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Association–Dissociation Equilibrium of an Amphiphilic Polyelectrolyte in Aqueous Solution

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Summary: The association–dissociation equilibrium in micellar solutions of an amphiphilic statistical copolymer was investigated by three different experimental techniques of sedimentation equilibrium, dynamic light scattering, and fluorescence. To analyze these experimental data, a novel method was proposed to determine the aggregation number, association constant, intermolecular interaction, and micellar structure. The application of this method to data from the three experimental techniques provided us consistent results characterizing the association–dissociation equilibrium in the present amphiphilic copolymer solutions.

Keywords: aggregation number; amphiphilic polyelectrolyte; association constant; association–dissociation equilibrium; dynamic light scattering; fluorescence; sedimentation equilibrium

Introduction

Amphiphilic polyelectrolytes comprise ionizable and hydrophobic monomer units.[1] In aqueous media, while ionizable monomer units tend to separate each other by strong electrostatic repulsion, hydrophobic monomer units tend to associate each other by strong hydrophobic interaction. For random copolymers consisting of ionizable and hydrophobic monomer units, it is rather difficult to find their conformations fulfilling both requirements of ionizable and hydrophobic monomer units, so that they may take some frustrated conformations in aqueous media. Recently, Hashidzume et al.[2] have investigated the micellar structure formed by statistical copolymers of sodium 2-(acrylamido)-2-methylpropane sulfonate (AMPS) and *n*-hexyl methacrylate (C6) (p(AMPS/C6); cf. Figure 1) in aqueous solution by light scattering and fluorescence. They found that the copolymer formed star-like unicore micelles at low degree of polymerization or low hydrophobic content, and also that not all hydrophobic side chains were incorporated into the hydrophobic core.

The previous study, however, did not explicitly consider the association–dissociation equilibrium of the statistical copolymer. Strictly speaking, the aggregation number is dependent on the polymer concentration in the equilibrium, and the dependence must be considered in the structural analysis of the micelle formed. However, the consideration needs the analysis of solution properties of finite concentrations where the intermolecular interaction affects the properties. The analysis is not an easy task.

In this study, we have investigated the association–dissociation equilibrium of the statistical copolymer p(AMPS/C6) in aqueous solution by sedimentation equilibrium, dynamic light scattering, and fluorescence. The experimental data obtained have been analyzed by a novel method to determine the aggregation number, association constant, and intermo+lecular interaction parameter of p(AMPS/C6).

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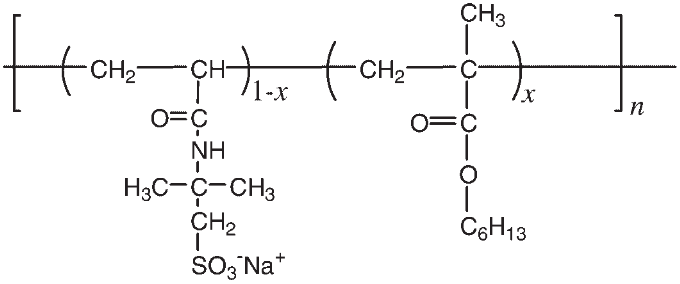

Figure 1.
Chemical Structure of p(AMPS/C6).

Experimental Part

A sample of the copolymer p(AMPS/C6) was prepared by the RAFT copolymerization.[2] The hydrophobic content (the mole fraction x) was estimated to be 0.32 by H^1 NMR and the weight-average molecular weight M_1 of the unimer was determined to be 2.2×10^4 in methanol with 0.2M $LiClO_4$ by sedimentation equilibrium. Sedimentation equilibrium measurements were also performed for 0.1 M aqueous NaCl solutions of the p(AMPS/C6) sample over a wide concentration c range at 25 °C to obtain the concentration distribution $c(r)$ under the centrifugal field; r is the radial distance from the center of revolution. From $c(r)$, the apparent molecular weight M_{app} and also $Z(r_{\mathrm{m}})$ defined by[3,4]

$$M_{\mathrm{app}}^{-1} = \frac{\omega^2 (r_{\mathrm{b}}^2 - r_{\mathrm{a}}^2) c_0 (\partial\rho/\partial c)}{2RT(c_{\mathrm{b}} - c_{\mathrm{a}})} \text{ and}$$

$$Z(r_{\mathrm{m}}) = \frac{\omega^2 (\partial\rho/\partial c)}{2RT(d\ln c/dr^2)_{r_{\mathrm{m}}}} \qquad (1)$$

were calculated as functions of c. Here, ω is the angular velocity of the rotor, r_{b}, r_{a}, and r_{m} are the radial distances from the center of revolution to the cell bottom, the meniscus, and the middle of the solution, respectively, c_{b} and c_{a} are the polymer mass concentrations at r_{b} and r_{a} respectively, and c_0 is the solution concentration under no centrifugal field; RT is the gas constant multiplied by the absolute temperature, and $(\partial\rho/\partial c)$ is the specific density increment of the dialyzed solution.

Fluorescence decays emitted from pyrene solubilized in 0.1 M aqueous NaCl solutions of p(AMPS/C6) were measured to estimate the average number $\bar{n}$ of pyrene molecules within a hydrophobic core formed by hexyl groups.[2] The detailed procedure and an example of the decay curve are shown in ref 2. The number ν_{c} of the hydrophobic core per chain in the solution was estimated from this $\bar{n}$ using $\nu_{\mathrm{c}} = ([\mathrm{Py}]/\bar{n})/(1000c/M_1)$ with the molar concentration [Py] of solubilized pyrene.[5]

Dynamic light scattering measurements were made to estimate the first cumulant Γ of the fast-relaxation component as a function of the magnitude of the scattering vector k.[2] An example of the relaxation spectrum is given in ref. 2. The apparent hydrodynamic radius $R_{\mathrm{H,app}}$ divided by the weight average molar mass M_{w} was obtained from Γ by the equation[4,6]

$$\Gamma = \frac{k_{\mathrm{B}} T (1 - \bar{\upsilon} c)}{6\pi\eta_0 M_{\mathrm{app}}} \frac{M_{\mathrm{w}}}{R_{\mathrm{H,app}}} k^2 + O(k^4) \qquad (2)$$

with the Boltzmann constant k_{B}, the partial specific volume $\bar{\upsilon}$ [$= \rho_0^{-1}(1 - \partial\rho/\partial c)$; ρ_0: the solvent density] of the polymer, the solvent viscosity η_0, and the apparent molecular weight M_{app} determined by sedimentation equilibrium.

Results

Figure 2 shows by filled circles the concentration dependence of M_{app} for 0.1 M aqueous NaCl solutions of our p(AMPS/C6) sample at 25 °C. Although not shown

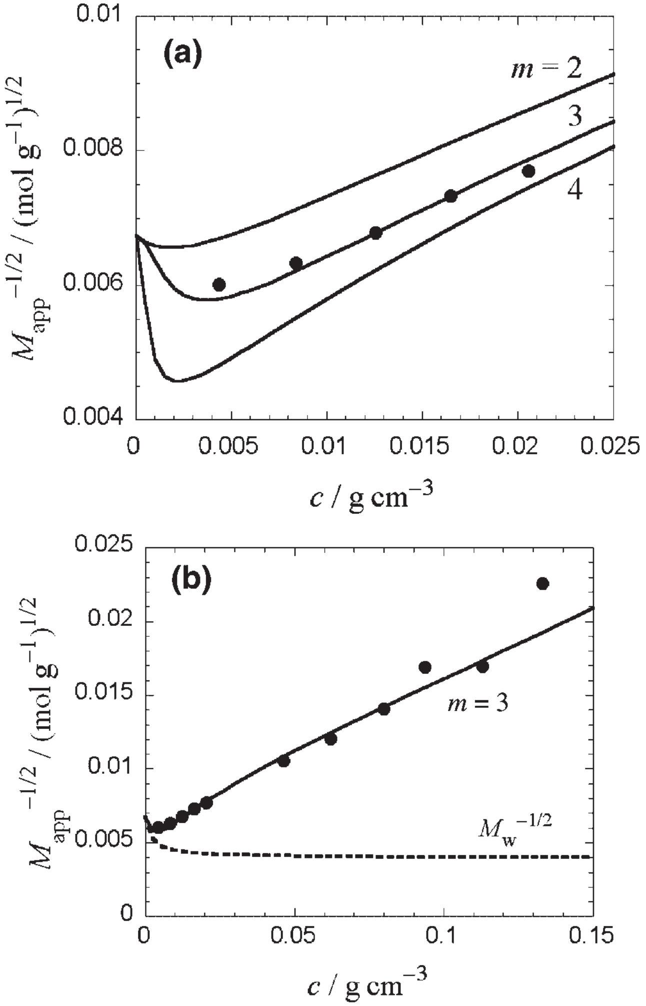

Figure 2.
Concentration dependence of the apparent molecular weight M_{app} for the p(AMPS/C6) sample in 0.1 M aqueous NaCl at 25 °C in a dilute region (a) and in a wide concentration range (b); circles, experimental data; solid curves, theoretical values calculated by eq 3.

here, the quantity $Z(r_m)$ (cf. eq 1) for the same solutions agreed with M_{app} within experimental errors. The simple extrapolation of data in a dilute region shown in Panel a gives the weight average molar mass M_w of 3.2×10^4 (cf. eq 3 given below). If the solution contains aggregate and unimer, this M_w value is not for the aggregate but for the mixture, so that M_w/M_1 ($\sim$1.5) for the partially associated polymer sample does not necessarily provide the proper aggregation number.

Fluorescence decay curves for pyrene-solubilized p(AMPS/C6) solutions give us the average number $\overline{n}$ of pyrene molecules within a hydrophobic core formed by hexyl groups and then the number ν_c of the hydrophobic core per chain in 0.1 M aqueous NaCl solution. Figure 3 displays the polymer concentration dependence of ν_c in a dilute region (filled circles). The c dependence is rather weak. It was difficult to estimate $\overline{n}$ for more concentrated solutions because we had to increase the

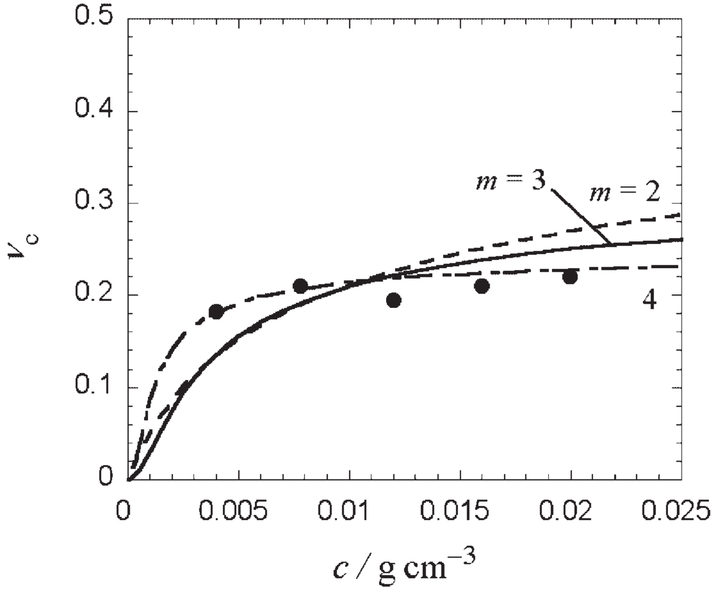

Figure 3.
Concentration dependence of the number ν_c of the hydrophobic core per chain of the p(AMPS/C6) sample in 0.1 M aqueous NaCl at 25 °C.

pyerene concentration [Py] too high to determine [Py] by UV absorption spectroscopy. If the asymptotic value of ν_c at high c is 0.2 and p(AMPS/C6) forms a uni-core micelle, the aggregation number of the micelle may be 5 (=1/0.2), but we will make more rigorous argument in the next section to estimate the aggregation number using both sedimentation equilibrium and fluorescence data.

The dynamic light scattering results are shown in Figure 4, where $R_{\mathrm{H,app}}/M_{\mathrm{w}}$ is plotted against c by filled circles. The quantity $R_{\mathrm{H,app}}/M_{\mathrm{w}}$ is affetced by the interparticle hydrodynamic interaction, so that we have to argue the micellar conformation with $R_{\mathrm{H,app}}/M_{\mathrm{w}}$ data considering this effect. This argument is also made in the next section.

Discussion

To estimate the aggregation number m of the micelle, we consider the following association–dissociation equilibrium[7]

$$\text{unimer} \overset{K_m}{\longleftrightarrow} m\text{–mer}$$

where K_m is the association constant of m-mer. We here do not consider the polydispersity in the aggregation number of the micelle. The weight fraction w_1 of the unimer can be calculated by the equation $1 - w_1 = mK_m w_1^m (1000c/M_1)^{m-1}$ where M_1 is the molecular weight of the unimer (=2.2×10^4 in the present case).

The apparent molecular weight M_{app} [or $Z(r_{\mathrm{m}})$] is then calculated by

$$M_{\mathrm{app}}^{-1} = [w_1 + (1 - w_1)m]^{-1} M_1^{-1} + 2(\Gamma_{2,0} + A_{2,\mathrm{w}})c \qquad (3)$$

where $\Gamma_{2,0}$ is the apparent second virial coefficient for the hard-core potential, and $A_{2,\mathrm{w}}$ is the second virial coefficient with respect to the electrostatic plus hydrophobic interactions. Sato et al.[4] proposed a thermodynamic perturbation theory based on the scaled particle theory for polydisperse spherocylinders. Utilizing their theory, we write

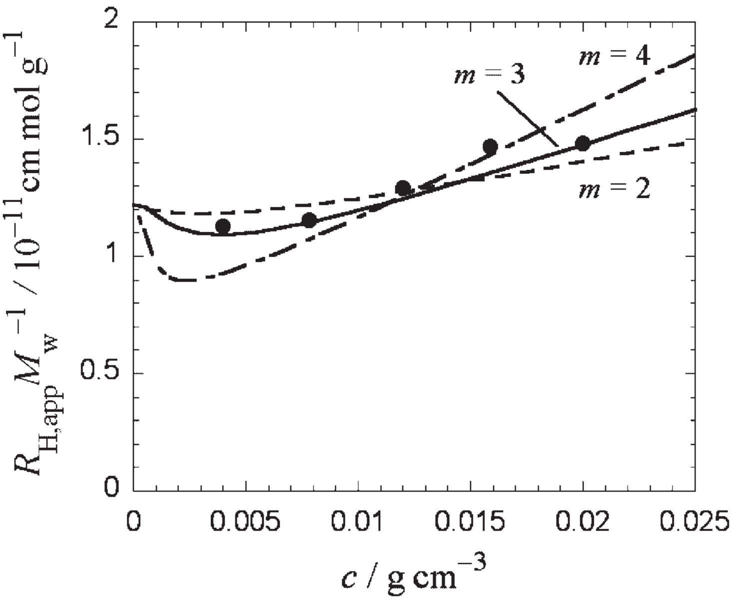

Figure 4.
Concentration dependence of $R_{H,app}/M_w$ for the p(AMPS/C6) sample in 0.1 M aqueous NaCl at 25 °C.

$$\Gamma_{2,0} = \frac{\pi d^2 N_A}{4M_L}\left[\frac{F_6^\circ}{dM_L} + \frac{F_2^\circ}{M_w} + \frac{F_7^\circ}{M_n} - \left(\frac{1}{M_n} - \frac{1}{M_w}\right)F_2^\circ(1+\phi F_2^\circ)\right] \tag{4}$$

where d, M_L, and ϕ are the hard-core diameter, the molar mass per unit contour length, and volume fraction ($= \pi d^2 N_A c / 4M_L$) of spherocylinders, and M_n is the number average molar masse; N_A is the Avogadro constant. The functions F_2, F_6, and F_7 are defined by

$$F_2^\circ \equiv \frac{8 - 7\phi + 3\phi^2}{3(1-\phi)^3}, F_6^\circ \equiv 2\frac{1+\phi}{(1-\phi)^4},$$
$$F_7^\circ \equiv \frac{2}{3}\left[\frac{4+\phi+\phi^2}{(1-\phi)^4}\right] \tag{5}$$

On the other hand, $A_{2,w}$ for amphiphilic copolymers may be calculated by

$$A_{2,w} = \frac{N_A}{2\overline{M}_0^2}\left[(1-x)^2\beta_{el} + x^2\beta_v\right] \tag{6}$$

where $\overline{M}_0$ is the average monomer molar mass, x is the content of hydrophobic monomer unit, and β_{el} and β_v are the binary cluster integrals with respect to the electrostatic and hydrophobic interactions, respectively. The theory of polyelectrolytes[8,9] provides us the following equation of β_{el}

$$\beta_{el} = \frac{2h^2}{\kappa}R(y) \tag{7}$$

Here, h is the contour length per monomer unit, κ is the reciprocal of the Debye screening length, and $y \equiv 2\pi\nu_{eff}^2 Q e^{-\kappa d}/\kappa$ with the effective charge density ν_{eff} and the Bjerrum length Q. In this study, ν_{eff} was calculated based on the Philip-Wooding theory[10] for charged cylinder model. The function $R(y)$ is given in ref.[8] Taking into account the contribution of counter ions of the copolymer to the ionic strength, we calculate κ by

$$\kappa^2 = 8\pi Q N_A\left(\frac{C_s}{1000} + \frac{1-x}{2\overline{M}_0}c\right) \tag{8}$$

The above equations for $\Gamma_{2,0}$ and $A_{2,w}$ do not include the effect of multiple contacts nor the branching effect. Both effects do not contribute to $\Gamma_{2,0}$ and $A_{2,\mathrm{w}}$ if we apply the single-contact approximation. As shown previously,[11] this approximation is good even for a linear flexible polymer (polystyrene) when the molecular weight is not so high. In what follows, we use this approximation to calculate $\Gamma_{2,0}$ and $A_{2,\mathrm{w}}$, assuming that the error is minor.

The number ν_c of the hydrophobic cores per chain may be calculated by

$$\nu_\mathrm{c} = w_1 n_\mathrm{c}^{(1)} + w_m n_\mathrm{c}^{(m)} \Big/ m \tag{9}$$

where $n_\mathrm{c}^{(1)}$ and $n_\mathrm{c}^{(m)}$ are the number of cores of the unimer and m-mer (micelle), respectively. In the following, we assume $n_\mathrm{c}^{(1)} = 0$ and $n_\mathrm{c}^{(m)} = 1$.

The above equations contains many parameters: m, K_m, d, M_L, h, ν_eff, and β_v. Among them, we have $h = 0.25$ nm for vinyl polymers,[12] $M_\mathrm{L} = \overline{M}_0/h = 835$ g/(mol·nm), and $d = (4\bar{\upsilon}M_\mathrm{L}/\pi N_\mathrm{A})^{1/2} = 1.14$ nm; ν_eff can be calculated with the linear charge density $[= (1-x)/h = 2.7\ \mathrm{nm}^{-1}]$ and d. Thus the unknown parameters are m, K_m, and β_v. If m is given, K_m can be determined so as to give the best fit of eq 9 (independent of β_v) to the ν_c data shown in Figure 3. The fitting results are shown by three curves in Figure 3 for $m = 2$, 3, and 4, among which the dot-dashed curve for $m = 4$ seems to fit the data points most successfully. Using those m and K_m, we have searched for a β_v value which fits eq 3 to the experimental M_app at high concentrations. Values of K_m and β_v so determined for $m = 2$, 3, and 4 are listed in Table 1, and theoretical values of M_app calculated by eq 3 with those parameter values are indicated by solid curves in Figure 2 for $m = 2$, 3, and 4. In the dilute region shown in Figure 2(a), the curve for $m = 3$ gives the best fit to the data points.

Table 1.
Values of K_m and β_v determined from M_app and ν_c data.

m	$K_m/\mathrm{M}^{-(m-1)}$	$\beta_\mathrm{v}/\mathrm{nm}^3$
2	1.4×10^3	−0.9
3	2.0×10^7	−0.9
4	5.5×10^{12}	−0.9

The quantity $R_\mathrm{H,app}/M_\mathrm{w}$ obtained by dynamic light scattering may be calculated by[6,13]

$$\frac{R_\mathrm{H,app}}{M_\mathrm{w}} = \frac{R_\mathrm{H}}{M_\mathrm{w}}\left(1 + k'_\mathrm{H} c/c^*\right) \tag{10}$$

where R_H is the true hydrodynamic radius, k'_H is the strength of the intermolecular hydrodynamic interaction (corresponding to the Huggins coefficient for the polymer solution viscosity), and c^* is the overlap concentration. For a mixture of unimer and m-mer, R_H can be calculated by

$$\frac{R_\mathrm{H}}{M_\mathrm{w}} = \left[\frac{w_1 M_1}{R_\mathrm{H,1}} + \frac{w_m M_m}{g_{\mathrm{H},m} R_{\mathrm{H},m}(\mathrm{linear})}\right]^{-1} \tag{11}$$

with $R_\mathrm{H,1}$ and $R_{\mathrm{H},m}$(linear) being hydrodynamic radii of linear chains with the molecular weights M_1 and M_m, respectively, and the g-factor $g_{\mathrm{H},m}$ of m-mer with branched architecture. Using this R_H, c^* may be written in the form

$$c^* = 3M_\mathrm{w} \big/ 4\pi N_\mathrm{A} R_\mathrm{H}^3 \tag{12}$$

As mentioned in the previous paper,[2] $R_\mathrm{H,1}$ and $R_{\mathrm{H},m}$(linear) for p(AMPS/C6) in 0.1 M aqueous NaCl may be identified with R_H of linear AMPS homopolymer with the same degree of polymerization N_0 in 5 M aqueous NaCl, which can be calculated by $R_{\mathrm{H},m}(\mathrm{linear})/\mathrm{nm} = 0.20 N_0^{0.57}$.[2,14] For m-arm star-shaped polymers, Douglas et al.'s[15] empirical equation for $g_{\mathrm{H},m}$ is available. Using a suitable value for each m as the adjustable parameter k'_H, we have calculated $R_\mathrm{H,app}/M_\mathrm{w}$. The results are shown by dashed, solid, and dot-dashed curves in Figure 4 for $m = 2$, 3, and 4, respectively. The values of k'_H chosen were 6 ($m = 2$), 10 ($m = 3$), and 16 ($m = 4$). The solid curve for $m = 3$ is most favorably compared with the experimental data, although k'_H for $m = 3$ is slightly larger than typical values (4–6)[13] of k'_H for neutral flexible polymers in good solvents.

In the previous study,[2] we have estimated the aggregation number m of a p(AMPS/C6) sample $\mathrm{M2}_{x=0.3}$ with $M_1 = 2.0 \times 10^4$ and $x = 0.36$ in 0.1 M aqueous NaCl to be 3.7 without consideration of the association-dissociation equilibrium. This result is almost consistent with that of the present study, i.e., $m = 3$ from sedimetation equilibrium and dynamic light scattering and $m = 4$ from fluorescence. However, if the previous static light scattering result of the sample $M2_{x=0.3}$ is analyzed by the method mentioned above, we obtain K_m much larger than the present result in the same solvent condition. That is, the star-like micelle formed by the previous sample $M2_{x=0.3}$ is indicated to be much stabler than that of the present sample. The differences in M_1 and x seem not to be enough to explain the difference in K_m. Moreover, we may not expect a large difference in the monomer sequence in the copolymer chains because both p(AMPS/C6) samples were prepared in the same method. At present we have no clear explanation of the difference in micellar stabilization

Conclusions

We have successfully applied the new method to analyze the association–dissociation equilibrium of an amphiphili statistical copolymer of sodium 2-(acrylamido)-2-methylpropane sulfonate and n-hexyl methacrylate in 0.1 M aqueous NaCl at 25 °C. The analysis has led to the conclusion that the aggregate formed by our copolymer sample with $M_1 = 2.2 \times 10^4$ and $x = 0.32$ is a star-like micelle comprising three copolymer chains. This conclusion is consistent with that obtained in the previous study.[2]

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