary particles or precipitated chains, resulting in facilitated aggregation. The aggregation process would be affected by difference in an electrolyte concentration. At higher electrolyte concentrations, the precipitated chains are prone to aggregate, resulting in larger particle size. In the present system, the electrolyte concentration injected into a polymerization medium was not so much high and thus the LCST of the growing polymer chains was independent of an electrolyte concentration. Figure 8 also shows that the electrolyte concentrations would not be high

enough to aggregate PNIPAM mature particles, suggesting that aggregation reaction of smaller particles would be induced by precipitated PNIPAM chains. The precipitated chains may cause the particles to aggregate due to a bridging flocculation process. Van der Waals interactions or hydrophobic interactions may be involved for the interactions between the precipitated polymer chains and PNIPAM particles. In summary, the higher the electrolyte concentration is, the larger the particle size is.

High Swelling Mechanism

The volume of the conventional PNIPAM particles, which were prepared in pure water, increased approximately 10 times when temperature was decreased from 40 $^{\circ}\text{C}$ to room temperature. On the other hand, micrometer-sized thermosensitive particles swelled about 40 times with a decrease in temperature. Swelling ratio is known to be dependent upon the concentration of cross-linker, with a decrease in cross-linker concentration resulting in an increased swelling ratio.²³ In the present study, however, the cross-linker concentration was constant. Let us consider now the reason micrometer-sized PNIPAM particles have a higher swelling capacity. The above particle formation and growth mechanism indicate that larger PNIPAM particles are the assemblies of nanometer-sized smaller particles. This is supported experimentally by the results in Figure 6. Smaller particles would be linked each other through non-covalent forces (Van der Waals interactions or hydrophobic interactions) and these particle-particle interaction would be relatively weak. As a result, larger PNIPAM particles have a higher swelling capacity compared to smaller ones. In addition, MBAAm is consumed more quickly than NIPAM and thus the particles obtained are unlikely to have a uniform composition.³² This may be concerned with high swelling capacity. But, micrometer-sized PNIPAM hydrogel particles have a very quick response to temperature changes because the size of PNIPAM hydrogel particles is relatively small. Heterogeneous swelling/deswelling dynamics, then, could not be observed by measurement with dynamic light scattering. Future work will be devoted to analyze the particle internal structure.

CONCLUSIONS

Aqueous precipitation polymerization of NIPAM with MBAAm by varying electrolyte NaCl concentration leads to the following conclusions:

1. Particle size can be controlled by varying electrolyte concentrations in the range of 0.55 to 1.60 μm , indicating that micrometer-sized PNIPAM particles can be obtained by the addition of an electrolyte in polymerization media.
2. Micrometer-sized PNIPAM particles have a higher swelling capacity compared to conventional nanometer-sized PNIPAM particles. This would be because larger PNIPAM particles are the assemblies of nanometer-sized smaller particles linked each other through weak non-covalent forces.

Received: February 12, 2009

Accepted: June 8, 2009

Published: July 23, 2009

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