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Micellization Protocols for Amphiphilic Polyelectrolytes in Water. How Do Polymers Undergo Intrapolymer Associations?

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ABSTRACT: Copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate and *N*-dodecylmethacrylamide (C_{12}MAm) undergo intrapolymer hydrophobic association in water, but depending on micellization procedures, the polymers may or may not form completely unimolecular micelles. Various protocols for the preparation of aqueous solutions of the polymers were examined to clarify how the polymers form preferentially unimolecular micelles. In solid polymer samples purified by reprecipitation followed by lyophilization, kinetically-frozen multimolecular micelles, formed by hydrophobic associations in entangled polymer chains during purification, may already exist. When a solid polymer sample is added to water, kinetically-frozen micelles are simply re-dissolved in water as such. Virtually unimolecular micelles were obtained when the solid sample was first dissolved in pure water at an elevated temperature (≥90℃), followed by addition of salt at the same temperature. Micelles formed from the copolymers with C_{12}MAm content ≥40 mol% were not equilibrium micelles but kinetically-frozen. The micelles were not completely unimolecular. The number of polymer chains comprising a micelle increased with C_{12}MAm content.

KEY WORDS Protocol / Entanglement / Sodium 2-(Acrylamido)-2-methylpropanesulfonate / *N*-Dodecylmethacrylamide / Interpolymer Association / Intrapolymer Association / Kinetically-Frozen Micelle /

Water-soluble amphiphilic copolymers, consisting of hydrophilic and hydrophobic monomer units, have been extensively studied in recent years for potential applications as well as academic interest in self-assembling phenomena.[1-5]

The self-association of amphiphilic polymers in water is mainly driven by hydrophobic interactions, yielding micellelike nanostructures. A large number of studies have been reported on polymer micelles formed from amphiphilic block and random copolymers in aqueous solutions.[6-10] In the case of water-soluble **AB** and **ABA** type amphiphilic block copolymers, where **A** and **B** represent hydrophilic and hydrophobic blocks, respectively, core-corona type micelles are normally formed by multipolymer associations.[11-20] In the case of amphiphilic random copolymers, self-association behavior is more complicated than that of block copolymers because large numbers of hydrophilic and hydrophobic segments are connected in random distribution on a polymer chain and therefore the association of polymer-bound hydrophobes occurs on the same polymer chain and between different polymer chains. If polymer-bound hydrophobes associate between different polymer chains, crosslinked structures are formed. When hydrophobe content in the copolymer is low, interpolymer association may lead to a large increase in solution viscosity. When hydrophobe content is sufficiently high, extensive interpolymer associations may lead to gelation arising from infinite network structures. In contrast, if polymer-bound hydrophobes associate completely within the same polymer chain, single-macromolecular micelles (unimolecular micelles) are formed.

Random copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS) and methacrylamides substituted with a bulky hydrophobe at the *N*-position undergo micellization within a single polymer chain in water to form unimolecular micelles.[8,21-23] In such unimolecular micelles, all polymer-bound hydrophobes are incorporated in intrapolymer domains (*i.e.*, micelle cores) surrounded by electrolyte segments and thus hydrophobes are no longer available for association with other hydrophobes on different polymer chains. In other words, unimolecular micelles possess a "closed" structure[24-26] and exist as such even at very high concentrations without undergoing interpolymer hydrophobic association.[8,21,22] However, it remains unanswered when and how polymer-bound hydrophobes undergo such highly preferential intrapolymer association dominating over interpolymer association.

Intrapolymer micellization of amphiphilic polyelectrolytes in water may be governed by free energy if micellization occurs under an equilibrium condition. An important question to be asked is whether intrapolymer micellization occurs at equilibrium when a solid polymer sample is directly dissolved in water or a micelle structure has already been formed in the solid sample and a kinetically-frozen micelle is simply re-dissolved in water. In the latter case, the micelle structure found after direct dissolution of a solid polymer sample in water should depend on the history of the sample. If that is the case, structures of polymer micelles may be found to differ depending on protocol for the preparation of polymer solutions.

To answer this question, we examined how the association behavior of copolymers of AMPS and *N*-dodecylmethacrylamide (C_{12}MAm) in water depends on protocol for the preparation of polymer solutions. C_{12} alkyl chains in these copolymers show a strong tendency for intrapolymer self-association, leading to the formation of unimolecular micelles.[22,23,27] These copolymers were

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prepared by free radical copolymerization in *N,N*-dimethylformamide (DMF) and purified by reprecipitation from a methanol solution into excess diethyl ether, followed by dialysis of an aqueous solution against pure water. A solid polymer sample was recovered from a dialyzed aqueous solution by freeze-drying. During polymerization and purification, polymers may exist as entangled chains. If polymer chains remain entangled in a solid sample, they may undergo "intrapolymer" micellization in an entangled state when dissolved in water, resulting in multipolymer micelles even if the polymer *per se* has a strong preference for intrapolymer self-association. Therefore, to obtain purely unimolecular micelles, we should eliminate chain entanglement before polymers are subjected to micellization. If polymers undergo self-association in an entangled state, polymer chains may not spontaneously disentangle upon simple dilution in water because the entanglement would be "locked" by hydrophobic associations of polymer-bound hydrophobes. In other words, hydrophobes on different polymer chains entangled would associate as if on the same polymer chain.

Hydrophobic interactions are driven by positive entropy resulting from a decrease in structured water molecules on the periphery of hydrophobes.[28,29] However, water structures are destroyed at elevated temperatures (≥90℃), if not completely, and hence hydrophobic interactions are greatly diminished at temperatures ≥90℃. Therefore, we tested mainly the following two protocols to "cancel" out the history of solid polymer samples and "unlock" entanglements if any; (i) a solid polymer sample was dissolved in an organic solvent miscible with water, and the polymer solution was dialyzed against pure water such that micellization occurs under equilibrium conditions according to change in solvent composition (*i.e.*, ratio of organic solvent to water) during dialysis, and (ii) a solid polymer sample was dissolved in pure water and the solution was heated at temperatures ≥90℃. In amphiphilic polyelectrolytes, hydrophobic attractive interactions balance with electrostatic repulsive interactions. Therefore, if hydrophobic interactions are disrupted at an elevated temperature, electrostatic repulsive interactions may facilitate the disentanglement of polymer chains. Such electrostatic effect on chain disentanglement would be more favorable in the absence of salt than its presence because electrostatic forces are shielded by added salt. Therefore, if we need to prepare a unimolecular micelle solution with added salt, it should be important when to add salt.

Micelles of AMPS–C_{12}MAm copolymers in aqueous solutions prepared by various protocols were carefully characterized by gel permeation chromatography (GPC), static light scattering (SLS), quasielastic light scattering (QELS), and nonradiative energy transfer (NRET) techniques. For characterization by NRET, the polymers were singly labeled with naphthalene (Np) or pyrene (Py) or doubly labeled with Np and Py on the same polymer chain (Chart 1).

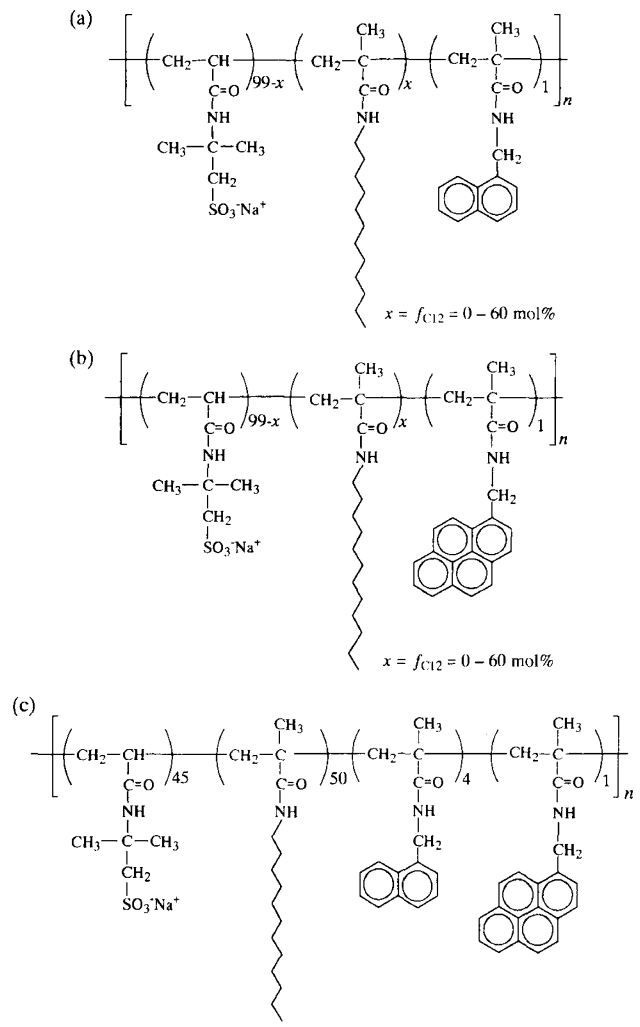


Chart 1.

EXPERIMENTAL

Monomers

N-Dodecylmethacrylamide (C_{12}MAm),[30] *N*-(1-naphthylmethyl)methacrylamide (1NpMAm),[31] and *N*-(1-pyrenylmethyl)methacrylamide (1PyMAm)[32] were synthesized as reported previously. 2-(Acrylamido)-2-methylpropanesulfonic acid (AMPS) was purchased from Wako Pure Chemicals and used without further purification. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized from ethanol prior to use.

Singly-Labeled Polymers

The polymers singly-labeled with naphthalene (Np) or pyrene (Py) were prepared by terpolymerization of AMPS, C_{12}MAm (0, 10, 20, 30, 40, 50, 55, or 60 mol%), 1NpMAm (1 mol%) or 1PyMAm (1 mol%), and 0.1 mol% (based on the total monomers) of AIBN in *N,N*-dimethylformamide (DMF) at 60℃ for 15 h. The details of the polymerization procedures were reported elsewhere.[22] The polymers were purified by reprecipitations from methanol (concentration is *ca.* 100 g L^{-1}) into excess diethyl ether, and polymer solutions in a dilute aqueous NaOH (polymer concentration is *ca.* 5 g L^{-1}) were dialyzed against pure water for a week and finally recovered by freeze-drying from aqueous solution. The compositions of the terpolymers were determined by N/C

in elemental analysis and UV absorbance. We previously confirmed that AMPS–C_{12}MAm,[32] AMPS–1NpMAm,[31] and AMPS–1PyMAm[32] copolymerizations result in copolymer compositions equal to monomer feed compositions and completely random distribution of monomer units (*i.e.*, "ideal copolymerization"). Therefore, the compositions of terpolymers are considered the same as monomer compositions in the feed and the distribution of the three monomer units in the terpolymer is completely random.

Doubly-Labeled Polymers

Polymers doubly-labeled with Np and Py were prepared and purified in a manner similar to the preparation of the singly-labeled polymers using AMPS (45 mol%), C_{12}MAm (50 mol%), 1NpMAm (4 mol%), and 1PyMAm (1 mol%) in monomer feed. The composition of the doubly-labeled polymer was determined by N/C in elemental analysis and UV absorbance.

Other Materials

NaCl and $LiClO_4$ were purchased from Wako Pure Chemicals and used without further purification. HPLC grade methanol and acetonitrile were purchased from Wako Pure Chemicals and used without further purification. Milli-Q water was used for all measurements.

Measurements

Absorption spectra were recorded with a JASCO V-550 spectrophotometer. Steady-state fluorescence spectra were measured on a Hitachi F-4500 fluorescence spectrophotometer using a 1-cm path length quartz cuvette.

Sample solutions were excited at 290 nm and fluorescence spectra were recorded in the wavelength range 300—550 nm. The intensity of pyrene fluorescence due to NRET from singlet excited naphthalene was estimated at 376 nm. Contribution from direct pyrene excitation was corrected by subtracting from each spectrum the emission spectrum of the corresponding Py-labeled polymer (Chart 1(b)) of the same Py concentration.

GPC measurements were performed at 40℃ with a JASCO GPC-900 system equipped with Shodex Asahipak GF-7M HQ columns in combination with a JASCO UV-975 detector. A 0.2 M $LiClO_4$ solution in methanol was used as eluent. Flow rate was maintained at 1 mL min^{-1} during measurement. Molecular weight distribution (M_w/M_n) of polymer samples was calibrated with poly(ethylene glycol) standards (Scientific Polymer Products, Inc.).

Hydrodynamic radii (R_h) and their distributions were measured with an Otsuka Electronics Photal DLS-7000 light scattering spectrometer equipped with a 60-mW Ar laser lamp and detector optics. An ALV-5000E digital multiple-τ correlator (Langen-GmbH) was used for data collection. Autocorrelation function was measured at different angles (50°—130°) at 25℃. Sample solutions were filtered with a 0.2 μm pore size membrane filter prior to measurement. The observed intensity autocorrelation function $g^{(2)}(t)$ was related to normalized autocorrelation function $g^{(1)}(t)$ by the Siegert relation,

$$g^{(2)}(t)=B[1+\beta|g^{(1)}(t)|^2] \quad (1)$$

where β is a constant parameter for an optical system used and $B$is a baseline term. To obtain relaxation time distribution, $\tau A(\tau)$, inverse Laplace transform (ILT) analysis for $g^{(2)}(t)$ was performed using the algorithm REPES[33] according to the equation,

$$g^{(1)}(t)=\int \tau A(\tau)\exp(-t/\tau)d\ln\tau \quad (2)$$

where τ is the relaxation time. Relaxation time distribution is presented as $\tau A(\tau)$ *versus* $\log\tau$ profile with equal area. Apparent diffusion coefficient, D, was calculated from the ILT moments as

$$D=(\Gamma/q^2)_{q\to 0} \quad (3)$$

where Γ is the relaxation rate and q is the magnitude of scattering vector expressed as,

$$q=\frac{4\pi n}{\lambda}\sin(\theta/2) \quad (4)$$

R_h is given by the Einstein–Stokes equation,

$$R_h=\frac{k_B T}{6\pi\eta D} \quad (5)$$

where k_B is the Boltzmann constant, T is the Kelvin temperature, and η is the solvent viscosity.

SLS data were obtained at 25℃ with an Otsuka Electronics Photal DLS-7000 light scattering spectrometer equipped with an Ar laser (50 mW at 488 nm). Sample solutions of polymers were filtered with a 0.2 μm pore size membrane filter prior to measurement. Under these conditions, measurement was performed at 25℃ and optical constant K was calculated from the relation,

$$K=\frac{4\pi^2 n^2(dn/dc)^2}{N_A\lambda^4} \quad (6)$$

where n is the refractive index of solution, N_A is the Avogadro number, λ is the wavelength, and dn/dc is the refractive index increment against concentration. Values of dn/dc were determined with a Shimadzu improved Schulz–Cantow type differential refractometer. Apparent weight-average molecular weights (M_w) were estimated as,

$$\frac{K_c}{R_\theta}=\frac{1}{M_w}(1+16\pi^2 n^2/3\lambda^2\langle S^2\rangle\sin^2\theta/2+L)+2A_2 c+L \quad (7)$$

where c is the polymer concentration, R_θ is the Rayleigh ratio, $\langle S^2\rangle$is the mean-square radius of gyration, θ is the scattering angle, and A_2 is the second virial coefficient.

RESULTS AND DISCUSSION

Characterization of Polymers

Because hydrophobic associations are absent in methanol, we estimated weight-average molecular weights (M_w) of Np-labeled polymers with C_{12}MAm content (f_{C12}) at 0—60 mol% (Chart 1(a)) by SLS in methanol containing 0.1 M $LiClO_4$ and molecular weight distribution (M_w/M_n) by GPC using methanol containing 0.2 M $LiClO_4$ as eluent. SLS measurements were performed in a polymer concentration range 1.0—5.0 g L^{-1}. An example of Zimm plots for the polymer with f_{C12}=

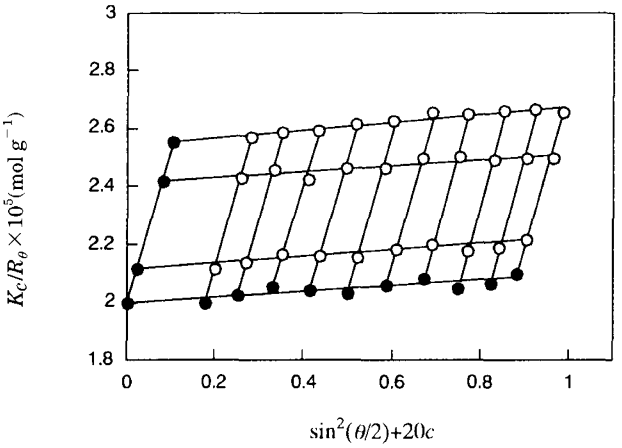


Figure 1. Zimm plots for the Np-labeled polymer with f_{C12}= 10 mol% in methanol containing 0.1 M $LiClO_4$ at 25℃.

10 mol% is presented in Figure 1. Under these conditions, reasonably good Zimm plots were obtained also for the other polymers with varying contents of C_{12}MAm. From Zimm plots, M_w were estimated by extrapolating θ and c to zero. The results are listed in Table I along with M_w/M_n roughly estimated by GPC. M_w in methanol solutions was (3.9—6.5)$\times 10^4$ and M_w/M_n 1.9—2.4. There is no particular dependence of M_w and M_w/M_n on f_{C12}.

Polymer Solution Preparations

To assess the dependence of polymer micellization on preparation of micelle solutions, QELS measurements were performed on sample solutions of the doubly-labeled polymer with f_{C12}=50 mol% (Chart 1(c)) at a polymer concentration of 1.5 g L^{-1} in 0.2 M NaCl aqueous solution, as summarized in Scheme 1. Apparent hydrodynamic radii (R_h) obtained at a scattering angle of 90° are indicated in Scheme 1.

Six solutions (**Solutions 1—6**) were prepared as follows (Scheme 1):

Solution 1: A dry polymer sample obtained as in the Experimental Section was dissolved in methanol and dialyzed against pure water for 1 week. The polymer was recovered by freeze-drying from an aqueous solution after dialysis. The polymer was dissolved in a 0.2 M NaCl aqueous solution at room temperature and allowed to stand for 2 days (**Process 1**). R_h for this solution (polymer concentration adjusted to 1.5 g L^{-1} prior to measurement) was 20.1 nm.

Solution 2: A dry polymer sample was dissolved in a water/acetonitrile mixed solvent (3/7, v/v) and dialyzed against pure water for 1 week. The polymer was recovered by freeze-drying from aqueous solution after dialysis. The polymer was dissolved in 0.2 M NaCl aqueous solution at 90℃ (**Process 2**). R_h for this solution was 8.9 nm.

Solution 3: The same freeze-dried polymer was dissolved in pure water at 90℃. After cooling to room temperature, a predetermined amount of NaCl was added to the solution (**Process 3**). R_h was 7.9 nm, slightly smaller than that for **Solution 2**.

Solution 4: A dry polymer sample was dissolved in 0.2 M NaCl aqueous solution at room temperature and allowed to stand for 2 days (**Process 4**). R_h was 25.5 nm.

Solution 5: The same dry polymer was dissolved in 0.2 M NaCl aqueous solution at 90℃ (**Process 5**). R_h was 7.5 nm, much smaller than for **Solution 4**.

Solution 6: Dry polymer was dissolved in pure water at 90℃. After cooling to room temperature, a predetermined amount of NaCl was added (**Process 6**). R_h was 6.1 nm, significantly smaller than for **Solution 5**.

R_h for **Solution 6** was smaller than for any other solutions. R_h for solutions prepared without heating at 90℃ (**Solutions 1** and **4**) are much larger than for solutions prepared by heating at the elevated temperature. We examined whether sonication during heating at 90℃ affects R_h (data not shown) but found virtually no effect.

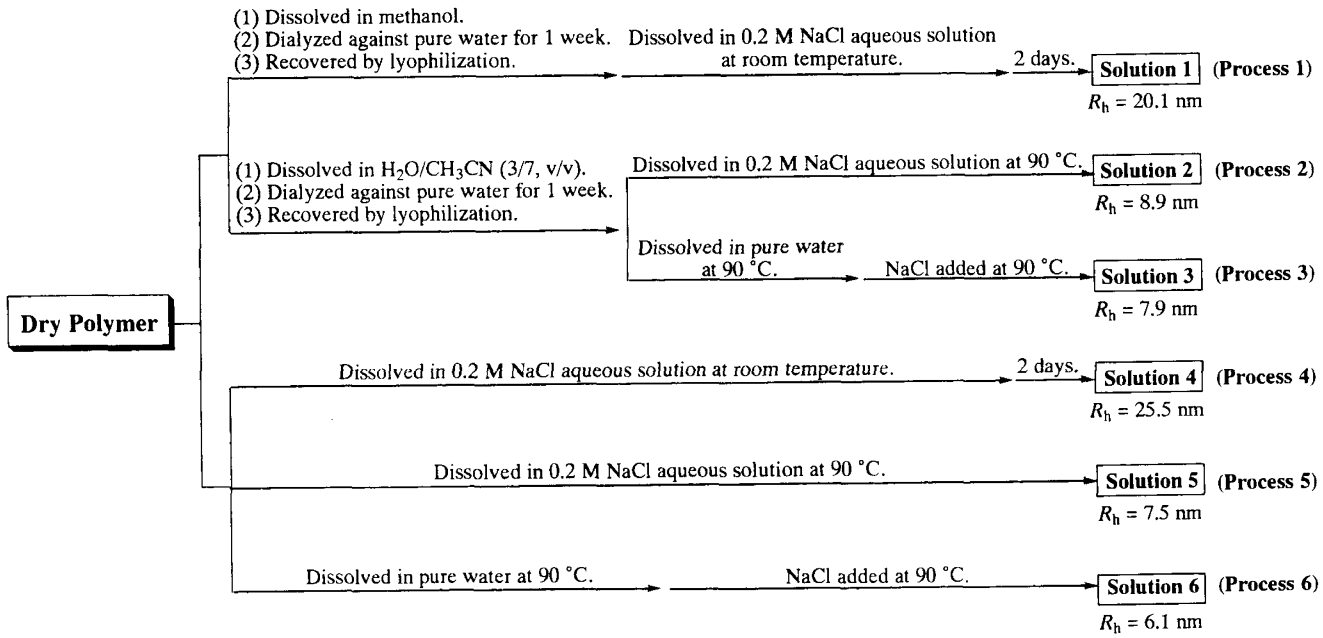
Micelle structures, formed when the reprecipitated polymers were first dissolved in water for dialysis, are retained in dry polymer samples recovered by freeze-drying. This micelle may be a kinetically-frozen micelle formed not only by intrapolymer hydrophobic association but also interpolymer association between polymer chains entangled in the dry sample. When the dry sample is dissolved in water at room temperature, the already-formed multipolymer micelles are simply redissolved without reorganization of micellar structure, and therefore hydrodynamic size is larger than that for the corresponding unimolecular micelle. If polymer solution in pure water is heated at 90℃, a temperature at which hydrophobic interactions diminish, the micellar structure may be disrupted allowing polymer chains to disentangle. Such disentanglement may be facilitated by interpolymer electrostatic repulsion. Upon cooling the solution to room temperature, each polymer chain undergoes completely intrapolymer self-association, yielding unimolecular micelles, as illustrated in Figure 2.

The distributions of relaxation times in QELS at θ=90° for **Solutions 4, 5,** and **6** are compared in Figure 3. Relaxation time distribution for **Solution 4** is bimodal with a small fast relaxation mode peak attributable mainly to unimers (a single polymer state) and a much larger slow mode peak to multipolymer aggregates. Relaxation time distribution for **Solution 6**, which shows the smallest R_h, is narrower than for **Solution 5**. We may thus conclude that heating polymer solution without added salt is most important to eliminate interpolymer hydrophobic association possibly brought about through chain entanglements in solid polymer samples recovered by freeze-drying. Dialysis of organic solutions of the polymer against pure water and sonication of polymer aqueous solution have little or not effect on the elimination of chain entanglements.

Nonradiative Energy Transfer (NRET)

NRET between an energy donor and energy acceptor covalently attached to the same polymer chain or separate polymer chains is useful to probe conformational changes.[34-38] Naphthalene and pyrene are often used as a donor–acceptor pair because this pair has large spectral overlap, and naphthalene can be selectively excited near 290 nm. NRET is indicated by increase in the intensity of pyrene fluorescence with excitation of naphthalene.[27]

Interpolymer NRET measurements were performed with polymer solutions prepared as follows:



Scheme 1. Preparation of aqueous solutiopns of polymer.

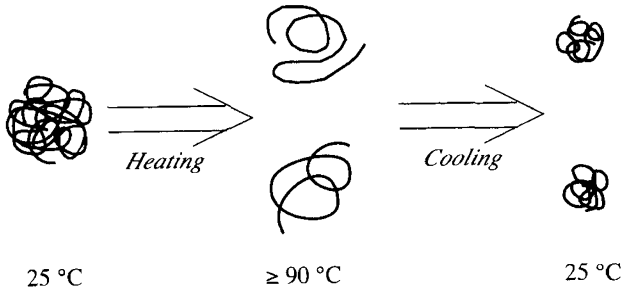


Figure 2. Conceptual illustration of the formation of unimolecular micelles when multimolecular micelles in pure water were heated at an elevated temperature, followed cooling.

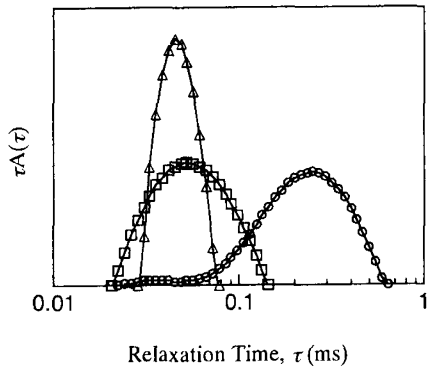


Figure 3. Distribution of the relaxation times in QELS measured at $\theta = 90°$ for 1.5 g L^{-1} doubly-labeled polymer solutions containing 0.2 M NaCl at 25℃. **Solution 4** (○), **Solution 5** (□), and **Solution 6** (△).

Solution (i): Polymer solutions (1 g L^{-1}) of the Np-labeled polymer and Py-labeled polymer of the same f_{C12} were separately prepared according to **Process 5** (except NaCl concentration of 0.05 M) and the two aqueous solutions were mixed at 4 : 1 (Np-labeled polymer : Py-labeled polymer) after cooling the solutions to room temperature.

Solution (ii): The Np-labeled and Py-labeled polymers with the same f_{C12} were dissolved in methanol (polymer concentration is *ca.* 10 g L^{-1}), and poured into excess diethyl ether to precipitate the polymers. After removing diethyl ether by centrifugation, the polymers were dissolved in water and recovered by freeze-drying from aqueous solution of the mixture of the Np-labeled and Py-labeled polymers. A polymer solution of the recovered polymer sample was prepared according to **Process 5** (except NaCl concentration of 0.05 M).

Solution (iii): The same freeze-dried polymer mixture was dissolved in pure water at 90℃ according to **Process 6** (except NaCl concentration of 0.05 M adjusted at room temperature).

Using these three solutions of polymers with f_{C12}= 0—60 mol%, we performed interpolymer NRET measurements at polymer concentration of 1.0 g L^{-1}. Figure 4 shows the ratio of intensity for Py fluorescence to that for Np fluorescence (I_{Py}/I_{Np}) plotted as a function of f_{C12} for the three solutions. I_{Py}/I_{Np} for **Solutions (ii)** and **(iii)** obviously increase when f_{C12}>40 mol%. This increase is due to an increase in the number of Np and Py labels close to each other within the Förster radius (R_0= 2.86 nm for transfer from 1-methylnaphthalene to pyrene[39]). These results suggest that when hydrophobe content is higher than 40 mol%, interpolymer associations during the reprecipitation and dialysis are retained to some extent even after treating the polymer sample according to **Processes 5** and **6**. The number of polymer chains associated together clearly increased with f_{C12} at f_{C12}>40 mol%. I_{Py}/I_{Np} for **Solution (ii)** are larger than for **Solution (iii)** at the same f_{C12} at f_{C12}> 40 mol%. This suggests a larger number of polymer chains to be associated in **Solution (ii)** than in **Solution (iii)**. This is an indication that chain disentangle-

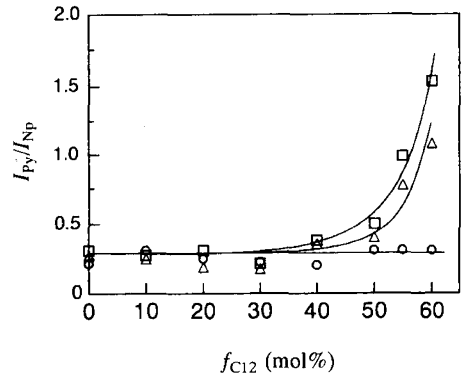


Figure 4. Intensity of fluorescence from Py to Np labels (I_{Py}/I_{Np}) as a function of f_{C12} for mixed solutions of Py- and Np-labeled polymers containing 0.05 M NaCl with excitation at 290 nm at 25℃: Polymer concentration; 1 g L^{-1}. **Solution (i)** (○), **Solution (ii)** (□), and **Solution (iii)** (△).

Table I. Molecular weights, molecular weight distribution, and hydrodynamic radii for Np-labeled polymers

f_{C12}(mol%)	M_w ($\times 10^4$)				M_w/M_n[c]	R_h(nm)[d]
	In methanol	[a]Solution A[b]	Solution B[b]	Solution C[b]		
0	5.5	6.1	5.6	—	2.1	7.3
10	5.0	6.0	6.7	—	2.2	7.1
20	6.5	6.7	7.1	—	2.2	4.9
30	4.3	5.3	8.5	—	2.4	3.9
40	4.9	—	8.8	7.6	2.2	4.0
50	3.9	—	20.7	19.5	1.9	4.3
55	4.4	—	81.7	47.5	2.1	5.7
60	5.2	—	381.5	281.2	1.8	8.1

[a] Determined by SLS in methanol containing 0.1 M $LiClO_4$ at 25 ℃. [b] Determined by SLS in 0.2 M NaCl aqueous solutions at 25℃. See text for preparation of **Solutions A—C**. [c] Determined by GPC using methanol containing 0.2 M $LiClO_4$ as eluent at 40℃. [d] Determined by QELS in 0.05 M NaCl aqueous solution at 25℃.

ment occurs more easily in pure water than in salt water.

In sharp contrast, I_{Py}/I_{Np} for **Solution (i)** are virtually constant at a small value independent of f_{C12}, indicating no interpolymer NRET at any f_{C12} in **Solution (i)**. This does not indicate the absence of interpolymer association in **Solution (i)**. When a solution of Np- and Py-labeled polymers was prepared separately from a solid sample of each polymer, some polymer chains in the solution may exist as interpolymer associates formed during precipitation and dialysis. When the two polymer solutions were mixed, the two polymers exist independently, and neither rearrangement of the association state nor further association occurs. Micelles formed from the AMPS–C_{12}MAm copolymers in aqueous solutions are thus kinetically-frozen.

I_{Py}/I_{Np} in interpolymer NRET measurement are much smaller than in intrapolymer NRET of doubly-labeled polymers. I_{Py}/I_{Np} for the doubly-labeled polymers (*i.e.*, intrapolymer NRET efficiencies) in **Solutions 4, 5,** and **6** were 10.5, 9.4, and 9.2, respectively. These large ratios indicate strong preference of intrapolymer association of the polymer.

AMPS–C_{12}MAm copolymers may thus show strong intrapolymer association but at hydrophobe content higher than *ca.* 40 mol%, some polymer chains associate in a interpolymer fashion, that may arise from already existing chain entanglements.

Quasielastic Light Scattering (QELS)

Using Np-labeled polymers with f_{C12} of 0—60 mol% (Chart 1(a)), aqueous solutions at polymer concentration of 1 g L^{-1} in 0.05 M NaCl were prepared according to **Process 6** and relaxation time distribution for the solutions was measured at varying scattering angles from 50° to 130°. The relaxation rate (Γ) (*i.e.*, reciprocal of relaxation time (τ)) at each peak top of relaxation time distribution was plotted against q^2, giving a straight line passing through the origin (data not shown). From the diffusion coefficient (D) determined from the slope of the plot, apparent R_h were calculated from eq 5 along with the viscosity and refractive index of 0.05 M NaCl aqueous solution at 25℃. R_h thus estimated are listed in Table I. R_h decreased gradually with increasing f_{C12}, showing a minimum value at $f_{C12}=30$ mol%, gradually increasing with increasing f_{C12} up to 50 mol%, followed by sharp increase in R_h at $f_{C12}>50$ mol%. Thus, at $f_{C12}<50$ mol%, the polymer main chain takes a more collapsed conformation at higher f_{C12} because hydrophobe associations of the C_{12} alkyl chains preferentially occur in the same polymer chain in this f_{C12} region. Not only intra- but also interpolymer associations occur at f_{C12} beyond 50 mol%.

Static Light Scattering (SLS)

SLS measurements were performed on the Np-labeled polymers (Chart 1(a)) with $f_{C12}=0-60$ mol% at 25℃ at 1.0—5.0 g L^{-1} polymer concentration in 0.2 M NaCl aqueous solution.

Polymer solutions for the SLS measurements were prepared as follows:

Solution A: Solutions of varying polymer concentrations (1.0—5.0 g L^{-1}) in 0.2 M NaCl were prepared according to **Process 6**.

Solution B: A 5.0 g L^{-1} polymer solution in 0.2 M NaCl was first prepared according to **Process 5** and aliquots of this solution were diluted with 0.2 M NaCl aqueous solution at 90℃ to prepare solutions of varying polymer concentration (1.0—4.0 g L^{-1}).

Solution C: A 5.0 g L^{-1} polymer solution in pure water was first prepared and NaCl was added (0.2 M NaCl) according to **Process 6**. Aliquots of the solution were diluted with a 0.2 M NaCl aqueous solution at 90℃ to prepare solutions of varying polymer concentration (1.0—4.0 g L^{-1}).

Apparent M_w estimated from Zimm plots for **Solution A** for the polymers with f_{C12} of 0—30 mol% are listed in Table I. Apparent M_w for **Solution A** agree fairly well with M_w in methanol (containing 0.1 M $LiClO_4$) in this f_{C12} range. The polymers with $f_{C12}\leq 30$ mol % thus exist as unimers in **Solution A**. For the polymer with $f_{C12}\geq 40$ mol%, K_c/R_θ at $\theta=0$ for **Solution A** increased with decreasing polymer concentration in Zimm plots. An example of such anomalous Zimm plots for the polymer with $f_{C12}=55$ mol% is presented in Figure 5. Polymer chains may aggregate and the number of polymer chains associating together (*i.e.*, aggregation num-

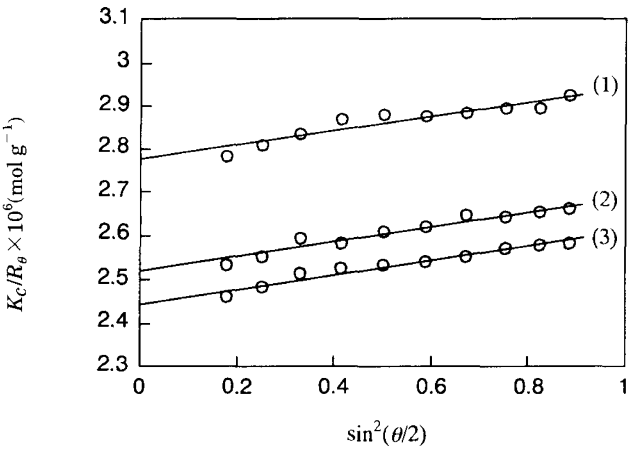


Figure 5. Zimm plots for the Np-labeled polymer with f_{C12}= 55 mol% in 0.2 M NaCl aqueous solution at 25℃. Polymer concentrations: (1) 1 g L^{-1}, (2) 2 g L^{-1}, and (3) 3 g L^{-1}.

ber) may increase with polymer concentration. Thus, it can be concluded that not only intra- but interpolymer associations occur in **Solution A** when $f_{C12} \geq 40$ mol%.

In contrast, normal Zimm plots were obtained with **Solution B** for all f_{C12}. Apparent M_w estimated from the Zimm plots for **Solution B** are listed in Table I. Even at $f_{C12} < 40$ mol% where hydrophobic association predominantly occurs in an intrapolymer fashion, apparent M_w for **Solution B** are larger than in methanol (containing 0.1 M $LiClO_4$) at the same f_{C12}. Entanglements between polymer chains may thus not be completely eliminated in **Solution B** prepared by **Process 5**. Apparent M_w slightly increased with f_{C12} up to 40 mol%, but increased with f_{C12} at $f_{C12} > 50$ mol%, suggesting interpolymer associations to be more pronounced at $f_{C12} > 50$ mol%.

In contrast to **Solution A**, K_c/R_θ at $\theta = 0$ for **Solution C** for f_{C12} of 40 to 60 mol% increased with polymer concentration, exhibiting normal Zimm plots. Apparent M_w estimated from Zimm plots for **Solution C** are listed in Table I. M_w for **Solution C** are smaller than for **Solution B** but larger than in methanol. Assuming M_w in methanol are of the unimer state, the aggregation number of polymer chains in **Solution C** may be estimated from M_w for **Solution C** and methanol solution. Aggregation numbers thus estimated for the Np-labeled polymers with f_{C12}=40, 50, 55, and 60 mol% in **Solution C** are *ca.* 2, 5, 10, and 50, respectively. Thus, virtually unimolecular micelles are formed at $f_{C12} < 40$ mol% if polymer solutions are prepared *via* **Process 6**. Strictly speaking, some polymer micelles for f_{C12}=40 mol% may consist of 2 or more polymer chains. As f_{C12} increases beyond 50 mol%, the number of polymer chains involved in a polymer micelle increases rapidly. SLS for **Solutions A** and **B** indicate association of the polymers with $f_{C12} \geq$ 40 mol% is not an equilibrium state, *i.e.*, micelle structures formed from hydrophobic associations are kinetically-frozen in aqueous solution with added salt.

CONCLUSION

Copolymers of AMPS and C_{12}MAm undergo preferentially intrapolymer hydrophobic association in water, but it depends on the protocol for micellization procedures whether the polymers end up with uni- or multimolecular micelles. Various preparations of aqueous solutions of the polymers were tested to clarify how polymers undergo micellization. In the solid polymer sample purified by reprecipitation followed by lyophilization, kinetically-frozen micelles may already exist. The micelles are not unimolecular but multimolecular, and formed by hydrophobic associations in entangled polymer chains depending on their history. When the solid polymer sample is added to water, kinetically-frozen micelles are simply re-dissolved in water as multimolecular micelles. Virtually unimolecular micelles may be obtained if the solid sample is first dissolved in pure water and the solution is heated to ≥90℃, followed by the addition of a predetermined amount of NaCl at the same temperature to adjust ionic strength of the micelle solution. This was found most effective for eliminating preexisting interpolymer associations. However, micelles formed from polymers with $f_{C12} \geq 40$ mol% were not completely in a single molecular state. The numbers of polymer chains consisting of a micelle for the polymers with f_{C12}=40, 50, 55, and 60 mol% were found 2, 5, 10, and 50, respectively, and increased with f_{C12}. The micelles of polymers with $f_{C12} \geq 40$ mol% are not in equilibrium but kinetically-frozen in aqueous solution with added salt.

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