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Reversible pH-Induced Formation and Disruption of Unimolecular Micelles of an Amphiphilic Polyelectrolyte

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ABSTRACT: Reversible pH-induced formation and disruption of a unimolecular micelle (unimer micelle) of a random copolymer of sodium 2-(acrylamido)-2-methylpropanesulfonate and 11-acrylamidoundecanoic acid (50 mol %) were investigated by static light scattering (SLS), quasi-elastic light scattering, viscometric, ^{1}H NMR spin−spin relaxation, and fluorescence probe techniques. The weight-average molecular weight (M_w) value of the copolymer determined by SLS in a 0.1 M NaCl aqueous solution at pH 7 was virtually the same as that determined in a methanol solution, M_w in 0.1 M NaCl being practically independent of pH in the range $3 < pH < 9$. These findings indicate that the copolymer exists as a single molecular state (unimer) in 0.1 M NaCl over the range $3 < pH < 9$. At $pH < 5$, the copolymer exhibited very small values of the radius of gyration, hydrodynamic radius, reduced viscosity, and spin−spin relaxation time, indicative of the formation of a unimer micelle under acidic conditions. All these values increased significantly with increasing pH in the range $5 < pH < 8$, reaching saturated values near pH 8. These observations indicate that the unimer micelle is disrupted into an open chain conformation at basic pHs. This is the first example of pH-responsive unimer micelles whose formation and disruption are controlled by the selective protonation and deprotonation of carboxyl groups attached at the terminal of polyelectrolyte-bound hydrophobes.

Introduction

Amphiphilic polyelectrolytes undergo self-association in aqueous media driven mainly by hydrophobic interactions competing with electrostatic repulsions within the same polymer chain and/or between different polymer chains.[1−3]

Amphiphilic polyelectrolytes have attracted considerable attention partly because they can be regarded as a simple model for the understanding of the behavior of more complicated biological polymers, given all the important biological macromolecules are polyelectrolytes with amphiphilic nature. A characteristic feature of amphiphilic polyelectrolytes, depending greatly on their molecular architecture, is that a polymer chain can fold back on itself due to completely intrachain hydrophobic associations to form a unimolecular micelle ("unimer micelle") with a higher-order structure, resembling the most common example of proteins adopting a globular-folded tertiary structure.[4,5] This is the case of random copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (NaAMPS) and *N*-dodecylmethacrylamide (C_{12}MAm) with C_{12}MAm contents in the copolymer range 20−50 mol %.[6−8] In the NaAMPS/C_{12}MAm copolymer, hydrophobic associations of C_{12} chains occur completely within the same polymer chain forming intrachain hydrophobic microdomains that undergo secondary association to form a unimer micelle with a third-order structure.[9] This unimer micelle is unique in that it is a compact assembly with a stable structure, existing as such independent of its concentration (even at very high concentrations) without undergoing any change in its association structure. Moreover, the unimer micelle can be "functionalized" by incorporating functional small molecules of hydrophobic nature into the micelle, which can be done either by covalent attachment of target molecules to the polymer chain[10−13] or by direct dissolution of free molecules in the micelle in aqueous media.[14] Such a functionalized unimer micelle provides an opportunity to study physicochemical behavior of the target molecule in unusual microenvironments of the unimer micelle. For example, in the case of chromophore-functionalized unimer micelles, unlike conventional molecular assembly systems, a large modification of photophysical and photochemical behavior of the incorporated chromophores has been observed.[10−13]

Stimuli-responsive polymers have attracted considerable interest because of their potential ability to capture and deliver materials, which may find a variety of pharmaceutical or environmental applications.[15,16] The unimer micelle is so stable that it is difficult to disrupt its micellar structure unless it is transferred from water into a polar organic solvent in which the polymer is soluble and adopts a random coil conformation. If, however, the disruption and formation of the unimer micelle can be controlled by external conditions, it may become a good candidate for a novel stimuli-responsive system. In the present work, we attempted to endow pH responsiveness to the unimer micelle.

For the self-association of amphiphilic polyelectrolytes, the balance of competing hydrophobic and electrostatic interactions is a critical factor, which is primarily a function of the numbers of hydrophobes and charges in the polymer as well as the size of the hydrophobe. In the case of polycarboxylic acids with amphiphilic nature, the number of charged segments relative to the number of hydrophobic segments in the polymer chain varies with the degree of dissociation of carboxyl groups, and hence a pH-induced conformation change is commonly observed.[17−22] In contrast, polysul-

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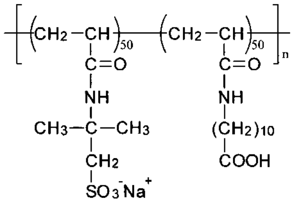
Chart 1. Amphiphilic Copolymer Used in This Study



poly(NaAMPS/AmU)

fonic acids with amphiphilic nature show no such pH dependence of their conformation because they are fully ionized even at very low pHs.[23,24]

McCormick and co-workers[25] reported that a block copolymer of sodium 4-vinylbenzoate and sodium 4-styrenesulfonate, prepared by reversible addition−fragmentation chain transfer polymerization (RAFT), underwent reversible pH-induced micellization in water. This block copolymer is readily soluble in water at intermediate and high pHs and exists as unimeric chains. At low pHs, however, it forms multipolymer micelles due to selective protonation of the poly(4-vinylbenzoic acid) block.

In the present study, we synthesized a random copolymer of NaAMPS and 50 mol % of 11-(acrylamido)-undecanoic acid (AmU) (poly(NaAMPS/AmU) in Chart 1). Our use of AmU was suggested from the work by McCormick and co-workers[26,27] on the pH-dependent solution properties of the copolymers of acrylamide and AmU (≤ 10 mol %). Poly(NaAMPS/AmU) is similar to the NaAMPS/C_{12}MAm copolymer in structure except the hydrophobic group possesses a carboxyl group at the terminal of the hydrophobe. At low pHs, the carboxyl groups in the AmU unit are protonated while the sulfonate residues remain charged.

In this paper, we report on the pH-induced reversible disruption and formation of a unimer micelle formed from poly(NaAMPS/AmU) characterized using static light scattering (SLS), quasi-elastic light scattering (QELS), viscometric, ^{1}H NMR spin−spin relaxation time (T_2), and fluorescence probe techniques.

Experimental Section

Materials. Pyrene and 2,2′-azobis(isobutyronitrile) (AIBN) were recrystallized from methanol. Methanol was dried over 4 Å molecular sieves and distilled. Water was purified with a Millipore Milli-Q system. Other reagents were used as received. Sodium 11-acrylamidoundecanoate (AmU) was prepared according to the method of Gan and co-workers.[28]

Polymer. Copolymerization of sodium 2-(acrylamido)-2-methylpropanesulfonate (NaAMPS) and AmU was carried out by ordinary free-radical polymerization in the presence of AIBN in methanol. A procedure for the copolymerization is as follows: 2-(Acrylamido)-2-methylpropanesulfonic acid (4.14 g, 20 mmol) was neutralized with NaOH (0.89 g, 22 mmol) in 80 mL of methanol, and 5.55 g (20 mmol) of AmU and 16 mg (0.1 mmol) of AIBN were added to this solution. The methanol solution was outgassed on a vacuum line by six freeze−pump−thaw cycles. Copolymerization was carried out at 60 °C for 12 h. The polymerization mixture was poured into a large excess of diethyl ether to precipitate the resulting polymer. The copolymer was purified by reprecipitation from a methanol solution into excess ether and then dissolved in water. The solution was dialyzed against a dilute NaOH aqueous solution (pH 8) for a week. The copolymer was recovered by a freeze-drying technique. The acid groups of the polymer thus obtained were sodium salts of the sulfonate and carboxylate. The copolymer composition was determined from the intensity ratio of ^{1}H NMR resonance bands associated with the methylene protons in the NaAMPS unit (3.32 ppm) and the methylene protons neighboring the amide bond in the AmU unit (2.83 ppm) in D_2O at 95 °C.

Measurements. Gel Permeation Chromatography (GPC). GPC analysis was performed with a Tosoh HLC-8020 equipped with a Tosoh RI-8020 refractive index detector and two TSKgel α-M (Tosoh) columns using a DMF/water (50/50, v/v) mixture containing 50 mM of LiBr as the eluent at a flow rate of 1.0 mL/min. Molecular weights of the copolymer were calibrated with sodium polystyrenesulfonate standard samples.

Static Light Scattering (SLS). SLS measurements were performed at 25 °C with an Otsuka Electronics Photal DLS-7000DL light scattering spectrometer equipped with an Ar^+ laser (50.0 mW at 488 nm). Weight-average molecular weight (M_w), *z*-average radius of gyration (R_g), and the second virial coefficient (A_2) values were estimated from the relation[29]

$$\frac{KC_p}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{1}{3}\langle R_g{}^2\rangle q^2\right) + 2A_2C_p \qquad (1)$$

where C_p is the polymer concentration, $K = 4\pi^2 n^2 (dn/dC_p)^2/N_A\lambda^4$ with *n* being the refractive index of solvent, N_A Avogadro's number, λ the wavelength (=488 nm), and dn/dC_p the refractive index increment against C_p, R_θ is the Rayleigh ratio, and $q = (4\pi n/\lambda)\sin(\theta/2)$ with θ being the scattering angle. By measuring R_θ for a set of C_p and θ, values of M_w, R_g, and A_2 were estimated from Zimm plots. Benzene was used for the calibration of the instrument. Values of dn/dC_p were determined with an Otsuka DRM-1020 differential refractometer.

Quasi-Elastic Light Scattering (QELS). QELS data were obtained at 25 °C with an Otsuka Electronics Photal DLS-7000DL light scattering spectrometer equipped with a multi-τ, digital time correlator (ALV-5000E). An Ar^+ laser (50.0 mW at 488 nm) was used as a light source. To obtain the relaxation time distribution, $\tau A(\tau)$, the inverse Laplace transform (ILT) analysis was performed using the algorithm REPES.[30,31]

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

Here, τ is the relaxation time and $g^{(1)}(t)$ is the normalized autocorrelation function. The relaxation rate, $\Gamma = \tau^{-1}$, is a function of C_p and θ.[32] The diffusion coefficient in the limit of zero angle, $D_z(C_p)$, was calculated from $D_z(C_p) = (\Gamma/q^2)_{q\to 0}$. The translational diffusion coefficient (D_0) was calculated from

$$D_0 = D_z(C_p)/(1 + k_d C_p) \qquad (3)$$

where k_d is the hydrodynamic virial coefficient. The hydrodynamic radius, R_h, is given by the Stokes−Einstein equation, $R_h = k_B T/(6\pi\eta D_0)$, where k_B is Boltzmann's constant, *T* is the absolute temperature, and η is the solvent viscosity. The details of QELS instrumentation and theory are described in the literature.[33−35]

Viscosity. Viscosity measurements were carried out at 25 °C using a modified Ubbelohde type viscometer.

^{1}H NMR. ^{1}H NMR spectra were measured using a Bruker DRX-500 spectrometer operating at 500 MHz in D_2O. Chemical shifts were determined by using 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid as the internal reference. ^{1}H NMR spin−spin relaxation times (T_2) were determined by the Carr−Purcell−Meiboom−Gill (CPMG) method using a $\{90°_x\tau(180°_y 2\pi)_n\}$ pulse sequence.[36] A 90° pulse of 13.85 μs was calibrated and used for the measurement. Peak intensities at 12 different numbers of the 180° pulse were measured.

Fluorescence. Fluorescence spectra were recorded on a Shimadzu RF-5000 fluorescence spectrophotometer. Pyrene was used as a fluorescence probe. A pyrene-saturated aqueous stock solution was prepared as previously.[37] The concentration of pyrene was determined from UV absorption at 334 nm using a molar extinction coefficient of 5.4×10^4 L mol^{-1} cm^{-1}. Emission spectra of pyrene were measured with excitation at 334 nm. Excitation and emission slit widths were maintained at 25.0 and 1.0 nm, respectively. Excitation spectra were monitored at 390 nm.

Table 1. Weight-Average Molecular Weights (M_w) and Polydispersity Index (M_w/M_n) for the Copolymer

$M_w{}^a$ (GPC) $\times 10^{-4}$	$M_w/M_n{}^a$	M_w(SLS) $\times 10^{-4}$			
		pH 3[b]	pH 7[b]	pH 9[b]	methanol[c]
9.9	2.26	11.4	12.8	14.8	11.5

[a] Determined by GPC using a mixed solvent of water and DMF (50/50, v/v) containing 50 mM LiBr as the eluent. [b] Determined by SLS in 0.1 M NaCl aqueous solutions. [c] Determined by SLS in methanol containing 0.1 M $LiClO_4$.

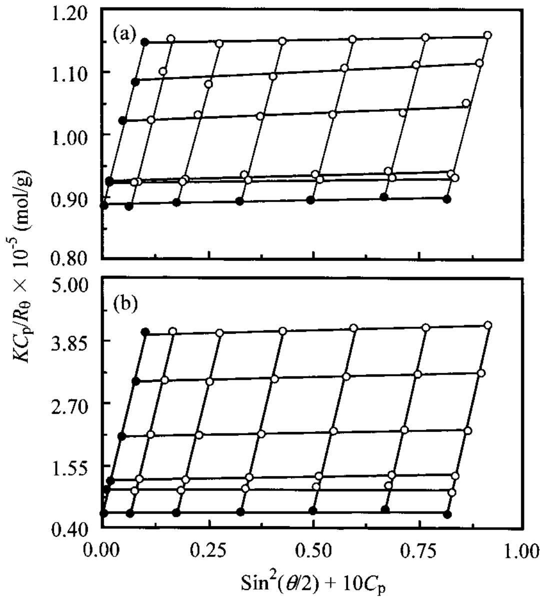


Figure 1. Representative examples of Zimm plots for the copolymer in 0.1 M NaCl at pH 3 (a) and 9 (b) at angles from 30° to 130° with a 20° increment. The polymer concentration was varied from 1.0 to 10 g/L.

Preparation of Sample Solutions. Sample solutions for light scattering and viscosity measurements were prepared as follows: A solid polymer sample (sodium salt form of the carboxylate) recovered by freeze-drying was dissolved in water containing 0.1 M NaCl or in methanol containing 0.1 M $LiClO_4$, and the solutions were allowed to stand for a day for equilibration. Sample solutions were filtered with a 0.2 μm pore size membrane filter prior to measurement. Sample solutions for fluorescence measurements were prepared by dissolving a solid polymer sample in a 0.1 M NaCl aqueous solution containing 2.2×10^{-7} M pyrene, and the solutions were allowed to stand for a day for equilibration. The solution pH was adjusted by adding a proper amount of aqueous NaOH or HCl. For ^{1}H NMR relaxation measurements, sample solutions of the polymer at $C_p = 5.0$ g/L were prepared in D_2O containing 0.1 M NaCl, and pD was adjusted with a D_2O solution of DCl or NaOD. The final pD value was determined from the relation pD = pH + 0.4.[38]

Results and Discussion

Basic Characteristics of the Copolymer. The copolymer obtained in the sodium salt form of the acid groups was soluble in 0.1 M NaCl aqueous solutions at all pHs studied (pH 3−9). The content of the AmU unit in the copolymer was determined to be 50 mol % by ^{1}H NMR. M_w and polydispersity index (M_w/M_n) of the copolymer estimated from GPC are listed in Table 1.

Light Scattering. Parts a and b of Figure 1 show representative examples of Zimm plots for poly(NaAMPS/AmU) in 0.1 M NaCl aqueous solutions at 25 °C at pH 3 and 9, respectively.

M_w values for poly(NaAMPS/AmU) determined by SLS at pH 3, 7, and 9 are fairly close to M_w estimated by GPC (Table 1). Furthermore, M_w values for poly(NaAMPS/AmU) determined by SLS in water (0.1 M NaCl) and in methanol (0.1 M $LiClO_4$) are close to each other (Table 1). In methanol, hydrophobic association is absent, and therefore each polymer chain should exist as a single molecule. These observations indicate that there is no interchain association in 0.1 M NaCl aqueous solutions. M_w values for the copolymer determined by SLS in 0.1 M NaCl aqueous solutions are plotted in Figure 2a against solution pH. In the whole range of pH examined ($3 \le \text{pH} \le 9$), M_w is practically independent of pH (i.e., $M_w = (1.24 \pm 0.24) \times 10^5$), indicating that there is no interchain association at any pHs studied.

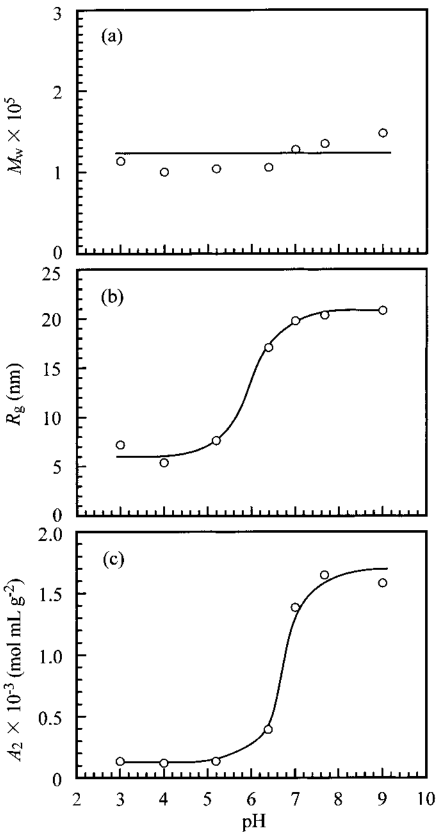


Figure 2. Weight-average molecular weight (M_w) (a), radius of gyration (R_g) (b), and the second virial coefficient (A_2) (c) for the copolymer as a function of pH in 0.1 M NaCl.

In Figure 2b, R_g values for poly(NaAMPS/AmU) in 0.1 M NaCl aqueous solutions estimated from SLS are plotted against pH. At low pHs, R_g values are very small but R_g starts to increase near pH 5 with increasing pH, reaching a constant value of $R_g = 20$ nm near pH 8. It is important to note that R_g increases with increasing pH in the region $5 < \text{pH} < 8$ whereas M_w remains almost constant (Figure 2a). At pH < 5, most of the pendent alkyl carboxylate groups are protonated, and therefore, the hydrophobic association of pendent alkyl groups occurs within the polymer chain, forming a highly compact conformation (i.e., a unimer micelle). At pH > 8, on the other hand, the degree of ionization for the pendent carboxyl group is sufficient to prevent electrostatically the hydrophobic interaction between the pendent alkyl chains, thus the polymer existing in an open chain conformation.

Morishima et al.[6−8] have shown that NaAMPS/C_{12}-MAm copolymers with C_{12}MAm contents (f_{C12}) ranging $20 < f_{C12} \le 50$ mol % have an extremely strong tendency

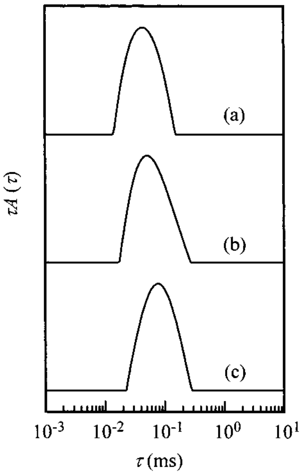


Figure 3. Examples of QELS relaxation time distributions for the copolymer at $C_p = 1.0$ g/L in 0.1 M NaCl at pH 3 (a), 6 (b), and 9 (c) at $\theta = 90°$.

for intrachain hydrophobic association in water. In particular, when f_{C12} is 50 mol %, unimer micelles with a highly compact third-order structure are formed.[9] Similar to the case of the NaAMPS/C$_{12}$MAm copolymer, poly(NaAMPS/AmU) in the present study forms a compact unimer micelle under acidic conditions.

Figure 2c shows A_2 values for poly(NaAMPS/AmU) plotted against solution pH. Values of A_2 are very small at pH < 5, but A_2 starts to increase near pH 5 with increasing pH, reaching a practically constant value near pH 8. This pH-dependent profile of A_2 is very similar to that of R_g (Figure 2b). The increase in the A_2 value with an increase in pH in the region 5 < pH < 8 is also an indicative of the chain expansion occurring as the pendent carboxyl groups are more ionized.

The pH-dependent conformational change is reflected in the hydrodynamic radius (R_h). The formation of unimer micelle of poly(NaAMPS/AmU) under acidic conditions was also observed by QELS measurements. Distributions of τ for the copolymer in 0.1 M NaCl aqueous solutions of $C_p = 1.0$ g/L were measured at different pHs at varying scattering angles ($\theta = 30-130°$). Figure 3 shows examples of the distributions observed at a scattering angle of 90°. The distributions are unimodal independent of pH. Γ at each peak top of the unimodal distributions was plotted against the square of the scattering vector (q^2) (data not shown). These plots yielded a straight line passing through the origin, indicating that the observed relaxation times are attributed to a diffusive mode. The $D_z(C_p)$ values estimated from the slope of the $\Gamma - q^2$ plots were found to be in good agreement with those calculated from Γ at the peak top of the QELS relaxation time distribution obtained at a fixed θ of 90°. Therefore, we used $D_z(C_p)$ values calculated from Γ at the peak top of the relaxation time distribution. As $D_z(C_p)$ depends on C_p (eq 3), we measured QELS at various polymer concentrations ($C_p = 1.0-10$ g/L) and estimated D_0 by extrapolating C_p to zero. Using D_0 thus estimated, we calculated R_h from the Stokes−Einstein relation. Figure 4a shows R_h as a function of pH. At pH < 5, R_h values are constant at ca. 6.1 nm. In the case of the NaAMPS/C$_{12}$MAm copolymer ($f_{C12} = 50$ mol %, $M_w = (1.5-3.0) \times 10^4$), an R_h value of ca. 5 nm is reported for a unimer micelle in a 0.05 M NaCl aqueous solution at $C_p = 1.0$ g/L.[39] Given M_w of poly(NaAMPS/AmU) is larger than that of the NaAMPS/C$_{12}$MAm copolymer by nearly 1 order of magnitude, the R_h of ca. 6.1 nm for poly(NaAMPS/AmU) at low pHs is a reasonable value for a unimer micelle formed from poly(NaAMPS/AmU).

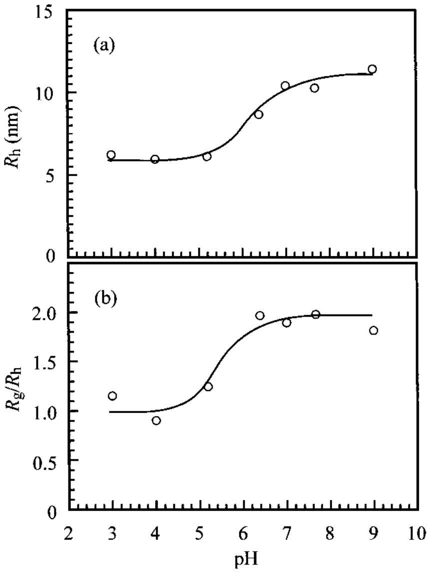


Figure 4. Hydrodynamic radius (R_h) (a) and R_g/R_h (=ρ) (b) for the copolymer as a function of pH in 0.1 M NaCl.

As pH is increased, the R_h value starts to increase near pH 5 (Figure 4a). This pH value for the onset of the R_h increase agrees with the cases of R_g and A_2 (Figure 2b,c). At pH 9, R_h for poly(NaAMPS/AmU) is 11 nm, being about 1.8 times larger than R_h values at pH < 5. This means that the specific hydrodynamic volume (V_h) for the copolymer varies about 6 times when pH is changed from 5 to 9 (because $V_h \propto R_h^3$).

The ratio of R_g/R_h (=ρ) at $C_p \rightarrow 0$ is an important parameter depending on the chain conformation. It is known that the theoretical value of the ρ parameter for a homogeneous sphere is 0.778, and it increases substantially for a less dense structure and polydisperse solution.[40−43] For example, a ρ value of 2.94 is reported for a copolymer of acrylamide and acrylic acid in 0.5 M NaCl at pH 7.3−7.5.[44] Also, ρ values of 2.16 and 1.59 are reported for poly(sodium sulfoethyl methacrylate) and poly(sodium sulfododecyl methacrylate) in 0.1 M NaCl aqueous solutions, respectively.[45] Figure 4b shows the pH dependence of ρ at $C_p \rightarrow 0$ for poly(NaAMPS/AmU). At acidic pHs, ρ values are relatively close to the theoretical value for a uniform hard sphere, suggesting that the unimer micelle of poly(NaAMPS/AmU) is nearly spherical and uniform. On the other hand, larger values ($\rho \approx 2$) were observed at basic pHs, being typical of nonspherical objects such as elongated species. These observations indicate that a transition from a contracted to an expanded conformation occurs as pH is increased from an acidic to basic region.

Viscosity Studies. To investigate the effect of pH on solution viscosity for poly(NaAMPS/AmU), the reduced viscosity (η_{sp}/C_p) at $C_p = 1.0$ g/L in 0.1 M NaCl was measured as a function of pH at 25 °C (Figure 5). At acidic pHs, the reduced viscosities are small, scarcely depending on solution pH, which indicates that the copolymer chain adopts a collapsed conformation in a low-pH region. In the case of the NaAMPS/C$_{12}$MAm copolymer ($f_{C12} = 50$ mol %, $M_w = 1.5 \times 10^4$, $M_w/M_n = 1.9$), the reduced viscosity in 0.1 M NaCl at $C_p = 1.0$ g/L is reported to be ca. 0.007 L/g.[6,46] The reduced viscosities for poly(NaAMPS/AmU) under acidic condi-

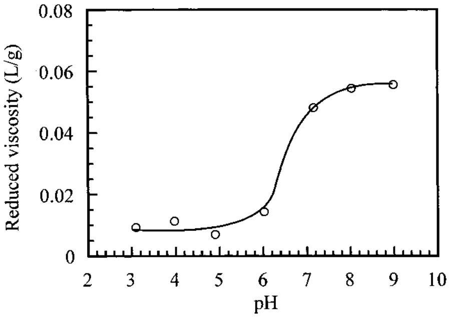


Figure 5. Reduced viscosity (η/C_p) at C_p = 1.0 g/L for the copolymer as a function of pH in 0.1 M NaCl at 25 °C.

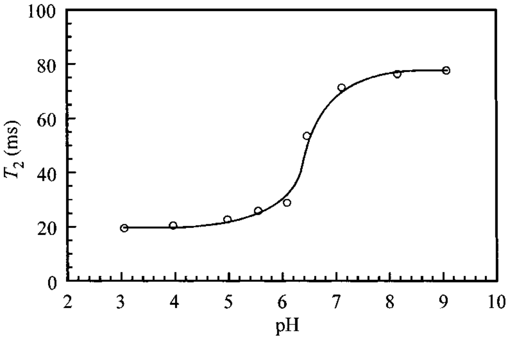


Figure 6. Spin−spin relaxation time (T_2) for the copolymer as a function of pH in D_2O containing 0.1 M NaCl at C_p = 5.0 g/L.

tions (η_{sp}/C_p = 0.007−0.011 L/g) are close to that for the NaAMPS/C_{12}MAm copolymer that forms a unimer micelle. The reduced viscosity for poly(NaAMPS/AmU) increases with increasing solution pH, reaching a constant value of ca. 0.055 L/g at pH > 8. The reduced viscosity in the high-pH region (pH > 8) is about 6 times larger than that in the low-pH region (pH < 5). The observation that the reduced viscosity sharply increases in the range 5 < pH < 8 indicates that the compact conformation of the polymer chain expands into an open conformation in this pH region. This finding in the reduced viscosity agrees well with the observations from the light scattering measurements.

^{1}H NMR Relaxation Time. Spectral changes in NMR, such as chemical shift and spin−lattice or spin−spin relaxation times, provide useful information about the association phenomena of surfactants and hydrophobically modified water-soluble polymers.[47,48] It is known that ^{1}H NMR relaxation times are influenced by changes in the dynamic motion of protons. T_2 decreases as the molecular motion decreases. To obtain information about motional restriction of alkyl chains when they form hydrophobic microdomains, we performed ^{1}H NMR relaxation time measurements with poly(NaAMPS/AmU) in D_2O under various pH conditions. For the determination of T_2, we chose a resonance peak at ca. 1.3 ppm assigned to the methylene ($-CH_2-$) protons in the pendent alkyl chains of the AmU unit because the peak is relatively intensive and isolated from the other peaks. Figure 6 shows the variation of T_2 as a function of pH. At pH < 5, T_2 shows small values of ca. 20 ms, but it starts to increase near pH 5 with increasing pH. This observation is interpreted as the onset of the disruption of a unimer micelle into an open chain conformation. At pH 8, T_2 reaches a constant value of ca. 77 ms. This longer T_2 at pH ≥ 8 is indicative of the increased motional freedom of the pendent alkyl groups in the AmU unit in an open chain conformation.

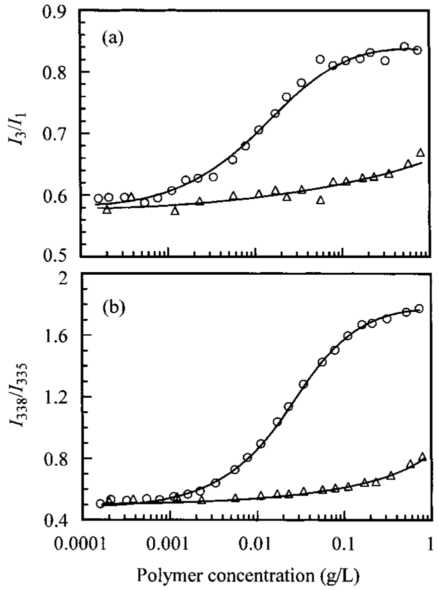


Figure 7. Ratios of I_3/I_1 (a) and I_{338}/I_{335} (b) in pyrene fluorescence emission and excitation spectra, respectively as a function of polymer concentration in 0.1 M NaCl at pH 4 (○) and 8 (△).

Fluorescence. In Figure 7a, the intensity ratio of the third to first vibronic peaks (I_3/I_1) in pyrene fluorescence spectra for 2.2 × 10^{-7} M of pyrene solubilized in 0.1 M NaCl aqueous solutions at pH 4 and 8 in the presence of poly(NaAMPS/AmU) were plotted against C_p. It is well-established that I_3/I_1 is sensitive to the polarity around pyrene, i.e., I_3/I_1 being larger in less polar media.[49,50] At pH 4, the I_3/I_1 ratio in the presence of the copolymer is in the neighborhood of 0.59 at the lowest C_p (0.0002 g/L), which is practically the same as that for molecular pyrene in water. As C_p is increased, the I_3/I_1 ratio commences to increase significantly at C_p = 0.001 g/L, reaching a nearly constant value of 0.82 at C_p = 0.2 g/L. This observation implies that poly(NaAMPS/AmU) is able to incorporate pyrene molecules into hydrophobic microdomains formed from the self-association of the pendent alkyl carboxylic groups under acidic conditions and that the number of the hydrophobic microdomains is large enough to incorporate all pyrene molecules present in the solution when C_p is larger than 0.2 g/L at pH 4. On the other hand, at pH 8, the I_3/I_1 ratio in the presence of the copolymer is almost constant at 0.58 at C_p < 0.1 g/L, but the ratio starts to increase slightly at C_p = 0.1 g/L with increasing C_p. At pH 8, the pendent carboxyl groups in the copolymer are deprotonated. Since the content of the AmU unit in the copolymer is as high as 50 mol %, the polymer chain is crowded with undecanoate chains. In such a situation, all alkyl chains in the AmU unit may not be completely kept from associating together, but some alkyl chains may interact to form hydrophobic microenvironments along the polymer chain. Some pyrene molecules may be trapped in the hydrophobic microenvironment, thus showing a slight increase in I_3/I_1 with increasing C_p at pH 8.

Excitation spectra of 0.1 M NaCl aqueous solutions of pyrene probes in the presence of the copolymer at varying C_p were measured at pH 4 and 8 (data not shown). At pH 4, the excitation spectra showed peaks associated with the (0−0) band of pyrene at 335 nm in

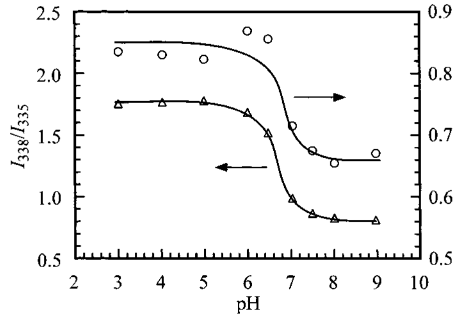


Figure 8. Ratios of I_{338}/I_{335} (△) and I_3/I_1 (○) in pyrene excitation and emission spectra, respectively, as a function of pH in the presence of the copolymer in 0.1 M NaCl at C_p = 1.0 g/L.

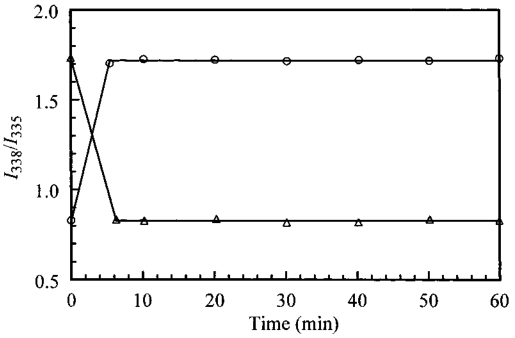


Figure 9. Plot of I_{338}/I_{335} in pyrene excitation spectra vs time in the presence of the copolymer at C_p = 1.0 g/L in 0.1 M NaCl. Solution pH 3 (△) and 9 (○) were changed rapidly to pH 9 (△) and 3 (○), respectively, and the solution was subjected to fluorescence measurement. The dead time for the measurement, including the pH shift, is about 6 min.

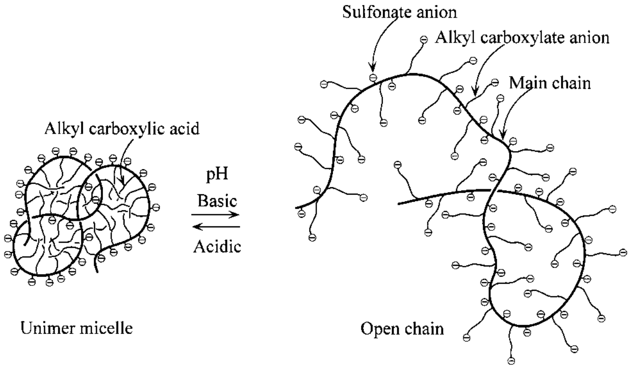


Figure 10. Schematic representation of pH-induced formation and disruption of a unimer micelle of the copolymer.

a low-C_p regime and the peak shifted to 338 nm at higher C_p. It is known that the (0−0) band in pyrene excitation spectra in water shifts to longer wavelengths when pyrene is solubilized in hydrophobic microdomains.[51−54] Thus, we estimated the ratio of the intensity at 338 nm relative to that at 335 nm (I_{338}/I_{335}) for 0.1 M NaCl aqueous solutions of the copolymer and plotted in Figure 7b as a function of C_p at pH 4 and 8. As C_p is increased, the I_{338}/I_{335} ratio commences to increase near C_p = 0.001 g/L at pH 4. At pH 8, the I_{338}/I_{335} ratio shows a slight tendency to increase with C_p when C_p > 0.1 g/L. The I_{338}/I_{335} ratio changes parallel with the I_3/I_1 ratio as can be seen in Figure 7a,b.

In Figure 8, the I_{338}/I_{335} and I_3/I_1 ratios estimated from excitation and emission spectra, respectively, for pyrene probes solubilized in 0.1 M NaCl aqueous solutions in the presence of the copolymer at C_p = 1.0 g/L are plotted against pH. The I_{338}/I_{335} ratio in the presence of the copolymer is practically constant at 1.74 in a lower pH region (pH < 5), but the I_{338}/I_{335} ratio decreases near pH 5 with increasing pH, reaching a small value (0.81) at pH = 8. When the solution pH was decreased from 9 to 3 and subsequently increased back to 9, the pH-induced emission and excitation spectral changes were found to be completely reversible without hysteresis.

The time dependence of the pH-responsive behavior of poly(NaAMPS/AmU) was studied by monitoring changes in the I_{338}/I_{335} ratio upon pH changes from 3 to 9 and back from 9 to 3 (Figure 9). The addition of aqueous NaOH to a copolymer solution of an initial pH of 3 (I_{338}/I_{335} = 1.73) to pH 9 results in a fast decrease in the I_{338}/I_{335} ratio, reaching a constant value of 0.81. This decrease in I_{338}/I_{335} occurs within the dead time of the experiment (ca. 6 min), and no further change in the I_{338}/I_{335} ratio was observed for hours (not all plots are shown in Figure 9). Upon decreasing pH from 9 to 3 by adding aqueous HCl, a similarly fast change in the I_{338}/I_{335} ratio from 0.81 to a final value of 1.73 was observed within the dead time of the experiment. These observations indicate that pyrene probes are released upon disruption of unimer micelles (i.e., hydrophobic microdomains) and solubilized again upon formation of the micelle and that this disruption and formation of unimer micelles occur within 6 min or shorter. Thus, it is reasonable consider that a conformational change from a unimer micelle to an open chain and vice versa occur within 6 min or shorter, reaching an equilibrium conformation. It should be noted that the rate of the conformational change for poly(NaAMPS/AmU) is considerably faster than in the case of micelles formed from a block copolymer of polystyrene (PS) and poly(methacrylic acid) (PMA) where PS and PMA form a kinetically frozen core and shell, respectively, in water.[55]

A hypothetical model for the pH-induced disruption and formation of a unimer micelle due to the deprotonation and protonation of the AmU units is conceptually illustrated in Figure 10. At basic pHs, the electrostatic repulsion between the pendent alkyl carboxylate anions causes the polymer chain to adopt an open chain conformation. At acidic pHs, on the other hand, the intrachain association of the protonated pendent alkyl carboxyl groups causes the polymer chain to collapse into a unimer micelle.

Conclusions

The random copolymer of NaAMPS and AmU (50 mol %) forms a unimer micelle in 0.1 M NaCl aqueous solutions at low pHs whereas the copolymer adopts an open chain conformation at high pHs. Thus, the copolymer exhibits pH-induced disruption of the unimer micelle into an open chain conformation. These conclusions are based on the observations that (1) M_w determined by SLS for a 0.1 M NaCl aqueous solution at pH 7 was virtually the same as that for a methanol solution, (2) M_w values in 0.1 M NaCl were practically independent of pH, (3) at pH < 5, the copolymer showed small values of R_g, R_h, η_{sp}/C_p, and T_2, and (4) all these values increased significantly upon increase in pH in the range 5 < pH < 8, reaching saturated values near pH 8. The transition from the unimer micelle to the open chain and vice versa were found to occur within 6 min or less. This is the first example of pH-responsive unimer micelles, and the copolymer may provide a novel pH-responsive system based on a unimer micelle.

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