Hydroxylated Poly(*N*-isopropylacrylamide) as Functional Thermoresponsive Materials

Tomohiro Maeda,† Tomohide Kanda,† Yuuki Yonekura,† Kazuya Yamamoto,† and Takao Aoyagi*,†,‡

Department of Nanostructure and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, 1-21-40, Korimoto, Kagoshima 890-0065, Japan

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In this study, we developed a poly(*N*-isopropylacrylamide)-based thermoresponsive polymeric material with a high content of hydroxyl groups. We newly designed the functional monomer, *N*-(2-hydroxyisopropyl)acrylamide (HIPAAm), considering maintaining the continuous and repeated structure of the isopropylamide group after copolymerization and the monomer reactivity ratios. The thermoresponsive polymer was derived by conventional radical copolymerization of HIPAAm with *N*-isopropylacrylamide (NIPAAm) in high yield. Estimation of monomer reactivity ratios, r_1 and r_2, supported the almost random sequence of the comonomers. The obtained copolymers showed a very sensitive phase transition and/or separation in response to temperature in aqueous media although they have many hydrophilic parts, and their thermoresponsive behavior was not affected by the pH. Furthermore, the cloud points of these copolymers closely depended on the HIPAAm content and could be easily controlled by adding salts. HIPAAm is expected to regulate the phase transition and/or separation temperature of the NIPAAm-based copolymers while maintaining their desirable sensitive thermoresponse. Differential scanning calorimetric analysis showed that dehydration of the polymer chains occurring in phase transition became incomplete with increasing HIPAAm content. Moreover, it was found that poly(NIPAAm-*co*-HIPAAm) having a high content of the HIPAAm unit showed liquid−liquid phase separation involving coacervation. The sizes of the coacervate droplets were relatively monodisperse and very minimal. Poly(NIPAAm-*co*-HIPAAm) is valuable for use in biomedical fields such as bioseparation.

Introduction

Stimuli-responsive materials possess a high potential for biomedical use. Especially, temperature-responsive polymers have been expected to be a promising material for temperature-modulating controlled release, because of their sensitive phase transition behavior. Among these polymers, one of the most representative examples is poly(*N*-isopropylacrylamide) (PNIPAAm). PNIPAAm is hydrophilic and exists in a random coil in water below 31 °C, which corresponds to a lower critical solution temperature (LCST). Above the LCST, however, the PNIPAAm becomes hydrophobic and changes its conformation from a random coil to a globule, then aggregates due to the hydrophobic interaction among the isopropyl groups.[1−3] Because PNIPAAm shows a unique solution property in this way, it has been used in many biomedical fields.[4−8] To introduce functional groups onto the PNIPAAm chains, for example, for conjugation with bioactive molecules, conventional radical copolymerization is generally carried out using a functional comonomer such as acrylic acid.[9] However, the phase transition of the corresponding copolymer becomes insensitive with increasing comonomer content. We then considered that the continuous and repeated structure of the isopropylamide group is an important factor in achieving a sensitive phase transition. Therefore, we have newly designed a novel carboxylic monomer, *N*-(2-carboxyisopropyl)-acrylamide (CIPAAm), considering the continuous monomer alignment like a homopolymer structure.[10] As expected, poly-(NIPAAm-*co*-CIPAAm) as well as the corresponding hydrogels showed a very sensitive phase transition even under an isotonic condition at pH 7.4.[11,12] Moreover, Ebara et al. evidenced that the continuous and repeated structure of the isopropylamide group is very important to retain thermally sensitive properties in introducing the functional comonomer in comparing CIPAAm to 3-carboxyl-*n*-propylacrylamide (CNPAAm).[13] Additionally, to synthesize a cationic copolymer, we also designed *N*-(2-aminoisopropyl)acrylamide (AIPAAm) based on the same concept, and the preparation of poly(NIPAAm-*co*-AIPAAm) was reported.[14] As expected, poly(NIPAAm-*co*-AIPAAm) also demonstrated a clear phase transition. These observations strongly suggested that the same isopropylacrylamide groups, the contributed random sequence, and the resulting copolymer would maintain a sensitive phase transition, even though it contained many hydrophilic side chains.

To use thermoresponsive polymers in a biological system, their phase transition profile should be unaffected by the pH. However, because poly(NIPAAm-*co*-CIPAAm) and poly-(NIPAAm-*co*-AIPAAm) are electrically charged, the thermoresponse and cloud points of these copolymers are influenced by the pH in aqueous media. In this study, we designed a new monomer, *N*-(2-hydroxyisopropyl)acrylamide and prepared functional temperature-responsive materials by conventional radical copolymerization with NIPAAm. Because poly(NIPAAm-*co*-HIPAAm) is nonionic, their phase transition and/or separation behavior would not be affected by the pH.

Experimental Section

Materials. D,L-2-Amino-1-propanol and acryloyl chloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and used as received. Triethylamine was purified by distillation over KOH. 2,2′-

* Corresponding author. Phone/Fax: +81-99-285-7794. E-mail: aoyagi@eng.kagoshima-u.ac.jp.

† Kagoshima University.

‡ PRESTO, Japan Science and Technology Agency.

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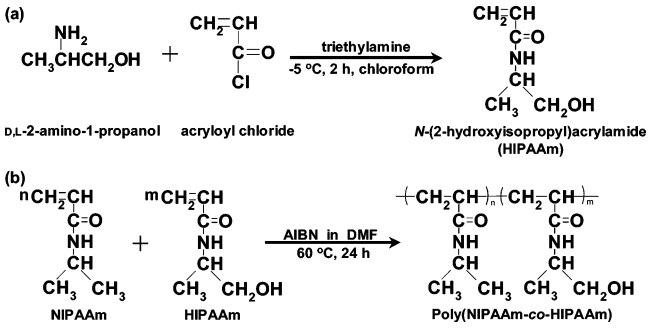
Scheme 1. Preparation of (a) HIPAAm Monomer and (b) Poly(NIPAAm-*co*-HIPAAm)



Azobis(isobutyronitrile) (AIBN) was obtained from Wako Pure Chemical Industries (Tokyo, Japan) and recrystallized from ethanol. *N*-Isopropylacrylamide was kindly supplied by Kohjin (Tokyo, Japan) and recrystallized from benzene−hexane. Other reagents and solvents were commercially available and used without further purification.

Synthesis of HIPAAm Monomer. D,L-2-Amino-1-propanol (0.15 mol) and triethylamine (0.15mol) were dissolved in anhydrous chloroform, and this solution was stirred at −5 °C under a nitrogen atmosphere for 20 min. Acryloyl chloride (0.15 mol) was slowly added to the solution under a nitrogen atmosphere, and the solution was then stirred at −5 °C for 2 h. The solution was evaporated, and the resulting viscous liquid was dissolved in 2-propanol. The mixture solution was kept at −20 °C for 24 h to precipitate triethylamine hydrochloride, and suction filtration was then carried out to remove the salts. The filtrate was concentrated and purified by column chromatography with ethyl acetate as an eluent. The HIPAAm monomer was obtained as a colorless viscous liquid (Scheme 1a). The yield was 72.6%. The chemical structure of HIPAAm was confirmed by ^{1}H NMR (JEOL JNM-GSX400, 400 MHz spectrometer); δ (D_2O, ppm) = 1.17 (d, 3H, CH_3CHCH_2OH), 3.61 (m, 2H, CH_3CHCH_2OH), 4.04 (m, 1H, CH_3CHCH_2OH), 5.78 (d, 1H, CH_2=C*H*), 6.22(m, 2H, CH_2=CH).

Preparation of Poly(NIPAAm-*co*-HIPAAm). NIPAAm, HIPAAm monomers and AIBN (0.1 mmol, 1.0 mol % relative to monomers) as an initiator were dissolved in DMF. After oxygen was removed thoroughly, polymerization was carried out at 60 °C for 24 h (Scheme 1b). We then removed unreacted monomers completely by dialysis against water at ambient temperature for 1 week. After lyophilization for 3 days, we obtained the copolymers as a white powder. The HIPAAm content in the copolymer was calculated by ^{1}H NMR. The number-average molecular weight (M_n) and polydispersity index (M_w/M_n) of the copolymers were determined by gel permeation chromatography (GPC, Jasco LC-2000 Plus, Tokyo, Japan). DMF containing 10 mM LiBr as an eluent and poly(ethylene glycol) were used as the standard for calibration. Table 1 shows the characterization of the obtained copolymers.

Measurements of Cloud Point. The cloud points of poly(NIPAAm-*co*-HIPAAm) aqueous solutions (1.0 w/v %) were determined by transmittance measurements using a UV−vis spectrometer (Jasco V-550 spectrometer, Tokyo, Japan) equipped with a temperature controller.

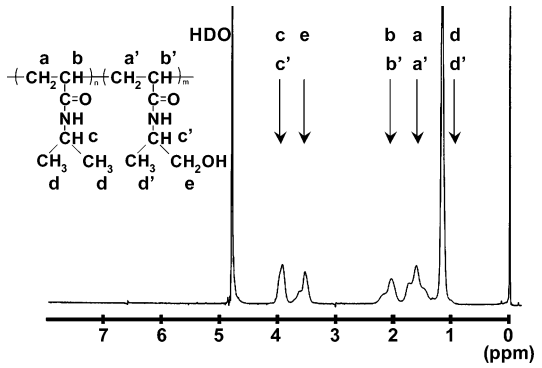


Figure 1. ^{1}H NMR spectrum of NH-50 in D_2O at room temperature: polymer concentration, 1.0 w/v %.

The transmittance of the copolymer solutions was recorded as a function of temperature. The temperature was raised at 1.0 °C/min, and the wavelength was fixed at 500 nm. The cloud points of the polymer solutions were defined as the temperatures when the transmittance was 50%.

Differential Scanning Calorimetry Measurements of Poly(NIPAAm-*co*-HIPAAm) Aqueous Solution. Differential scanning calorimetry (DSC, EXSTAR 6000, Seiko Instruments, Tokyo, Japan) was used to estimate the thermoresponsive profile of poly(NIPAAm-*co*-HIPAAm). DSC measurements were carried out between 0 and 120 °C at a scanning rate of 2.0 °C/min in the heating process. Copolymers with a variety of HIPAAm compositions were dissolved in water (10 w/v %). The obtained copolymer solutions were placed in silver pans, and these pans were completely sealed. We then carried out the DSC measurements.

Microscopic Observation of Poly(NIPAAm-*co*-HIPAAm) Aqueous Solution. Optical images of poly(NIPAAm-*co*-HIPAAm) aqueous solutions were recorded above and below each cloud point of the copolymers using a Nikon ECLIPSE microscope equipped with Digital Sight DS-5M, Nikon (Tokyo, Japan).

Dynamic Light-Scattering Measurements of Poly(NIPAAm-*co*-HIPAAm) Aqueous Solution. Dynamic light-scattering (DLS) measurements were carried out with a light-scattering spectrometer (FPAR-1000HL, Otsuka Electronics Co., Ltd., Osaka, Japan). The samples were measured above the cloud points of the copolymers at a concentration of 0.10 w/v %.

Results and Discussion

Characterization of Poly(NIPAAm-*co*-HIPAAm). Scheme 1 indicates the synthesis route of HIPAAm monomer and poly(NIPAAm-*co*-HIPAAm), and Table 1 summarizes the characterization of the copolymers. The HIPAAm contents of the resulting copolymers were almost the same as that in the feed. A typical ^{1}H NMR spectrum of the copolymer (NH-50) is shown in Figure 1. The copolymer composition was estimated by comparing the peak area of methine protons (c, c′) with methylene protons (e) in the same figure.

Table 1. Preparation of Poly(NIPAAm-*co*-HIPAAm)

	in feed (mol %)		in copolymer (mol %)[a]				
code	NIPAAm	HIPAAm	NIPAAm	HIPAAm	yield (%)	$M_n \times 10^{-4}$ [b]	M_w/M_n [b]
NH-0	100	0	100	0	94	2.2	2.9
NH-10	90	10	91	9	87	2.5	2.5
NH-20	80	20	78	22	96	2.8	2.5
NH-30	70	30	73	27	94	3.0	2.6
NH-40	60	40	61	39	92	3.6	2.5
NH-50	50	50	49	51	97	2.6	2.0
NH-80	20	80	18	82	90	3.3	2.2
NH-90	10	90	12	88	89	3.2	2.2
NH-100	0	100	0	100	89	4.3	1.8

[a] Determined by ^{1}H NMR. [b] Determined by GPC.

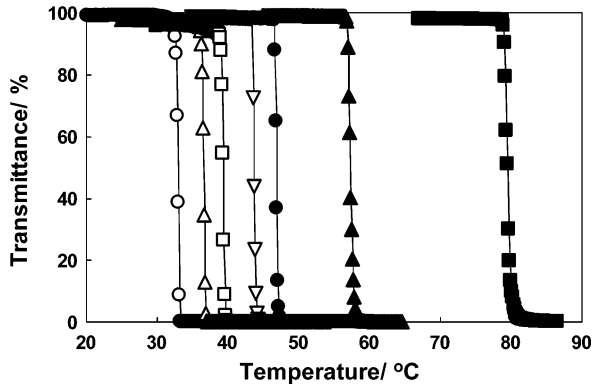


Figure 2. Transmittance changes for 1.0 w/v % aqueous solutions of poly(NIPAAm-*co*-HIPAAm) as a function of temperature: NH-0 (○), NH-10 (△), NH-20 (□), NH-30 (▽), NH-40 (●), NH-50 (▲), NH-80 (■).

Table 2. Temperature Ranges Showing Transmittance Changes in Poly(NIPAAm-*co*-HIPAAm)

code	temperature range (°C)[a]
NH-0	0.43
NH-10	0.64
NH-20	0.52
NH-30	0.36
NH-40	0.51
NH-50	0.90
NH-80	0.86

[a] Temperature range indicates the temperature difference between 90% and 10% in transmittance.

In our concept, the monomer alignment of the polymer chains and uniformity of the comonomer content are essential for preparing the functional polymer with a sensitive temperature response. Therefore, we confirmed the monomer reactivity ratios, r_1, r_2, of the NIPAAm (1) and HIPAAm (2). According to the Kelen−Tüdös method, the determined values were $r_1 = 1.08$, $r_2 = 0.60$, which indicated the highly random sequence of the comonomers in every copolymer chain and the homogeneity of the comonomer content. Furthermore, because the structure of the HIPAAm monomer is very similar to that of NIPAAm, poly(NIPAAm-*co*-HIPAAm) can retain the continuous and repeated structure of the isopropylamide group after copolymerization. Therefore, poly(NIPAAm-*co*-HIPAAm) is expected to exhibit a very sensitive thermoresponse. Previously, Xue et al. reported that the monomer reactivity ratios, r_1, r_2, of the NIPAAm (1) and acrylic acid (2) were 14.0 and 0.07.[15] That suggests that poly(NIPAAm-*co*-acrylic acid) would comprise a mixture of the copolymer with different contents of the comonomers, when the copolymer was obtained in high yield. A designed functional monomer such as HIPAAm would enable fabrication of acrylamide-based polymeric materials with well-defined structure and high yield.

Temperature-Responsive Property of Poly(NIPAAm-*co*-HIPAAm). We estimated the cloud points of the copolymers in water by means of transmittance measurements of the polymer aqueous solutions. Figure 2 shows the transmittance change in poly(NIPAAm-*co*-HIPAAm), and Table 2 presents the temperature range indicating the transmittance change in poly(NIPAAm-*co*-HIPAAm). All the copolymer solutions showed a very sensitive and discontinuous transmittance change in a distinctly narrow temperature range. It should be noted that even the copolymers with a high content of hydrophilic comonomer (HIPAAm) exhibited a clear transmittance change. It was found that the polymer chains would possess a strong intra- or intermolecular association force even in the copolymer containing a high content of hydroxyl groups. These results strongly suggested that three factors, (1) preserving the continuous and repeated structure of the isopropylamide group after copolymerization, (2) random monomer alignment of the polymer chains, and (3) uniformity of the comonomer content, are very important for constructing a functional polymer with a sensitive temperature response. As expected, poly(NIPAAm-*co*-HIPAAm) did not demonstrate pH dependency in the phase transition and/or separation, showing a sensitive thermoresponse under the condition of acidic or basic solution (data are not shown). Figure 3 shows the relationship between the cloud points of the copolymers and the HIPAAm content. The cloud points of the copolymer solutions shifted to a higher temperature with increasing HIPAAm content because the hydrophilicity of the copolymers increased. Moreover, on the basis of the results in Figure 3, it was revealed that the cloud points of the copolymers could be easily controlled by varying the feed ratio of the comonomers. Phase transition and/or separation temperature control is very important for drug delivery, as well as in other biomedical applications. It is well-known that the LCST of NIPAAm polymers can be easily modulated by means of copolymerization or addition of an additive such as surfactants, urea, and salts.[16−19] Hoffman's group has reported that NIPAAm copolymers with acrylamide-type comonomers could show a phase transition in a limited temperature range from 0 to 65 °C.[17] However, in this study, the NIPAAm copolymers having 80 mol % of HIPAAm monomers showed a sharp thermosensitivity at 80 °C. This suggests that the HIPAAm monomer is surely useful for controlling the cloud point of the acrylamide-based copolymers over a wide temperature range without losing their sensitive thermoresponse using only simple copolymerization.

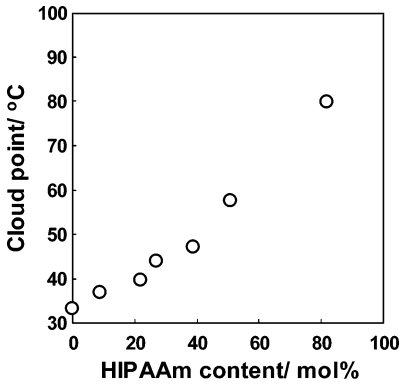


Figure 3. Cloud points for 1.0 w/v % aqueous solutions of poly(NIPAAm-*co*-HIPAAm) as a function of HIPAAm content.

In terms of the temperature-responsive property of the polymeric material with hydroxyl groups, Aoki et al. reported the preparation of an optically active *N*-(1-hydroxymethyl)-propylmethacrylamide polymer and its phase transition behavior.[20] According to their paper, the corresponding polymer demonstrated a very clear phase transition with hysteresis that meant that the transition behavior was different between the heating and cooling processes. This might be due to an inflexible main chain and an additional specific conformation based on the optical activity of the polymers. In our study, we used D,L-2-amino-1-propanol and acryloyl chloride as starting compounds; therefore, the poly(NIPAAm-*co*-HIPAAm) contained racemic propylamide groups as side chains and a more flexible acryloyl backbone. Actually, hysteresis was not observed under the measurement conditions in this study.

Salting-Out Effect on the Phase Transition and/or Separation Profile of Poly(NIPAAm-*co*-HIPAAm). It is well-

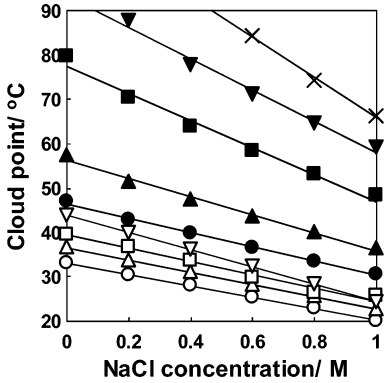


Figure 4. Cloud points for 1.0 w/v % aqueous solutions of poly(NIPAAm-*co*-HIPAAm) as a function of NaCl concentration: NH-0 (○), NH-10 (△), NH-20 (□), NH-30 (▽), NH-40 (●), NH-50 (▲), NH-80 (■), NH-90 (▼), NH-100 (×).

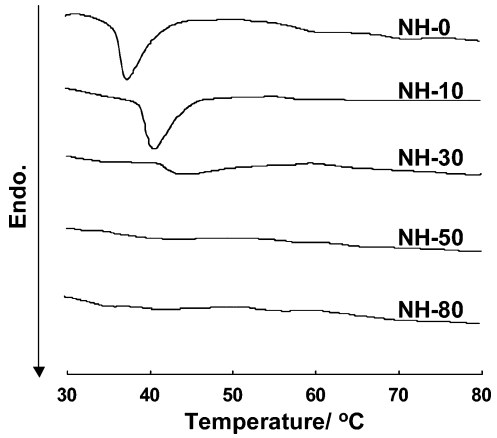


Figure 5. DSC curves for 10 w/v % aqueous solutions of poly(NIPAAm-*co*-HIPAAm).

known that cloud points of water-soluble polymers, for example, poly(ethylene glycol), are considerably influenced by adding salts.[16,21−24] Figure 4 shows the salting-out effect on the phase transition and/or separation behavior of poly(NIPAAm-*co*-HIPAAm). These results suggested that the cloud points of the copolymers also could be modulated by adding salts. From Figure 4, it was found that the copolymer showing a thermoresponse at ∼37 °C under isotonic condition ($I = 0.15$) was NH-20. Interestingly, even the HIPAAm homopolymer indicated a clear transmittance change on adding the salts. In all copolymers, the cloud points of the polymer solutions shifted to a lower temperature with increasing NaCl concentration, indicating that the hydrophobicity of the copolymers increased with increasing NaCl concentration due to the salting-out effect and would cause dehydration more easily. As seen in Figure 4, the salt-adding effect on the phase transition and/or separation profile of poly(NIPAAm-*co*-HIPAAm) becomes more sensitive with increasing HIPAAm content. These phenomena are very similar to that of the poly(ethylene glycol) aqueous system.[21−23]

Dehydration Occurred in the Phase Transition of Poly(NIPAAm-*co*-HIPAAm). To investigate the thermoresponsive profile of poly(NIPAAm-*co*-HIPAAm) in more detail, we evaluated the phase transition behavior of copolymers with differential scanning calorimetry. Figure 5 shows the DSC thermograms of copolymer aqueous solutions. In PNIPAAm and NH-10, NH-30, the endothermic peak due to dehydration of the polymer chains that occurred in the phase transition was observed at the temperature which corresponds to each cloud point of the copolymers determined by transmittance measurements. However, the endothermic peak gradually became smaller with

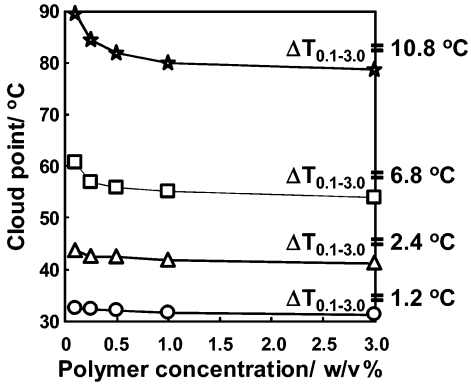


Figure 6. Cloud points for aqueous solutions of poly(NIPAAm-*co*-HIPAAm) as a function of polymer concentration: NH-0 (○), NH-30 (△), NH-50 (□), NH-80 (☆). $\Delta T_{0.1-3.0}$ indicates the difference of the cloud point between 0.1 and 0.3 w/v %.

increasing HIPAAm composition and could not be observed above NH-50. These results suggested that dehydration of the polymer chains occurring in the phase transition became insufficient with increasing HIPAAm content. An increment in the HIPAAm composition raises the hydrophilicity of the copolymers, and the interaction between the polymer chains and water becomes strong. Consequently, dehydration of the polymer chains would become more difficult with increasing HIPAAm content.

Polymer Concentration Dependency of Cloud Points in Poly(NIPAAm-*co*-HIPAAm) Aqueous Solutions. In the transmittance measurements, copolymers with a high content of HIPAAm showed a sensitive transmittance change, but the endothermic peak resulting from dehydration of the polymer chains was not shown in the differential scanning calorimetric analysis. It was then considered that copolymers with a high content of HIPAAm showed a liquid−liquid phase separation accompanied by coacervation. It is well-known that the cloud point of a thermoresponsive polymer which shows liquid−liquid phase separation involving coacervation depends on the polymer concentration.[25,26] Therefore, we examined the polymer concentration dependency of the cloud point in poly(NIPAAm-*co*-HIPAAm). As shown in Figure 6, the polymer concentration dependency of the cloud point became more predominant with increasing HIPAAm content. These results suggested that intermolecular interaction had begun to contribute to causing a thermoresponse with increasing HIPAAm composition, which demonstrated the possibility of coacervation in copolymers with a high content of HIPAAm. The cloud points of the thermoresponsive polymer showing a coil−globule transition such as the NIPAAm homopolymer seem to be relatively independent of polymer concentration. On the other hand, the copolymers causing coacervation would form coacervate droplets by association among the polymer chains; therefore, the cloud points of such copolymers would be affected by polymer concentration. On decreasing the polymer concentration, the cloud points of the copolymers shifted to a higher temperature because association among the polymer chains would become more difficult.

Thermoresponsive Coacervation of Poly(NIPAAm-*co*-HIPAAm). We performed microscopic observation of copolymer aqueous solutions to confirm the coacervation visually. Figure 7 shows the micrographs of NH-50 aqueous solution containing NaCl above and below the cloud point. From the observation, we could ascertain the presence of coacervate droplets above the cloud point. Coacervate droplets vanished as the temperature was lowered below the cloud point. However, the temperature was again raised above the cloud point, and the coacervate droplets then appeared again. This process was

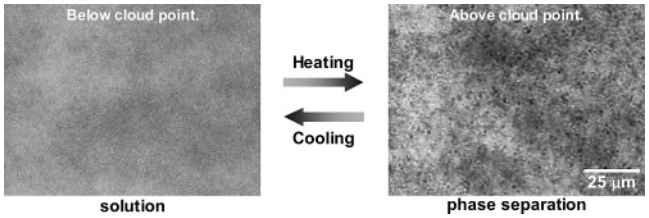


Figure 7. Micrograph of coacervate in NH-50 aqueous solution: polymer concentration, 0.10 w/v %; NaCl concentration, 0.50 M.

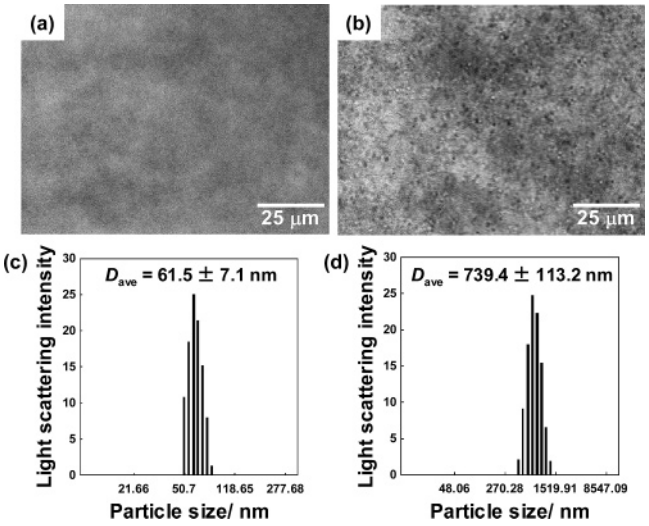


Figure 8. (a, b) Micrographs and (c, d) dynamic light-scattering measurements of 0.10 w/v % NH-50 aqueous solutions above the cloud points: NaCl concentration, (a, c) 0 M and (b, d) 0.5 M.

completely reversible. As well, we added NaCl to the copolymer aqueous solution in the microscopic observation; actually, we could not observe the coacervate droplets without adding the salt (Figure 8a). We then conducted dynamic light-scattering measurements and could confirm a single peak at ca. 60 nm diameter (Figure 8c). It is suggested that the formed coacervate droplets without adding the salts were very minimal and that the size of the coacervate droplets increased on adding the salts (Figure 8, parts b and d). As far as we know, the synthesized thermoresponsive polymers showing coacervate droplets having a size of several tens of nanometers have not been reported, and the majority of them are greater than micrometer scale.[25−28] It was noted that even though the poly(NIPAAm-*co*-HIPAAm) used in this study was prepared by free radical copolymerization, the size of the coacervate droplets formed above the cloud point was comparatively monodisperse. This seems to be attributed to three factors as mentioned above; therefore, the property of the produced coacervate droplets would become relatively uniform. These matters would be very valuable for applications. As a consequence, it became clear that poly(NIPAAm-*co*-HIPAAm) with a high content of HIPAAm units showed a liquid−liquid phase separation accompanied by coacervation. Coacervation is very attractive for separation or purification of bioactive molecules. The aqueous two-phase separation system with coacervate formation for dyes or proteins will be investigated in future studies.

Conclusions

We newly designed the novel monomer, *N*-(2-hydroxyisopropyl)acrylamide (HIPAAm), considering three factors: (1) preserving the continuous and repeated structure of the isopropylamide group after copolymerization, (2) random monomer alignment of the polymer chains, and (3) uniformity of the comonomer content. The functional temperature-responsive polymer, poly(NIPAAm-*co*-HIPAAm), was prepared by conventional free radical copolymerization with NIPAAm. As expected, the monomer reactivity ratios were almost the same value, and poly(NIPAAm-*co*-HIPAAm) showed a very sensitive and discontinuous transmittance change in aqueous media. In addition, the cloud points of the copolymers could be easily modulated by varying the feed ratio of the comonomers and adding salts. We found that poly(NIPAAm-*co*-HIPAAm) with a high content of the HIPAAm unit showed a liquid−liquid phase separation accompanied by coacervation. Even though the poly(NIPAAm-*co*-HIPAAm) was prepared by free radical copolymerization, the size of the coacervate droplets was very small and relatively monodisperse, which seems to be due to the three factors mentioned above. Because poly(NIPAAm-*co*-HIPAAm) has a hydroxyl group as a side chain, it is possible to immobilize bioactive molecules, such as peptides and proteins. We are now studying the phase transition and/or separation mechanism of poly(NIPAAm-*co*-HIPAAm) in relation to water structure, such as freezing or nonfreezing water, around the polymer.

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References and Notes

(1) Fujishige, S.; Kubota, K.; Ando, I. *J. Phys. Chem.* **1989**, *93*, 3311.
(2) Kubota, K.; Fujishige, S.; Ando, I. *J. Phys. Chem.* **1990**, *94*, 5154.
(3) Marchetti, M.; Pranger, S.; Cussler, E. L. *Macromolecules* **1990**, *23*, 1760 and 3445.
(4) Kost, J.; Langer, R. *Adv. Drug Delivery Rev.* **2001**, *46*, 125.
(5) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *120*, 12694.
(6) Matsumoto, A.; Kurata, T.; Shiino, D.; Kataoka, K. *Macromolecules* **2004**, *37*, 1502.
(7) Yakushiji, T.; Sakai, K.; Kikuchi, A.; Aoyagi, T.; Sakurai, Y.; Okano, T. *Langmuir* **1998**, *14*, 4657.
(8) Akiyama, Y.; Kikuchi, A.; Yamato, M.; Okano, T. *Langmuir* **2004**, *20*, 5506.
(9) Kim, S.; Healy, K. E. *Biomacromolecules* **2003**, *4*, 1214.
(10) Aoyagi, T.; Ebara, M.; Sakai, K.; Sakurai, Y.; Okano, T. *J. Biomater. Sci. Polym. Ed.* **2000**, *11*, 101.
(11) Ebara, M.; Aoyagi, T.; Sakai, K.; Okano, T. *Macromolecules* **2000**, *33*, 8312.
(12) Ebara, M.; Aoyagi, T.; Sakai, K.; Okano, T. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 335.
(13) Ebara, M.; Yamato, M.; Nagai, S.; Aoyagi, T.; Kikuchi, K.; Sakai, K.; Okano, T. *Surf. Sci.* **2004**, *570*, 134.
(14) Yoshida, T.; Aoyagi, T.; Kokufuta, E.; Okano, T. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 779.
(15) Xue, W.; Champ, S.; Huglin, M. B. *Polymer*, **2000**, *41*, 7575.
(16) Shild, H. G.; Tirell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352.
(17) Priest, J. H.; Murray, S. L.; Nelson, Hoffman, A. S. *ACS Symp. Ser.* **1987**, *350*, 255.
(18) Shild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
(19) Feil, H.; Bae, Y. H.; Kim, S. W. *Macromolecules* **1992**, *25*, 5528.
(20) Aoki, T.; Muramatsu, M.; Torii, T.; Sanui, K.; Ogata, N. *Macromolecules* **2001**, *34*, 3118.
(21) Materna, K.; Cote, G.; Szymanowski, J. *J. Colloid Interface Sci.* **2004**, *269*, 466.
(22) Kjellander, R.; Florin, E. *J. Chem. Soc., Faraday Trans.* **1981**, *77*, 2053.
(23) Florin, E.; Kjellander, R.; Eriksson, J. C. *J. Chem. Soc., Faraday Trans.* **1984**, *80*, 2889.
(24) Eeckman, F.; Moës, A. J.; Amighi, K. *Int. J. Pharm.* **2004**, *273*, 109.
(25) Miyazaki, H.; Kataoka, K. *Polymer* **1996**, *37*, 681.
(26) Yamamoto, K.; Serizawa, T.; Akashi, M. *Macromol. Chem. Phys.* **2003**, *204*, 1027.
(27) Yin, X.; Stöver, H. D. H. *Macromolecules* **2005**, *38*, 2109.
(28) Sugihara, S.; Kanaoka, S.; Aoshima, S. *Macromolecules* **2004**, *37*, 1711.

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