Analysis of the Formation Mechanism for Thermoresponsive-Type Coacervate with Functional Copolymers Consisting of *N*-Isopropylacrylamide and 2-Hydroxyisopropylacrylamide

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We now report the formation mechanism of the thermoresponsive-type coacervate with the novel functional temperature-sensitive polymer, poly(*N*-isopropylacrylamide-*co*-2-hydroxyisopropylacrylamide) (poly(NIPAAm-*co*-HIPAAm)), synthesized in our laboratory. The effects of introducing the hydrophilic comonomer (HIPAAm) into the copolymer chains and adding salts on the behaviors of the coacervate droplets induced in the poly(NIPAAm-*co*-HIPAAm) aqueous solutions were investigated. Not only the particle sizes of the coacervate droplets but also the cloud points of the copolymer solutions could be modulated by the HIPAAm content incorporated in the copolymers. Moreover, the particle sizes of the coacervate droplets were also changed by adding salts. Namely, the particle sizes increased with the decreasing HIPAAm composition and increasing NaCl concentration. In addition, the ^{1}H NMR and differential scanning calorimetric measurements suggested that as the HIPAAm content decreased or NaCl concentration increased, dehydration of the copolymers induced in the phase transition and/or separation became much easier. Therefore, on the basis of the findings obtained from these measurements, we determined that the particle sizes of the coacervate droplets induced in the temperature-sensitive polymers increased as the number of the water molecules, which are dissociated from the polymeric chains during the phase transition and/or separation, increased. Besides, to examine the separation of the model solutes, the aqueous two-phase separation with the coacervate droplets of poly(NIPAAm-*co*-HIPAAm) was carried out. The partitions of Methyl Orange as a model solute under both acidic (pH 2) and basic (pH 12) conditions were performed. The amount of Methyl Orange partitioned into the coacervate droplets at pH 12 is much greater than that at pH 2, which indicated that the coacervate droplets could recognize a slight difference in the polarity or structure between the model solutes.

Introduction

Numerous studies have already been devoted to stimuli-responsive materials, which respond to external stimuli such as temperature,[1−3] light,[4−6] electrical potential,[7] stretching,[8,9] solvent,[10] pH,[11−14] or chemical species,[15−17] in terms of both fundamental and practical aspects. Poly(*N*-isopropylacrylamide) (PNIPAAm) is well-known as a thermoresponsive polymer and has been applied in a variety of areas because of its high potential.[18−20] PNIPAAm gives rise to a coil-to-globule transition and undergoes exquisite dehydration behavior at its lower critical solution temperature (LCST).[21] In temperature-sensitive polymers, however, there are not only the polymers on the order of PNIPAAm showing a coil−globule transition but also the ones exhibiting a thermoresponsive-type coacervation accompanied by the liquid−liquid phase separation. Coacervation is a phase separation phenomenon which the colloid causes and can be observed by the formation of droplets comprised of a concentrated polymer phase. Coacervate is very valuable in bioscience due to its potential for the purifications of bioactive molecules, such as nucleic acids and proteins, without losing their functions. In case of temperature-sensitive polymers, coacervate droplets are formed above a certain temperature corresponding to the cloud point and vanish below the cloud point. Because this process is reversible and coacervate droplets are repeatedly formed by only modulating the temperature, intelligent polymers causing thermoresponsive-type coacervation are quite attractive. Although examples of reports concerning these kinds of polymers seem to be comparatively few, they have been investigated and reported. For instance, it has been reported that some biomacromolecules, such as elastin, show coacervation in response to temperature.[22,23] Moreover, synthetic polymers, such as poly(*N*,*N*-dimethylacrylamide-*co*-*N*-phenylacrylamide) and poly(*N*-vinylamide-*co*-vinyl acetate), have also been demonstrated to exhibit temperature-induced coacervate droplets.[24,25] In addition, Sugihara et al. synthesized three types of copolymers consisting of isobutyl vinyl ether and 2-hydroxyethyl vinyl ether by living cationic polymerization.[26] They are random copolymers, diblock copolymers, and “*block-and-random*” copolymers and demonstrated different behaviors in aqueous media. The random copolymers caused sharp phase separation behaviors and exhibited coacervate droplets above the cloud points. In contrast, “*block-and-random*” copolymers did not show clear phase separation behaviors; that is, their thermoresponses were dull and they demonstrated significant

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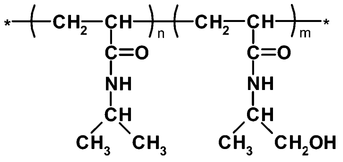


Figure 1. Chemical structure of poly(NIPAAm-*co*-HIPAAm).

hystereses during a heating and cooling process. Additionally, the diblock copolymers formed micelle-like structures and did not reveal a temperature response, which implied that the copolymers did not show a thermoresponsive-type coacervation. Accordingly, they suggested that random sequences of comonomers in each copolymer chain were essential to undergo a refined phase separation behavior.

Previously, we demonstrated that the copolymers obtained by the free radical copolymerization of NIPAAm with a novel functional monomer, 2-hydroxyisopropylacrylamide (HIPAAm) synthesized in our laboratory, produced very clear thermoresponsive properties in aqueous media despite including many hydrophilic groups (hydroxyl groups) on the side chains.[27,28] We insist that this interesting feature is due to the catenative structure of the isopropylacrylamide group and the similarity of the monomer reactivity ratios due to the similarity of the chemical structures of NIPAAm and HIPAAm (Figure 1). Furthermore, it has been revealed that poly(NIPAAm-*co*-HIPAAm) with a high HIPAAm unit content exhibits a thermoresponsive-type coacervation.[27] The particle sizes and the size distributions of coacervate droplets induced in poly(NIPAAm-*co*-HIPAAm) solutions in the absence of salts were small and comparatively monodispersed, even though the copolymers were prepared by free radical copolymerization. Typically, most of the coacervate droplets already reported are highly polydispersed in addition to their sizes of several tens or hundreds micrometer scale.[24,25,29] In addition, it seems difficult to regulate the size distribution of the coacervate droplets by only controlling the molecular weight distribution via living polymerization.[30] Thus, we consider coacervate droplets of poly(NIPAAm-*co*-HIPAAm) to be remarkably useful in certain applications, especially biotechnology such as the purification of bioactive molecules.

In this paper, we report the formation mechanism of the thermoresponsive-type coacervate for temperature-sensitive polymers using poly(NIPAAm-*co*-HIPAAm). The effects of the hydrophilic comonomer content and adding salts were investigated, and we additionally examined the correlativity between the temperature-induced phase separation behavior and the formation of coacervate. The studies concerning the physical property of thermoresponsive-type coacervate are still insufficient and further investigation is desired. In addition, the aqueous two-phase separation with coacervate droplets was carried out for the purpose of the separation and purification of model solutes.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAAm) was kindly provided by Kohjin (Tokyo, Japan) and purified by recrystallization from a mixture of benzene and hexane. The synthetic procedure of 2-hydroxyisopropylacrylamide (HIPAAm) was described in detail in our previous paper.[27] The copolymers used in this study were NH-30, NH-40, and NH-50, where NH-X denotes the copolymers containing X mol % of the HIPAAm unit. They were synthesized by free radical copolymerization and these methods were also previously described.[27] The HIPAAm contents introduced into the copolymers were determined by ^{1}H NMR spectroscopy (JEOL JNM-GSX400, 400 MHz spectrometer). The number-average molecular weights and the molecular weight distributions of the copolymers determined by gel permeation chromatography (GPC, Jasco LC-2000 Plus, Tokyo, Japan) were approximately $2.0-4.0 \times 10^4$ and 2.0−3.0, respectively. Methyl Orange was obtained from Wako Pure Chemical Industries (Tokyo, Japan) and used without further purification, which was employed as a model solute for the aqueous two-phase separation.

Dynamic Light Scattering and Transmittance Measurements of the Copolymer Aqueous Solutions. Dynamic light scattering (DLS) measurements were performed using a light scattering instrument (FPAR-1000HL, Otsuka Electronics Co., Ltd., Osaka, Japan) in order to estimate the particle sizes of the coacervate droplets induced in the poly(NIPAAm-*co*-HIPAAm) aqueous solutions. The copolymers were dissolved in ultrapure water and the sample solutions were prepared at a concentration of 0.10 w/v %. The copolymer solution measurements were then carried out above the cloud points, which were determined by transmittance measurements using a UV−vis spectrometer (Jasco V-550 spectrometer, Tokyo, Japan) equipped with a temperature controller.

Microscopic Observation. Optical images of the copolymer solutions were recorded below and above the cloud points of each copolymer using a Nikon ECLIPSE TS 100 microscope equipped with Digital Sight DS-5M, Nikon (Tokyo, Japan).

Analysis of Dehydration Behaviors of the Copolymer Solutions. To analyze the dehydration behaviors of the copolymers, ^{1}H NMR spectroscopy and differential scanning calorimetry (DSC, DSC 6100, Seiko Instruments, Tokyo, Japan) were used. The ^{1}H NMR measurements were performed as follows: The copolymer (NH-50) was dissolved in deuterium oxide (D_2O, Wako Co., Tokyo, Japan, 99%) at a concentration of 1.0 w/v %, and 3-(trimethylsilyl)propionic acid-d_4 sodium salt (Aldrich Chem. Co.) was added to the copolymer solutions as an internal standard. We then measured the ^{1}H NMR spectra of the sample solutions at 25 °C (below the cloud points) and 10 °C higher than each cloud point of the copolymer solutions (above the cloud points).

For the DSC analysis, the measurements were carried out in the temperature ranges of 0−120 °C while heating. The copolymer (NH-50) was dissolved in ultrapure water or a 1.0 M NaCl aqueous solution, and the sample solutions were prepared at a concentration of 10 w/v %. These copolymer solutions were placed in silver pans, which were then completely sealed to prevent the water from evaporating. The measurements were performed at the scanning rate of 2.0 °C/min.

Aqueous Two-Phase Separation with Coacervate Droplets of Poly(NIPAAm-*co*-HIPAAm). The copolymer (NH-50) was dissolved in 1.0 M NaCl aqueous solutions whose pH values were adjusted to pH 2 and pH 12 using hydrochloric acid and sodium hydroxide, respectively, and Methyl Orange (0.010 mM) as a model solute was added to the copolymer solutions. These sample solutions were incubated above the cloud points (50 °C) for 30 min and then separated into the two phases by centrifuging at 15000 rpm for 10 min. Subsequently, we determined the absorbance of the upper aqueous phase using a UV−vis spectrometer (Jasco V-550 spectrometer, Tokyo, Japan) to estimate the quantity of Methyl Orange enriched in the coacervate phase (lower phase).

Results and Discussion

Time Dependency for the Formation of Coacervate Droplets Induced in Poly(NIPAAm-*co*-HIPAAm) Aqueous Solution by Temperature. In general, the particle sizes of coacervate droplets become larger via a coalescence mechanism with increasing time and then precipitate to the bottom. First, using a DLS, we examined the time dependency of the hydrodynamic diameters of the coacervate droplets induced in poly(NIPAAm-*co*-HIPAAm) solutions by temperature (Figure

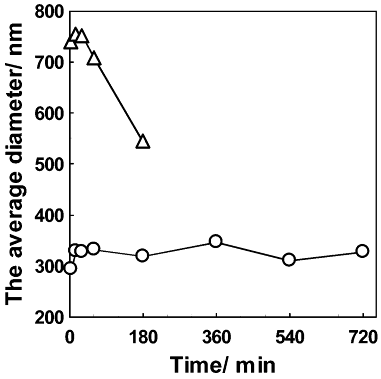


Figure 2. Time dependency for particle sizes of coacervate droplets induced in NH-30 aqueous solutions by temperature. The circle (○) and triangle (△) symbols denote the average diameters of coacervate droplets in the absence and presence (0.3 M) of NaCl, respectively. The DLS measurements were performed above the cloud points of the copolymer solutions (polymer concentration, 0.10 w/v %).

2). As for the NH-30 aqueous solution in the absence of salts, the diameter of the coacervate droplets was almost constant even after 12 h. On the other hand, in the NH-30 solution containing salts (0.3 M), although the diameter only slightly changed up to 1 h, it decreased at 3 h, and with more time, it was impossible to determine the particle size due to a decrease in the light scattering intensity of the copolymer solution. It seems that a decrease in the diameter at 3 h resulted from the selective precipitation of the coacervate droplets which became greater by coalescence. A decrease in the light scattering intensity of the copolymer solution is also due to precipitation of the coacervate droplets (in other words, precipitation of the polymeric chains). By the way, a decrease in the light scattering intensity was hardly observed in the NH-30 aqueous solution without adding salts. These results suggest a good dispersibility and stability of the coacervate droplets induced in the poly(NIPAAm-*co*-HIPAAm) solutions, and it was realized that the droplets tend to precipitate by adding salts.

HIPAAm Composition and Salt Concentration Dependency of Coacervate Droplet Particle Size. Figure 3a shows the transmittance curve and DLS histogram of the NH-30 solution, and Figure 3b indicates the hydrodynamic diameters of the coacervate droplets and the cloud points of the copolymer aqueous solutions as a function of the HIPAAm content. A single peak was observed in the DLS histogram and the size distribution was comparatively monodispersed. Moreover, the particle sizes of the coacervate droplets were very small in each copolymer. Besides, it became clear that the particle size and the cloud point significantly depended on the HIPAAm composition; i.e., the particle size decreased and the cloud point shifted to a higher temperature with the increasing HIPAAm content. An increase in the cloud points of the copolymers is due to an increase in the hydrophilicity of the copolymers, and the consideration concerning a decrease in the particle size was discussed and is described below. Consequently, we demonstrated that the particle size of the droplets as well as the cloud point of the copolymer solution can be easily controlled by the contents of the HIPAAm comonomer in the acrylamide-type thermoresponsive polymers.

Figure 4b shows a micrograph of the NH-50 aqueous solution above the cloud point. Although the copolymer aqueous solution became opaque above the cloud point, coacervate droplets could not be observed using an optical microscope (magnification; 400×), indicating that the droplets induced in the NH-50 aqueous solution are too small to be visually observed. (Indeed, as shown in Figure 3b, the particle size of the coacervate droplets induced in the NH-50 solution was several tens of nanometers.) However, we were successful in visualizing the coacervate droplets using a microscope by adding salts to the copolymer solutions (Figure 4c), which implied the possible increase in the particle size. We then investigated the salt concentration dependency of the coacervate droplet hydrodynamic diameters and plotted the particle sizes as a function of the NaCl concentration (Figure 5). From Figure 5, we found that the particle size of the droplets increased with the increasing NaCl concentration. The discussion concerning these results is also described below.

Correlation between the Particle Size of Coacervate Droplets and Dehydration Behavior Induced in the Phase Separation. On the basis of the results obtained from Figure 3b and Figure 5, it was found that the particle sizes of the coacervate droplets induced in the poly(NIPAAm-*co*-HIPAAm) aqueous solutions increased with a decrease in the HIPAAm composition and an increase in the NaCl concentration. As already shown in Figure 3b, as the HIPAAm composition decreases, the cloud points of the copolymer solutions shifted to lower temperature. In addition, it has been exhibited that the cloud points of the copolymers similarly decreased with the increasing NaCl concentration, which could be due to the salting-out effect.[27] That is, these tendencies, such as a decrease in the HIPAAm content and an increase in the NaCl concentration, lead to a cloud point decrease. Previously, we indicated that the endothermic enthalpy, due to dehydration which the

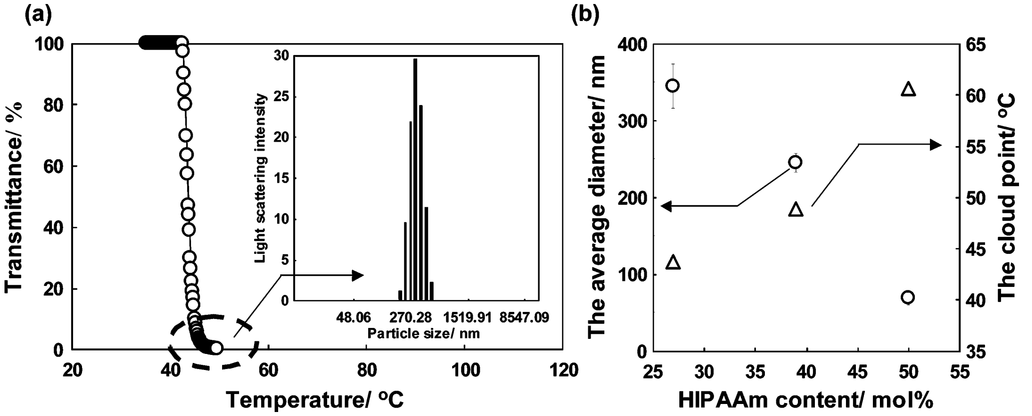


Figure 3. (a) Transmittance change in NH-30 aqueous solution (0.10 w/v %) as a function of temperature. The inset shows the dynamic light scattering measurement of NH-30 solution above the cloud point. (b) Particle sizes of coacervate droplets and the cloud points for poly(NIPAAm-*co*-HIPAAm) aqueous solutions (0.10 w/v %) with various HIPAAm compositions. Particle sizes were determined by DLS measurements above each cloud point of the copolymer solutions, and the cloud points were determined by UV−vis measurements.

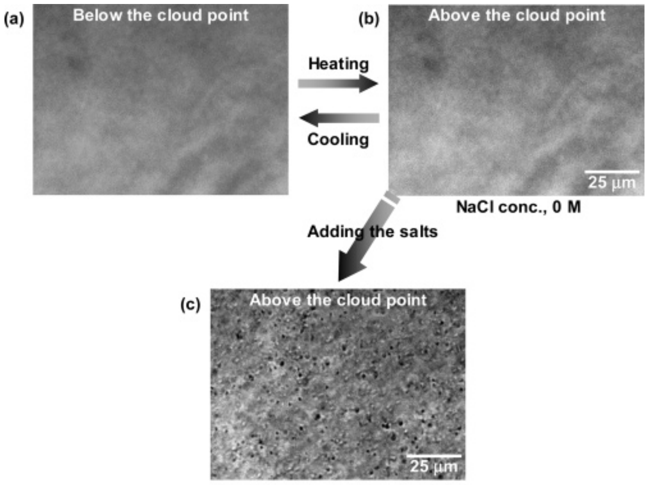


Figure 4. Micrographs of NH-50 aqueous solutions (0.10 w/v %) (a) below and (b, c) above each cloud point. The NaCl concentrations of the copolymer solutions were (b) 0 M and (c) 1.0 M, respectively.

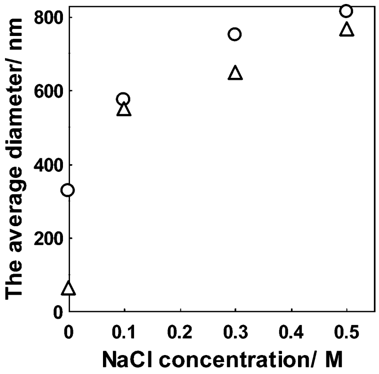


Figure 5. Particle sizes of coacervate droplets induced in poly(NIPAAm-*co*-HIPAAm) aqueous solutions (0.10 w/v %) by temperature as a function of NaCl concentration. The circle (○) and triangle (△) symbols denote the particle sizes of coacervate droplets induced in NH-30 and NH-50 solutions, respectively. The DLS measurements were performed above each cloud point of the copolymer solutions.

polymeric chains cause during the phase transition and/or separation, increased as the cloud points shifted to lower temperature,[28] and similar results have been reported by other research groups.[31,32] For this reason, we considered that there should be a dependence between the particle size of the coacervate droplets and dehydration behavior induced during the phase separation. We then examined the dehydration behavior of poly(NIPAAm-*co*-HIPAAm) in aqueous media using DSC and ^{1}H NMR. As for the ^{1}H NMR measurements, because the polymeric chains are capable of dissolving in deuterium oxide (D_2O) below the cloud points, the peaks derived from each of the copolymer protons are detected. Above the cloud points, however, the peak intensities of the copolymers might decrease or the peaks might be undetectable since dehydration of the polymer chains would occur to a greater or lesser degree. That being so, ^{1}H NMR spectroscopy informs us of the hydration states of the copolymers.

The HIPAAm composition dependence of the dehydration behavior for the poly(NIPAAm-*co*-HIPAAm) aqueous solutions has already been explored in our previous research.[28] Table 1 shows the endothermic enthalpy involving the phase transition and/or separation of the copolymers, which were obtained by DSC measurements. The endothermic enthalpy decreased with an increase in the HIPAAm composition, but they were too small to be estimated in the NH-50 and NH-80 aqueous solutions. These results strongly suggested that the number of water molecules dissociated from the polymeric chains decreases with the increasing HIPAAm composition. Additionally, in the ^{1}H NMR measurements, the drastic reductions of the peak intensities assigned to each proton of the copolymers were observed for the NH-0 (that is, PNIPAAm) and NH-10 solutions above each cloud point; however, although the peak intensities decreased to some degree, the peaks remained and could be observed in the copolymers containing more than 30 mol % HIPAAm units (i.e., NH-30, NH-50, and NH-80).[28] Hence, we demonstrated that the temperature-responsive polymers undergoing a coil−globule transition, such as PNIPAAm, give rise to an abrupt dehydration during the phase transition, while the polymers inducing coacervation show a partial dehydration and somewhat solvate even above the cloud points. Furthermore, the peak intensities of the copolymers above each cloud point became larger as the HIPAAm content increased, indicating that dehydration of the copolymer chains became insufficient with the increasing HIPAAm content. These results were consistent with the ones obtained by the DSC measurements.

Table 1. Enthalpy of Transition for Poly(NIPAAm-*co*-HIPAAm) Aqueous Solutions (10 w/v %) Determined by DSC Measurements

code	enthalpy of transition (kJ/unit-mol)
NH-0	5.75
NH-10	4.52
NH-30	1.86
NH-50	*a*
NH-80	*a*

[a] The enthalpy of transition in NH-50 and NH-80 was too small to be estimated.

Figure 6 shows the ^{1}H NMR spectra of the NH-50 solutions in the absence and presence of salts below and above each cloud point of the polymer solutions. In both polymer solutions, the peaks assignable to each proton of the copolymer remained despite heating to each cloud point; however, the remaining peaks for the copolymer solution in the absence of salts were larger than those in the polymer solution containing salts. We could also confirm that there was no difference in the ^{1}H NMR spectrum between those in the absence and presence of salts below the cloud points (Figure 6, parts a and c). Consequently, we determined that the dehydration of the polymer chains in the phase transition and/or separation is promoted by adding salts. Figure 7 indicates the DSC thermograms of the NH-50 aqueous solutions in the absence and presence of salts. The endothermic peak due to dehydration of the copolymer chains could not be observed in the NH-50 aqueous solution without adding salts (Figure 7a); on the other hand, the peaks were capable of being confirmed in the polymer solution containing salts (Figure 7b). These results also mean that dehydration, which occurs with the polymeric chains in the phase transition and/or separation, becomes easier to induce with the increasing NaCl concentration. This is in agreement with the results of ^{1}H NMR measurements (Figure 6). Therefore, the salting-out effect by adding salts promotes dehydration from the polymeric chains during the phase transition and/or separation.

Dehydration Behaviors of the Polymeric Chains Have an Influence on the Coacervate Formation as Well as Thermoresponsive Property. Based on the results obtained by several measurements such as DSC, ^{1}H NMR, DLS, UV−vis, and the microscopic observations, we concluded that the copolymers containing a low HIPAAm unit content, for example, NH-10, produced a coil−globule transition, while the copolymers, whose comonomer contents are relatively high,

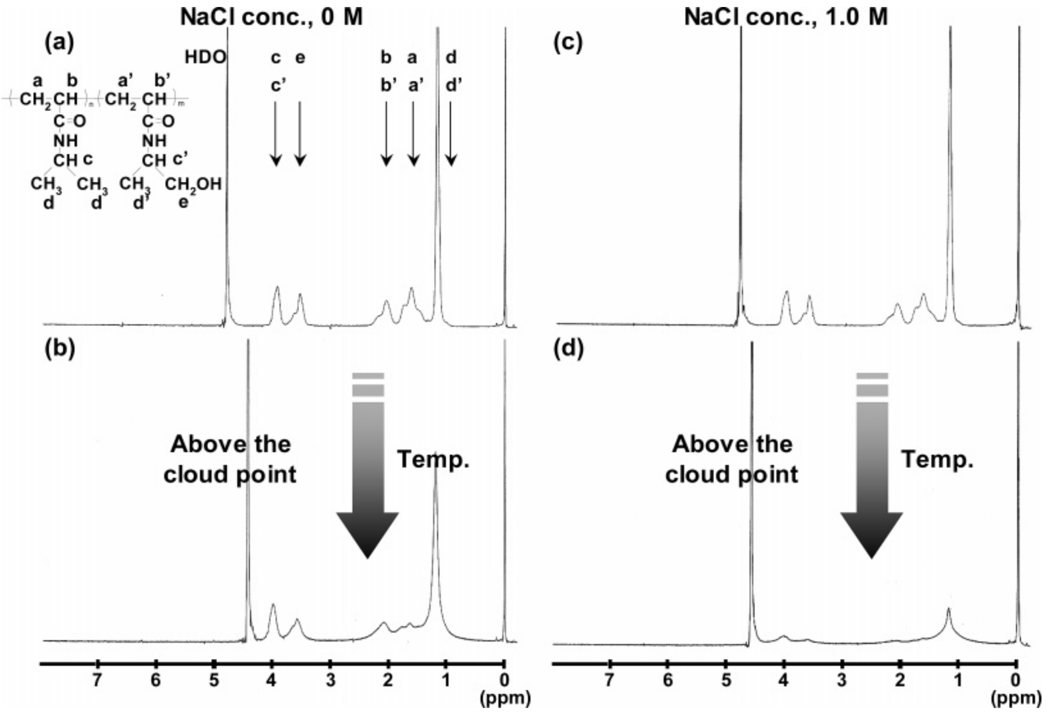


Figure 6. ^{1}H NMR spectra of NH-50 (1.0 w/v %) in D_2O. These measurements were performed at (a, c) 25 °C and (b, d) 10 °C higher than each cloud point of the copolymer solutions. The NaCl concentrations of the copolymer solutions are (a, b) 0 M and (c, d) 1.0 M, respectively.

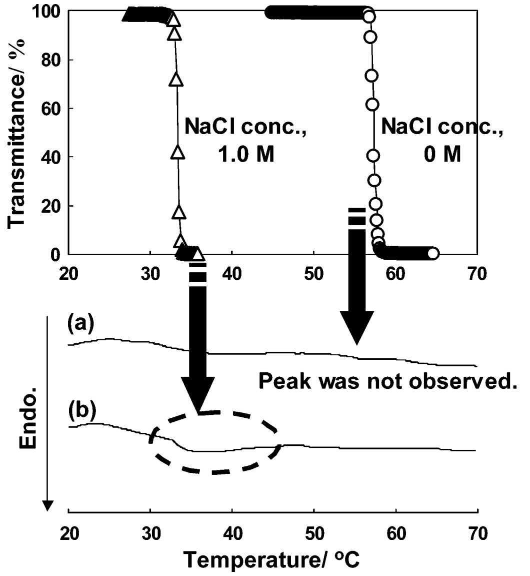
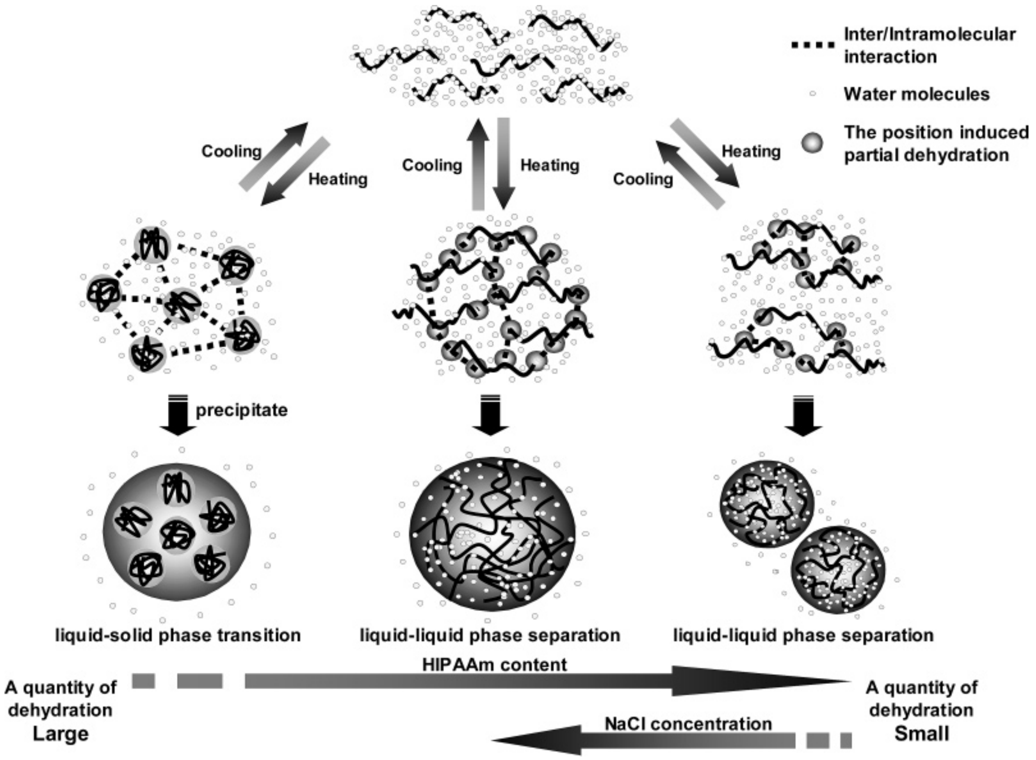


Figure 7. DSC thermograms of NH-50 (10 w/v %) aqueous solutions in the (a) absence and (b) presence of NaCl.

such as NH-30 and NH-50, give rise to coacervation in response to the temperature during the phase transition and/or separation.[27,28] In this study, furthermore, it was found that the particle size of the coacervate droplets increases as the HIPAAm content decreases or NaCl concentration increases. In addition, we suggested that the number of water molecules dissociated from the polymeric chains in the phase transition and/or separation increased with the decreasing HIPAAm content or increasing NaCl concentration. Based on these findings, we created the illustration as shown in Scheme 1, to demonstrate the correlation between the dehydration behaviors of poly(NIPAAm-*co*-HIPAAm) and the coacervate formation as well as the thermoresponsive behaviors of the copolymers in aqueous media. In Scheme 1, the number of water molecules disassociated from the polymeric chains decreases with heading for the right. The copolymers with a low HIPAAm (hydrophilic comonomer) composition cause a sharp dehydration behavior due to being comparatively hydrophobic. Since a gain of entropy due to the release of structured waters existing around the hydrophobic moieties satisfactorily compensates for the loss of entropy arising from the collapse of the polymeric chains, the polymers undergo a coil−globule transition.[32] Consequently, the collapsed chains aggregate with one another via a hydrophobic interaction, and the copolymers result in showing the liquid−solid phase transition. As the HIPAAm content increases, however, because hydrophilicity of the copolymers increases, the copolymers cause a partial dehydration, indicating that the number of structured waters released from the polymer chains decreases. For this reason, it becomes difficult to compensate for the loss of entropy due to the collapse of the polymer chains, and then the copolymers do not produce a coil−globule transition (i.e., intramolecular interaction becomes difficult to occur). However, since a partial dehydration occurs to a certain degree, the hydrophobic interactions among the hydrophobic moieties as well as the amide−amide and the amide−hydroxyl group hydrogen bonds occur at the dehydrated positions. In other words, the copolymer chains preferentially produce the intermolecular interactions among them by including a large quantity of water, resulting in the formation of the coacervate droplets. Thereby, the copolymers having a high content of HIPAAm units exhibit liquid−liquid phase separation involving coacervation. In this way, since the intermolecular interaction rather than the intramolecular one becomes dominant in the phase transition and/or separation with the increasing HIPAAm content, the cloud points of the copolymers with a high HIPAAm content are subject to the influence of the polymer concentration compared to those of the copolymers having a low HIPAAm content.[27] Moreover, with a further increase in the HIPAAm composition or decrease in NaCl concentration, partial dehydration becomes more difficult to induce, indicating that the probability of the intermolecular interactions among the polymeric chains decreases. As a consequence, the particle

Scheme 1. Schematic Illustration of Thermoresponsive Behavior and the Formation of Coacervate for Poly(NIPAAm-*co*-HIPAAm) in Water



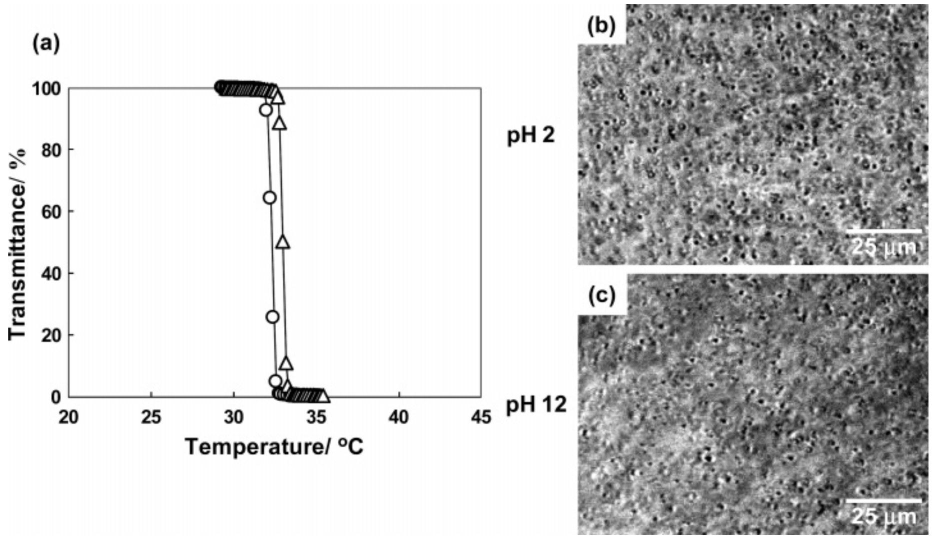


Figure 8. (a) Transmittance changes and (b, c) micrographs of coacervate droplets for NH-50 aqueous solutions (0.10 w/v %) containing NaCl (1.0 M). (a) The circle (○) and triangle (△) symbols show the transmittances at pH 2 and pH 12, respectively. (b, c) The microscopic observations were performed above the cloud points (50 °C) at (b) pH 2 and (c) pH 12.

size of the formed coacervate droplets becomes smaller with the increasing HIPAAm composition or decreasing NaCl concentration. Eventually, we clarified the correlation between the dehydration behaviors of poly(NIPAAm-*co*-HIPAAm) and the coacervate formation as well as thermoresponsive behaviors of the copolymers.

Aqueous Two-Phase Separation with Coacervate Droplets Induced in Poly(NIPAAm-*co*-HIPAAm) Aqueous Solution by Temperature. We also carried out the aqueous two-phase separation using coacervate droplets of poly(NIPAAm-*co*-HIPAAm). In this study, Methyl Orange was selected as the model solute. It is well-known as a pH indicator and its colored pH range is 3.1−4.4.[33] The aqueous two-phase separation was then performed under acidic (pH 2) and basic (pH 12) conditions in order to examine the difference in the amount of model solute enriched into the coacervate droplets by the difference in polarity or the chemical structure of the model solute.

Before the aqueous two-phase separation was carried out, the temperature-sensitive properties of poly(NIPAAm-*co*-HIPAAm) were estimated under acidic and basic conditions. Figure 8a shows the transmittance changes for the NH-50 aqueous solutions containing salts at pH 2 and pH 12, and Figure 8b and Figure 8c indicate the micrographs of the copolymer solutions at pH 2 and pH 12, respectively. A transmittance curve as well as the cloud point of the copolymer was not affected by the pH value, and the copolymers exhibited coacervate droplets in addition to similar particle sizes at both pH values. Thus, as might be expected, the thermoresponsive behaviors of poly(NIPAAm-*co*-HIPAAm) are not affected by the pH value because the copolymers are nonionic. Besides, we confirmed

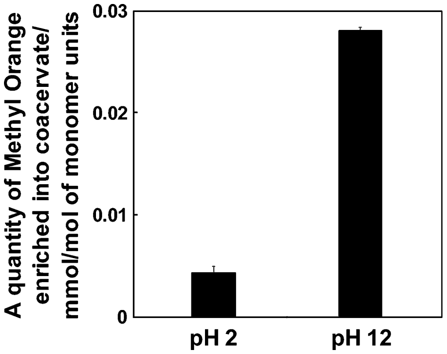


Figure 9. Quantity of Methyl Orange enriched into coacervate droplets induced in NH-50 aqueous solution (1.0 w/v %) containing NaCl (1.0 M) at pH 2 and pH 12. The initial concentrations of Methyl Orange were 0.010 mM at both pH values.

that the addition of Methyl Orange (0.010 mM) to the copolymer solutions did not influence the cloud point.

Figure 9 shows the amounts of the model solute enriched into the coacervate droplets at pH 2 and pH 12. The quantities of Methyl Orange partitioned into the coacervate droplets at each pH are significantly different from each other; that is, the amount at pH 12 is approximately 6.5 times greater than the one at pH 2. In other words, it could be possible for coacervate droplets induced in poly(NIPAAm-*co*-HIPAAm) solutions to recognize the slight difference in the polarity or structure between the model solutes and separate them. Besides this interesting property of coacervate droplets induced in poly(NIPAAm-*co*-HIPAAm), the droplets have small sizes and homogeneous size distributions. In addition, the particle size of the coacervate droplets as well as the cloud point can be controlled by the feed ratio of the HIPAAm. Accordingly, we suggest that the coacervate of poly(NIPAAm-*co*-HIPAAm) is of considerable use for applications in a variety of areas, such as biotechnology.

Conclusions

In this study, the thermoresponsive-type coacervate formation mechanism was investigated using poly(NIPAAm-*co*-HIPAAm). We demonstrated that both the hydrophilic comonomer contents in the copolymer chains and adding salts have a significant influence on the formation of the coacervate droplets. That is, the particle sizes of the droplets increased as the HIPAAm content decreased or the NaCl concentration increased. A decrease in the HIPAAm composition and an increase in the NaCl concentration tended to lower the cloud points of the copolymer solutions, and also made it easy for the water molecules existing around the copolymer chains to dissociate during the phase transition and/or separation, which was suggested by the DSC and ^{1}H NMR measurements. Hence, we revealed that the easier dehydration increases the sizes of coacervate droplets induced by the thermoresponsive polymers. In other words, it is demonstrated that the property of the thermoresponsive-type coacervate is governed by the dehydration behavior of the temperature-sensitive polymer.

The aqueous two-phase separation using Methyl Orange as a model solute was performed under acidic and basic conditions, making use of the coacervate droplets induced in the poly(NIPAAm-*co*-HIPAAm) solutions. Even though the cloud points of the copolymer solutions and particle sizes of the coacervate droplets were independent of the pH values of the solutions, the quantities of Methyl Orange enriched into the coacervate droplets under these conditions were noticeably and mutually different, suggesting the possibility of the high separative power of the coacervate droplets induced in poly(NIPAAm-*co*-HIPAAm) aqueous solutions by recognizing the slight difference in the polarity or chemical structure between the model solutes.

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