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Thermally Controlled Association in Aqueous Solutions of Diblock Copolymers of Poly[*N*-(2-hydroxypropyl)methacrylamide] and Poly(*N*-isopropylacrylamide)

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Introduction. Over the past few years thermosensitive gels, homo- and copolymers of *N*-isopropylacrylamide, have appeared in the literature with increasing frequency.[1,2] The main impetus for using these polymers has been their novel thermal behavior in aqueous media. They exhibit a lower critical solution temperature (LCST) in the temperature range of the human body and pronounced changes in the degree of hydration in aqueous media in response to changes in temperature. These properties as well as high sensitivity to other ambient stimuli such as electric field, ultrasound, or changes in pH open a broad range of potentials for application of thermosensitive polymers in many fields, such as membranes,[3] drug delivery systems,[4,5] cell culture,[6] isolation of biomolecules,[7,8] and enzyme activity control.[9] While the phase separation has been frequently studied in cross-linked aqueous gels (volume-phase separation)[1,10] and solutions of linear polymers,[2,11,12] little attention has been paid to the thermally sensitive association of copolymers containing blocks of poly(*N*-isopropylacrylamide) (poly(NIPAM)). Two types of copolymers have been studied so far: (i) block and graft copolymers of poly(NIPAM) with hydrophobic polymers and (ii) poly(NIPAM) with hydrophilic polymers. In the former case, micelles were observed below LCST and micellar aggregates above LCST.[13,14] The aggregation process can be reversible or irreversible. In the latter case, diblock copolymers of poly(NIPAM) with poly(ethylene oxide) (poly(NIPAM-*b*-EO))[15] were investigated. The thermally induced micellization of poly(NIPAM-*b*-EO) copolymers has been preliminarily reported.[15] The copolymers were molecularly soluble below LCST, whereas large particles having hydrodynamic radii (R_h) between 50 and 100 nm were observed above LCST.

In the present work, the effect of temperature on the association of diblock copolymers poly([*N*-(2-hydroxypropyl)methacrylamide]-*block*-poly(*N*-isopropylacrylamide) (poly(HPMA-*b*-NIPAM)) is investigated by light scattering methods in aqueous solutions. HPMA copolymers are nonimmunogenic and biocompatible polymers originally developed as drug carriers[16] and, at present, also studied as potential gene delivery systems.[17] Since poly(HPMA-*b*-NIPAM) are interesting new copolymers with potential application in thermoresponsive drug delivery and their solution behavior is unusual, we believe that it is worth reporting the results in a short communication.

Experimental Section. a. Copolymers. *N*-(2-Hydroxypropyl)methacrylamide was prepared by reaction of methacryloyl chloride with 1-aminopropan-2-ol, as described previously.[16] *N*-Isopropyacrylamide was purchased from Aldrich. All monomers were chromatographically pure.

Semitelechelic poly[*N*-(2-hydroxypropyl)methacrylamide] with carboxylic acid end group (poly(HPMA)-COOH) was synthesized by chain transfer radical polymerization.[18] The number-average molecular weights of poly(HPMA)-COOH ($M_n = 8500$ and 3900, $M_w/M_n = 1.7$) were determined by size exclusion chromatography (SEC) in 0.05 M TRIS buffer (pH 8.1) on a Superose12 column (Pharmacia). The column was calibrated using broad range poly(HPMA) standards properly characterized using low-angle laser light scattering. The number-average molecular weight ($M_n = 8400$ and 3800) was also calculated from the content of the end functional carboxylic groups assuming one functional end group per macromolecule. The content of the end carboxylic groups of poly(HPMA)-COOH was determined by titration with 0.05 M NaOH using automatic titration (Radiometer). The molecular weights of the polymer precursors determined by SEC are only slightly higher than those determined by the end group method.

Poly(*N*-isopropylacrylamide) with terminal amino groups was prepared by radical solution polymerization in the presence of chain transfer agent (2-aminoethane-1-thiol hydrochloride) as described in ref 19. Molecular weight ($M_n = 3200$) was determined by end group titration using 2,4,6-trinitrobenzenesulfonic acid by assaying the corresponding substituted trinitrophenylamine spectrophotometrically (1% polymer solution in 0.1 M sodium tetraborate, $\epsilon_{420} = 1.35 \times 10^4$ mol^{-1} cm^{-1}).

Block copolymers poly(HPMA-*b*-NIPAM) were prepared by reacting the succinimidyl ester of poly(HPMA)-COOH with poly(NIPAM)-NH$_2$ similarly to the method described in our previous paper.[18] Two copolymers were prepared having the same NIPAM blocks ($M_n = 3200$) and HPMA blocks with $M_n = 8400$ (copolymer 1) or 3800 (copolymer 2). Free poly(NIPAM)-NH$_2$ was removed from the copolymer by extraction with acetone. For the structure of copolymers, see Scheme 1.

b. Light Scattering. Solution properties of copolymer in aqueous solutions were characterized by static and dynamic light scattering methods. Both the static and dynamic light scattering were simultaneously measured with an ALV goniometer in vertically polarized light at wavelength $\lambda_0 = 532$ nm, angular range $\theta = 30° - 150°$, and temperatures from 25 to 45 °C. On every temperature change, the measurements were realized after reaching the steady state conditions, typically after tens of minutes in the phase separation region. Time evolution of the system was monitored by light scattering intensity at $\theta = 30°$.

For the static light scattering experiments, the apparatus was calibrated with benzene at $\theta = 90°$. The processed data are represented (unless otherwise stated) by apparent molecular weights $M_w{}^a = R(0,c)/Kc$, where K is the optical constant which includes the square of the refractive index increment dn/dc, $R(\theta)$ is the Rayleigh ratio, proportional to the intensity of the light

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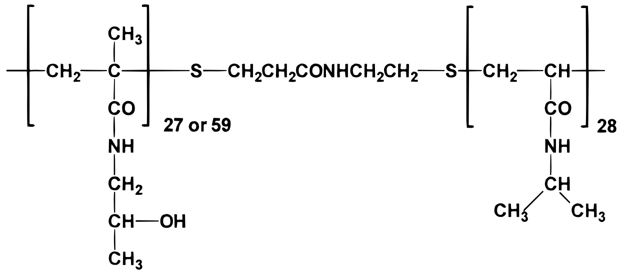
Scheme 1. Structure of Poly([*N*-(2-hydroxypropyl)methacrylamide]-*block*-poly(*N*-isopropylacrylamide) Copolymers



scattered from solutions, and c is the copolymer concentration in g/mL. A model of solid spheres in the Rayleigh approximation was used to obtain zero-angle limits of $R(\theta,c)/Kc$. In the case of very large particles, $M_w^a = R(30°,c)/Kc$ was used, which is a low-limit approximation of M_w.

The refractive index increments, dn/dc, for the copolymers were calculated as linear combinations of dn/dc values for participating blocks: dn/dc (at $\lambda = 546$ nm) = 0.179 and 0.174 for copolymers with low and high HPMA contents, respectively. The refractive index increments for poly(NIPAM) (0.194) and poly(HPMA) (0.167) in water were determined by a Brice-Phoenix refractometer at $\lambda = 546$ nm.

Polarized DLS measurements were made with an ALV 5000, multibit, multi-tau autocorrelator covering approximately 10 decades in the delay time τ. The time autocorrelation functions were fitted using the cumulant method. The apparent average hydrodynamic radius, R_h^a, was calculated from the zero-angle limit of the diffusion coefficient, $D^a(\theta)$, using the Stokes−Einstein relationship. In the case of very large particles, $R_h^a(30°)$ was used which is a low-limit approximation of R_h^a.

Results and Discussion. Since the copolymers are supposed to be thermosensitive, the equilibrium apparent hydrodynamic radius, R_h^a, and apparent molecular weight, M_w^a, of particles were measured as functions of temperature in aqueous solutions ($c = 5 \times 10^{-3}$ g/mL). The results are plotted for copolymers 1 and 2 in Figures 1 and 2, respectively. It can be seen in the figures that both R_h^a and M_w^a abruptly increase on heating at a temperature $t \approx 33$ °C, which is close to LCST of poly(NIPAM) (~32 °C).[2] M_w^a increases by several orders of magnitude, and R_h^a changes by more than 1 order of magnitude. The effect is more pronounced for copolymer 1 having longer poly(HPMA) blocks. The sizes of particles above the transition temperature are for copolymer 1 above the Rayleigh limit; therefore, apparent values obtained at $\theta = 30°$ were used in Figure 1. The decreases in $R_h^a(30°)$ and $M_w^a(30°)$ at temperatures above 40 °C in parts a and b of Figure 1, respectively, are due to multiple scattering in solutions. In both the samples, temperature hysteresis was observed on cooling, higher for copolymer 1. The width of distribution of the hydrodynamic radius for particles observed in the vicinity of 41 °C was smaller than 0.2 for both the solutions, being slightly smaller for copolymer 1. Thus, the observed particles cannot be regular aggregates but supramolecular structures formed by an association process of copolymers. Similar hysteresis in the heating and cooling circle was also observed in the coil-to-globule transition of individual NIPAM chains in water.[20] The effect was explained by the formation of intrachain structures, presumably by the intrachain hydrogen bonding in the globular state.[20,21] We suppose that the interchain and itrachain hydrogen bonding of NIPAM blocks in supramolecular structures is also responsible for the observed hysteresis.

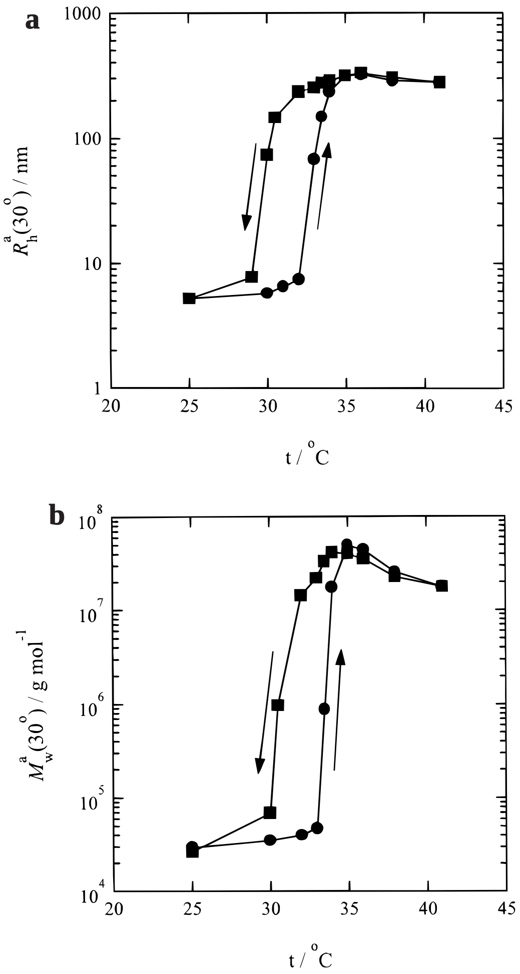


Figure 1. (a) Temperature dependence of the apparent hydrodynamic radius, $R_h^a(30°)$, of particles for copolymer 1. (b) Temperature dependence of apparent molecular weight, $M_w^a(30°)$, of particles for copolymer 1.

To characterize the particle structures, the mean weight fractions, w_c, of a copolymer in the particle (copolymer density) were calculated from the volumes of particles V (in cm^3) and from their corresponding molecular weights M_w^a (in g mol^{-1}); $w_c = M_w^a/N_AV$, where N_A is Avogadro's number. R_h^a values were used for estimation of V. The calculation was realized for copolymer 2, where better defined data are available, at $t = 41$ °C. Surprisingly, we found the value of w_c to be 4.3×10^{-3} g/mL, which is a value comparable with the copolymer concentration in solution ($c = 5 \times 10^{-3}$ g/mL) and approximately 5 times smaller than the weight fraction of copolymers in micelles observed in selective organic solvents.[22] This means with a high probability that the particles are not regular micelles. As a working hypothesis, we suggest for observed particles a vesicle-like structure. This interpretation is supported by the fact that the association number of particles at 41 °C is 2300 copolymer molecules, which is at least 1 order of magnitude higher than in micelles,[22] and the length of copolymer chains is by an order of magnitude shorter than observed hydrodynamic radii of particles. Particles having R_h between 50 and 100 nm, as observed in aqueous solutions of diblock

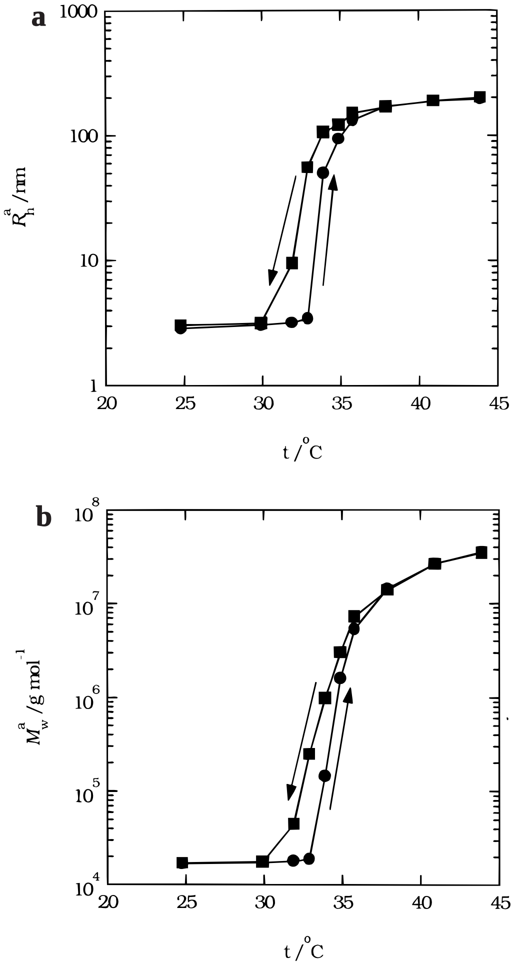


Figure 2. (a) Temperature dependence of the apparent hydrodynamic radius, $R_h{}^a$, of particles for copolymer 2. (b) Temperature dependence of apparent molecular weight, $M_w{}^a$, of particles for copolymer 2.

copolymers of low-molecular-weight poly(NIPAM-*b*-EO),[15] seem also larger than regular micelles.

For copolymer 2, the effect of concentration was estimated. While association of copolymers was observed at $c = 2 \times 10^{-3}$ g/mL, no formation of supramolecular structures was detected at $c = 1 \times 10^{-3}$ g/mL in the temperature range from 20 to 45 °C. This means that the critical association concentration should be somewhere between these concentrations. The onset of association (on heating) increases only slightly with decreasing copolymer concentration being 34 °C at $c = 2 \times 10^{-3}$ g/mL.

Conclusions. The thermosensitive block copolymers poly(HPMA-*b*-NIPAM) form large monodisperse particles on heating, probably vesicles, at temperatures higher than 33 °C. The size and molecular weight of supramolecular particles seem to decrease with decreasing molar ratio of hydrophilic HPMA polymer. Thus, the micelle formation we assume in aqueous solutions of the copolymers having only short HPMA blocks. New experiments verifying the hypothesis are in progress.

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