Water-Soluble Copolymers. 61. Microstructural Investigation of Pyrenesulfonamide-Labeled Polyelectrolytes. Variation of Label Proximity Utilizing Micellar Polymerization

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ABSTRACT: Synthesis of a series of pyrenesulfonamide-labeled polyelectrolytes with varied label proximities has been accomplished by a micellar polymerization technique. The solution properties of these terpolymers consisting of acrylamide (AM), acrylic acid (AA), and *N*-[(1-pyrenylsulfonamido)ethyl]-acrylamide (APS) are examined using viscometry, light scattering, and fluorescence spectroscopy. Classical light scattering indicates high molecular weights ($>1 \times 10^6$) and very good polymer solvation even at high NaCl concentrations ($A_2 \sim 10^4$ cm^3 mol/g^2). Viscometry and dynamic light scattering studies indicate no interpolymer aggregation of the hydrophobic APS labels. Also, no intermolecular associations are evident through measurement of I_E/I_M as a function of terpolymer concentration in 0.5 M NaCl or at pH 3 and 7. Further photophysical results are interpreted on the basis of intramolecular excimer formation. Steady-state fluorescence emission studies indicate that I_E/I_M scales with the surfactant to APS ratio (SMR) in polymerizations. Also, I_E/I_m is a linear function of n, the initial number of APS monomer molecules per micelle. Steady-state excitation and time-resolved fluorescence emission studies have been utilized to probe the microenvironment surrounding the APS labels at varying polymer concentration, electrolyte concentration, and pH values.

Introduction

Copolymers exhibiting associative thickening behavior in aqueous media have become increasingly important in the formulation of pharmaceuticals, cosmetics, agricultural chemicals, and coatings.[1-3] Not only are the rheological properties desirable, but also the opportunity for phase transfer of hydrophobic ingredients into microheterogeneous domains is intriguing. The commercial development of such systems is currently hindered by a lack of fundamental understanding of the parameters which control domain organization.

Of special interest to our group have been (1) the elucidation of the mechanism of associative thickening and (2) the tailoring of thickeners with reversible associations responsive to changes in pH, ionic strength, temperature, or shear stress. An especially attractive polymerization technique, termed "micellar" polymerization, first reported independently by Evani[4] and by Turner et al.,[5] utilizes a surfactant to solubilize a relatively low mole percent of a hydrophobic monomer in water for copolymerization with a hydrophilic monomer.

Among the attractive features of micellar polymerization is the relatively small concentration of hydrophobic monomer required for an efficient, thickening copolymer. For example, in copolymerization of *n*-alkylacrylamides in SDS/water with acrylamide (AM), concentrations as low as 0.5 mol % hydrophobic comonomer yield effective copolymers.[6] While attractive from a cost standpoint, analytical determination of the extent of comonomer conversion and incorporation into the copolymer is difficult for such low concentrations. These problems were solved by Valint et al.,[7] who utilized chromophore-containing acrylamido comonomers such as *N*-(4-butylphenyl)acrylamide (BPAM), which may be examined analytically by UV spectroscopy. However, direct evidence of specific microstructural placement is not available in these systems.

Recently, we have conducted experiments with chromophore-labeled hydrophobic monomers to further study the relationship between the copolymer microstructure and solution behavior utilizing pyrenesulfonamide-labeled monomers, in which the monomer served as both the hydrophobe and fluorescent reporter molecule.[8,9] Micellar polymerization resulted in a microblocky structure consisting of short runs of hydrophobic monomer randomly spaced between long runs of hydrophilic monomer. The excimer to monomer ratio (I_E/I_M) and apparent viscosity increased with copolymer concentration. No associative effects were observed for copolymers of identical composition with random placement of hydrophobic monomer units prepared by solution polymerization.

We have considered the difficulty of dissolution of the uncharged associative copolymers and have, therefore, introduced functionality responsive to changes in pH or ionic strength.[10-12] For example, we recently reported associative terpolymers of AM with noncharged hydrophobic comonomers and sodium acrylate (NaA), sodium 3-acrylamido-3-methylbutanoate (NaAMB), or sodium 2-acrylamido-2-methylpropanesulfonate (NaAMPS). In the group of terpolymers containing carboxylate functionality, viscosity in water was shown to be dependent on composition, microstructure, molecular weight, polymer concentration, ionic strength, temperature, and pH.[10,11] Also, we demonstrated the degree of associative thickening behavior of AM/NaA-based terpolymers to be a function of the surfactant to monomer ratio (SMR) in micellar polymerization.[12]

In this report, we examine the role of surfactant to monomer ratio (SMR) in the reaction medium on microstructure utilizing the *N*-[(1-pyrenylsulfonamido)-ethyl]acrylamide (APS) monomer as a fluorescent label. Comparison is made with previously reported terpolymers of identical AM/AA compositions with *N*-(4-de-

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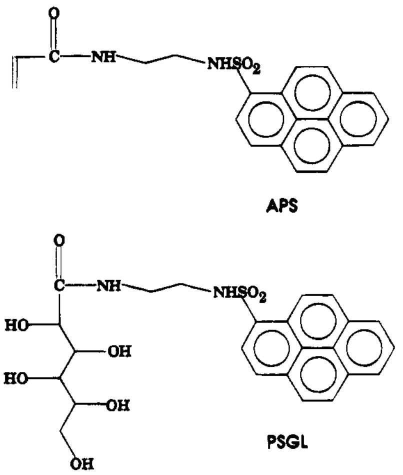

Figure 1. Structures of the fluorescent monomer, APS, and its water-soluble model compound, PSGL.

cylphenyl)acrylamide as the hydrophobic monomer. Unlike the uncharged copolymer of AM/APS, however, the AM/AA/APS terpolymers of this study do not show intermolecular associative thickening, apparently due to insufficient liaisons of hydrophobic microdomains even at high concentrations of terpolymer.

Experimental Section

Materials. The following materials were purchased from Aldrich and used as received unless otherwise noted: acrylamide, sodium dodecyl sulfate (SDS), and acrylic acid (vacuum distilled before use); $K_2S_2O_8$ (recrystallized from water). All other materials were purchased commercially and used as received.

Monomer and Model Compound Synthesis. The syntheses of *N*-[(1-pyrenylsulfonamido)ethyl]acrylamide (APS) and the water-soluble model compound 2,4-dimethyl-*N*-[(1-pyrenylsulfonamido)ethyl]gluconamide (PSGL) have been reported previously.[8]

Co- and Terpolymer Synthesis and Purification. The general procedures for terpolymer synthesis by micellar polymerization have been described earlier.[10−12] Polymerizations were conducted at 50 °C for 3.5 h in deionized water. A total monomer concentration of 0.44 M was used and [monomer]:[$K_2S_2O_8$] was 3000:1. Feed ratios for the terpolymers were as follows: AM, 0.695; AA, 0.300; APS, 0.005. The surfactant (SDS) to APS molar ratio, SMR, was varied in successive polymerizations at 40, 60, 80, and 100. A control polymer of AM and AA was also synthesized in the presence of SDS (0.132 M) with an AM:AA ratio of 0.70:0.30. The AA monomer was used in its acidic form (below pH 4 during polymerization) and neutralized during purification. Polymers were isolated by precipitation into acetone followed by drying and redissolution in water. After 1 week, the pH was adjusted to 7−8 and the polymers were dialyzed against deionized water using SpectraPor No. 4 dialysis tubing (MW cutoff 12−14 K) for an additional week. The samples were then lyophilized to a constant weight.

Solution Preparation. Stock solutions of the copolymer and terpolymers were prepared at 0.2−0.5 g/dL in deionized water. After dissolution, the pH of the polymer solutions was adjusted to 7.1−7.5 using microliter amounts of concentrated HCl or NaOH solutions unless otherwise noted. Dilutions were made with deionized water. For 0.5 M NaCl solutions, dry NaCl was added to solutions identical to the ones described here. Dilutions were made with 0.5 M NaCl.

Co- and Terpolymer Composition. Copolymer composition was determined by elemental analysis (M-H-W Laboratories, Phoenix, AZ). A previously published method using a combination of elemental analysis and UV spectroscopy was employed to determine terpolymer compositions.[11,12] UV spectra of dilute solutions (0.02 g/dL) of terpolymers in water were obtained and the amount of APS incorporation was determined at 352 nm (ϵ = 24000 M^{-1} cm^{-1}).[8]

UV/vis spectroscopy. UV/vis spectra were obtained on a Hewlett-Packard Model 8452A photodiode-array spectrophotometer. Polymer concentrations were 0.02 g/dL in water ([APS] $\approx 4 \times 10^{-6}$ mol/L).

Steady-State Fluorescence Spectroscopy. Steady-state fluorescence spectra were obtained with a Spex Fluorolog 2 fluorescence spectrophotometer equipped with a DM3000F data system. Slit widths were maintained at 1−2 mm. Emission spectra were obtained by excitation at 340 nm while monitoring the emission from 350 to 600 nm. Monomer intensities were recorded at 400 nm, and excimer intensities were recorded at 519 nm. Spectra were normalized at 400 nm. Excitation spectra were obtained by exciting from 250 to 400 nm while monitoring the emission intensity at either 400 nm (monomer emission) or 519 nm (excimer emission). Due to lower emission intensities for the excimer species, excimer excitation spectra were normalized to monomer excitation at 350 nm. All spectra were corrected for photomultiplier response by an internal correction provided by the manufacturer.

Time-Resolved Fluorescence Spectroscopy. Time-resolved fluorescence data were obtained with a Edinburgh Analytical Instruments FL900CDT single-photon-counting instrument equipped with an H_2-filled nanosecond flashlamp. A total of 10^4 counts in the maximum channel were taken. Fluorescence lifetimes were obtained from fits of the decay profiles utilizing software provided by the manufacturer, which employs the standard iterative deconvolution method.

Viscometry. Viscosity measurements were conducted on a Contraves LS-30 low-shear rheometer at 25 °C and a shear rate of 6 s^{-1}.

Classical and Dynamic Light Scattering. Refractive index increments for classical studies were obtained on a Chromatix KMX-16 laser differential refractometer at 25 °C. A Spectra-Physics 127 laser operating at 632.8 nm was used for all light scattering studies. Data points for classical studies were taken at multiple angles at 25 °C using a Brookhaven Instruments Model BI-200SM automatic goniometer interfaced with a Brookhaven Instruments personal computer. Zimm plots were constructed using software provided by the manufacturer. Polymer solutions of 2.0×10^{-4} g/mL at pH 7.3−7.5 in 0.5 M NaCl were filtered through Millipore 0.45 μm filters to remove dust and diluted with filtered 0.5 M NaCl. Dynamic light scattering studies were performed at 90°, and the signals were processed with a Brookhaven Instruments Model BI-2030AT autocorrelator. Data were analyzed using the algorithm CONTIN and associated software provided by the manufacturer. Polymer solutions of 2.0×10^{-4} g/mL at pH 7.3−7.5 in 0.5 M NaCl were filtered through Tygon tubing filter loops using Millipore 0.45 μm filters to remove dust. Typical filtration times were 14−48 h. Multiple analyses were performed to ensure reproducibility.

Results and Discussion

Terpolymer Synthesis. The fluorescently labeled polyelectrolytes of this study, **P2−P5**, were prepared from acrylamide (AM), acrylic acid (AA), and *N*-[(1-pyrenylsulfonamido)ethyl]acrylamide (APS). The structures of these monomers and the resulting terpolymers are shown in Figure 2. The synthesis of APS and the water-soluble model compound 2,4-dimethyl-*N*-[(1-pyrenylsulfonamido)ethyl]gluconamide (PSGL) (Figure 1) have been reported in an earlier study.[8] The polymerization procedure has also been detailed previously.[12] In this study AM is utilized as the major hydrophilic component since it is readily polymerized to high molecular weight in aqueous media. AA copolymerizes readily with AM and provides ionizable groups along the polymer backbone. The AM:AA:APS in the feed was 69.5:30.0:0.5. Potassium persulfate ($K_2S_2O_8$), a water-soluble initiator, was used in a ratio of 3000:1 [total monomer]:[initiator]. These feed ratios provided a series of high molecular weight, moderate charge density polyelectrolytes which contain small numbers (~35)

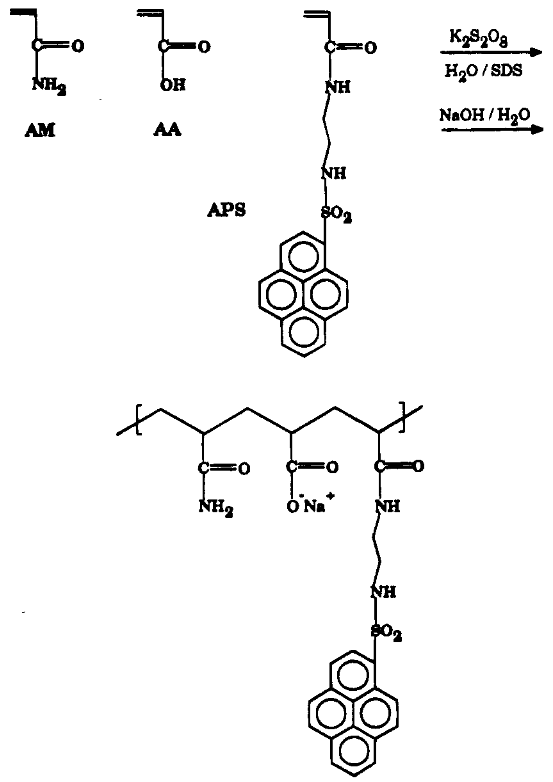

Figure 2. Synthesis of AM/AA/APS Terpolymers **P2**–**P5**.

of hydrophobic APS units per chain. Terpolymerizations were carried out in deionized water at 50 °C under micellar reaction conditions utilizing sodium dodecyl sulfate (SDS) as the surfactant to solubilize the hydrophobic APS comonomers. The SMR or surfactant to monomer ratio is defined by

$$\mathrm{SMR} = [\mathrm{SDS}]/[\mathrm{APS}] \qquad (1)$$

[SDS] is the molar concentration of surfactant, and [APS] is the hydrophobic APS comonomer molar concentration. The SMR may be varied to control the average number of hydrophobic monomers per micelle, *n*, as predicted from the Poisson distribution by

$$n = \frac{N[\mathrm{H}]}{[\mathrm{SDS}] - \mathrm{cmc}} \qquad (2)$$

where cmc is the critical micelle concentration of SDS in this system and *N* is the aggregation number of SDS.[13,14] At the polymerization temperature and monomer concentrations of this study, a cmc value of 6.5×10^{-3} mol/L was obtained for SDS.[12] An aggregation number of approximately 60 is generally accepted for SDS. Equation 2 is appropriate if the aggregation number of the micelle is not significantly altered by the presence of the hydrophobic monomers. This assumption should be quite valid at low values of *n*.

By setting the SMR values in successive polymerizations at 40, 60, 80, and 100, the initial number of hydrophobic molecules per micelle can be adjusted as indicated in Table 1. At SDS concentrations well above the cmc, eq 2 may be approximated by $n = N(\mathrm{SMR})^{-1}$. The relationship between *n* and SMR is shown in Figure 3. Decreasing SMR results in a higher number of hydrophobic comonomers per micelle. Note that an SMR of 60 predicts approximately 1 hydrophobe per micelle initially; below 60 there is an average of less than 1 hydrophobe/micelle. Persistent turbidity in polymerization feeds with an SMR of slightly below 40 (approximately 2 monomers per micelle) precludes the use of lower SMR values for the APS system. As indicated in Table 1, the range of *n* in this study is therefore limited to values of hydrophobic monomer per micelle of 0.62–1.62. Although this range of *n* is quite narrow, the data show that slight variation of the synthetic conditions can effect differences in terpolymer microstructure as evidenced by the photophysical properties of the terpolymers.

Table 1. Initial Micellar Parameters in Polymerizations

sample	SMR[a]	[SDS][b]	*n*[c]
P2	40	0.088	1.62
P3	60	0.132	1.05
P4	80	0.176	0.78
P5	100	0.220	0.62

[a] Calculated from eq 1. [b] Molar concentration of surfactant. [c] APS molecules/micelle calculated from eq 2.

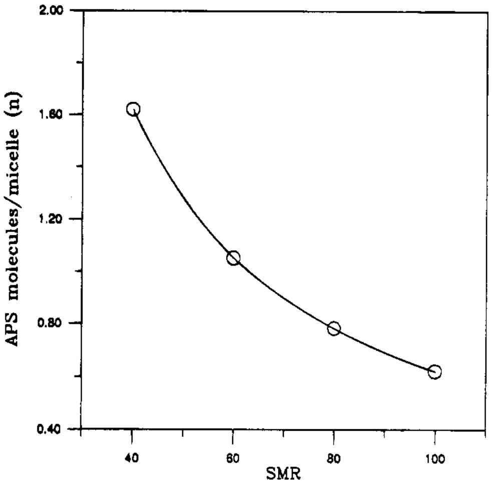

Figure 3. APS molecules/micelle (*n*) as a function of SMR (eq 1) calculated from the Poisson distribution.

Table 2. Co- and Terpolymer Composition and Conversion Data

sample	SMR[a]	feed ratio AM:AA:APS	mol % AM[b]	mol % AA[b]	mol % APS[c]	% conv[d]
P1		70:30:0.0	57.3	43.7	0.00	36
P2	40	69.5:30.0:0.5	60.3	39.5	0.20	23
P3	60	69.5:30.0:0.5	61.0	38.8	0.17	24
P4	80	69.5:30.0:0.5	61.7	38.1	0.16	27
P5	100	69.5:30.0:0.5	60.6	39.2	0.16	28

[a] Calculated from eq 1. [b] Determined from elemental analysis. [c] Determined from UV spectroscopy. [d] Percent conversion, determined gravimetrically.

A copolymer of AM and AA, **P1**, was synthesized in the presence of SDS (0.132 M SDS and AM:AA feed ratio of 70:30) as a control. Comparison of the bulk and molecular properties of the terpolymers with this copolymer is made before considering the microstructural properties of the terpolymers.

Terpolymer Composition. The compositions of terpolymers **P2**–**P5** as well as the control AM/AA copolymer, **P1**, are shown in Table 2. Terpolymers **P2**–**P5** are very similar in AM and AA composition, with approximately 60–62% AM and 38–40% AA in the resulting terpolymers. The AM:AA ratio of copolymer **P1** is 57:43. For **P2**–**P5** the degree of APS incorporation is less than half that expected from the feed concentrations. Decreased hydrophobe incorporation in micellar polymerizations with carboxylate monomers has been attributed to charge–charge repulsions during polymerization.[11] Conducting the polymerization below the pK_a of the ionizable group can alleviate such

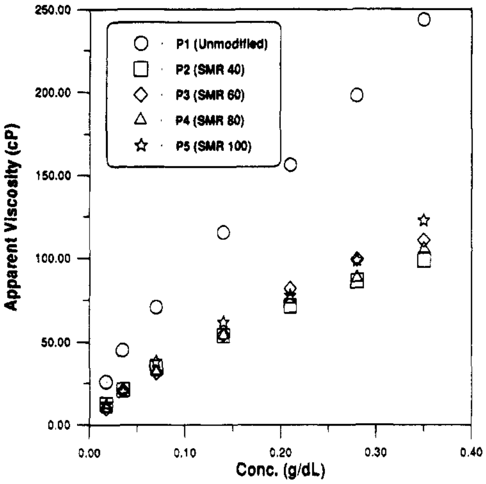

Figure 4. Apparent viscosity as a function of polymer concentration in deionized water for copolymer **P1** and terpolymers **P2–P5** at 25 °C and 6 s^{-1}.

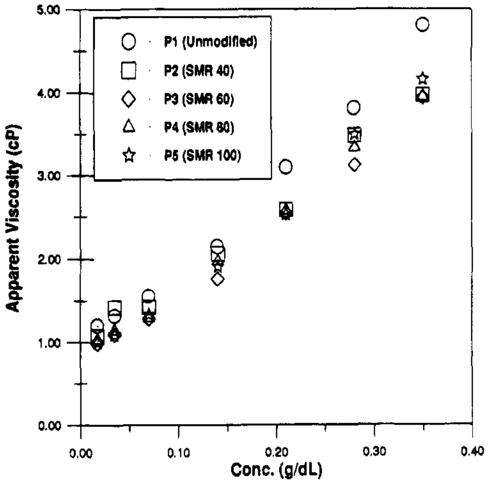

Figure 5. Apparent viscosity as a function of polymer concentration in 0.5 M NaCl for copolymer **P1** and terpolymers **P2–P5** at 25 °C and 6 s^{-1}.

problems.[12] Decreased APS incorporation has, however, also been noted in nonionic copolymers prepared by micellar polymerization[8] as well and may result from the location of APS monomer in SDS micelles.

Polymerizations were terminated at low conversion to limit compositional drift in the terpolymers. Also, mole percent APS in the terpolymers appears to be unaffected by increasing SMR. This is in contrast to studies utilizing smaller hydrophobic monomers which indicate enhanced hydrophobe incorporation at low surfactant levels.[7,15]

Viscometric Studies. Bulk or macroscopic solution properties of the copolymer **P1** and the terpolymers **P2–P5** were examined at neutral pH using viscometry. Apparent viscosities in deionized water (Figure 4) and 0.5 M NaCl (Figure 5) were measured as a function of polymer concentration.

Copolymer **P1** exhibits a linear increase in apparent viscosity with concentration in deionized water (Figure 4). Also the values of the viscosity are very high, typical of high molecular weight polyelectrolytes in low ionic strength media. Terpolymers **P2–P5** exhibit similar behaviors in deionized water at pH 7 (Figure 4), but the values are lower than for **P1** over the concentration range investigated. Lower viscosities for the terpolymers are most likely due to both lower molecular weights and reduced hydrodynamic volume due to intramolecular associations of the hydrophobic APS units.

Table 3. Classical Light Scattering Data in 0.5 M NaCl for P1–P5 at 25 °C

sample	SMR[a]	$M_w \times 10^{-6}$ [b]	$DP_w \times 10^{-4}$ [c]	$A_2 \times 10^4$ [d] (cm^3 mol/g^2)	R_g [e] (nm)
P1		1.7	2.4	10.0	100
P2	40	1.3	1.8	11.5	82
P3	60	1.2	1.7	14.1	89
P4	80	1.3	1.8	12.1	84
P5	100	1.2	1.7	6.7	82

[a] Calculated from eq 1. [b] Weight-average molecular weight. [c] Weight-average degree of polymerization. [d] Second virial coefficient. [e] Radius of gyration.

Little difference in viscosity is evident when comparing terpolymers **P2–P5** and copolymer **P1** in 0.5 M NaCl (Figure 5). Apparent viscosities are an order of magnitude lower when compared to those in water, attributed to shielding of the charge–charge repulsions of carboxylate anions along the polymer chain. Viscosities for **P1–P5** essentially lie on the same line and increase in a linear fashion with concentration. No break in the curves or rapid increase in viscosity is evident; therefore, intermolecular associations are not apparent at the macroscopic level.

This behavior differs from that observed for uncharged AM/APS copolymers of similar label content[8,9] in which significant intermolecular aggregation was observed. At neutral pH, the high charge densities (≈40 mol % carboxyl groups) of **P2–P5** would not be expected to promote intermolecular association, especially in deionized water. Studies on hydrophobically modified polyelectrolytes from our laboratories have correlated decreased intermolecular associations with high polyelectrolyte charge density,[11,12] particularly at low ionic strength. These observations are consistent with light scattering and fluorescence studies to be presented in subsequent sections.

Light Scattering Studies. Light scattering experiments were carried out in 0.5 M NaCl at 25 °C. The pH values of these solutions were 7.3–7.5. Refractive index increments (dn/dc values) were also obtained under these conditions. Values for dn/dc were found to be very similar for the five polymers, and an average value of 0.1703 ± 0.0124 was used for all calculations since the polymers differ very little in composition.

Classical light scattering with the standard Zimm analysis was used to determine the weight-average molecular weights (M_w), the second virial coefficients (A_2), and the mean radii of gyration (R_g) for **P1–P5** (Table 3). Weight-average degrees of polymerization (DP_w), calculated from M_w values using an average molecular weight per repeat unit of 71.5 (60:40, AM:AA), are also displayed in Table 3.

The molecular weight of copolymer **P1** is the highest, with a value of 1.7×10^6 ($DP_w = 2.4 \times 10^4$). Terpolymers **P2–P5** are of slightly lower molecular weight, with values of $(1.2-1.3) \times 10^6$ ($DP_w = (1.7-1.8) \times 10^4$). Candau and co-workers[15] have noted that surfactants such as SDS contain alcohols capable of chain transfer reactions as impurities. Since the labeled polymers are more hydrophobic and interact more effectively with the SDS micelles during polymerization, chain transfer reactions are more likely and molecular weights are lower. A_2 values ($(7-14) \times 10^{-4}$ cm^3 mol/g^2) indicate that **P1–P5** are very well solvated even in 0.5 M NaCl due to the high degree of ionization of the polymer chain at neutral pH. R_g values are consistent with M_w and A_2 data, with values for hydrophobically modified **P2–**

Table 4. Dynamic Light Scattering Data in 0.5 M NaCl for P1–P5 at 25 °C

sample	SMR[a]	$D_0 \times 10^8$ [b] (cm^2/s)	d_H[c] (nm)	R_H/R_G
P1		6.8	68	0.340
P2	40	6.5	72	0.439
P3	60	6.5	72	0.404
P4	80	7.4	63	0.375
P5	100	6.8	69	0.421

[a] Calculated from eq 1. [b] Translational diffusion coefficient. [c] Hydrodynamic diameter calculated from eq 3.

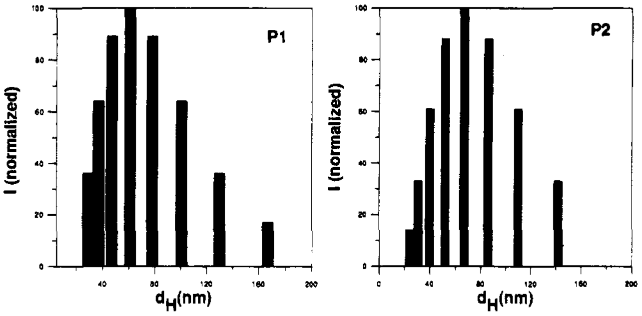

Figure 6. Examples of diameter distributions (in nm) of **P1** (left) and **P2** (right) from dynamic light scattering studies in 0.5 M NaCl at 25 °C.

P5 lower than that of **P1**, ranging from 82 to 89 nm. These lower values of R_g may be attributed to the lower molecular weights of the terpolymers as well as a slightly more collapsed state due to hydrophobic interactions of the APS groups.

Dynamic light scattering by the standard autocorrelation method allowed determination of diffusion coefficients (D_0) for **P1–P5**. The hydrodynamic diameter (d_H) of the polymer coil can be determined from D_0 by the Stokes–Einstein equation:

$$D_0 = \frac{kT}{3\pi\eta_0 d_H} \qquad (3)$$

in which k is Boltzmann's constant, T is temperature, and η_0 is the viscosity of the medium. Diffusion coefficients and hydrodynamic diameters are shown in Table 4. Values for D_0 ((6.5–7.4) × 10^{-8} cm^2/s) and d_H (63–72 nm) are very similar for the copolymer and the four terpolymers. Values of d_H are reproducible with a standard deviation of ±5 nm. The magnitudes of the diffusion coefficients and hydrodynamic diameters corroborate the classical light scattering data, indicating that **P1–P5** occupy large dimensions in 0.5 M NaCl. In all cases the distributions in 0.5 M NaCl solutions are broad, unimodal, and approximately symmetrical. Distributions of copolymer **P1** and terpolymer **P2** are shown in Figure 6. Also, the similarity of the distributions of **P2–P5** (not shown) to **P1** indicates that the presence of interpolymer aggregates is minimal, consistent with the viscosity data.

Photophysical Studies. The studies discussed above indicate that terpolymers **P2–P5** behave as unimolecular coils at neutral pH even at high NaCl concentration. On both the macroscopic and molecular level **P2–P5** behave much like copolymer **P1**, which contains no APS label.

Steady-State Fluorescence Emission Studies. Steady-state fluorescence emission spectra for **P2–P5** are shown in Figures 7 and 8 (concentration of 0.02 g/dL and a pH of 7.1–7.5). Emission spectra in water (Figure 7) and in 0.5 M NaCl (Figure 8) are qualitatively identical and exhibit both normal, or "monomer", fluorescence from approximately 360 to 450 nm and excimer fluorescence from 450 to 600 nm (see figure insets). These data are summarized in plots of I_E/I_M in deionized water (○) and 0.5 M NaCl (●) vs SMR (Figure 9). The dilute solution spectra in Figures 7 and 8 support the existence of "blocky" microstructures in terpolymers **P2–P5** at ≤0.2 mol % of the APS chromophore.

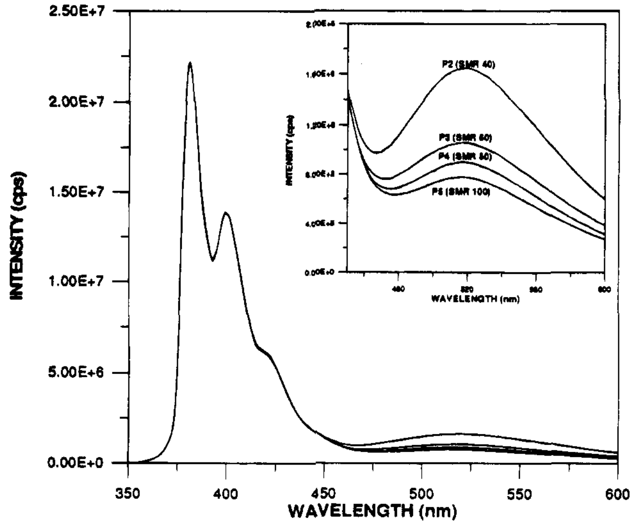

Figure 7. Fluorescence spectra of terpolymers **P2–P5** in water. Inset: Excimer region from 450 to 600 nm for **P2** (SMR 40), **P3** (SMR 60), **P4** (SMR 80), and **P5** (SMR 100).

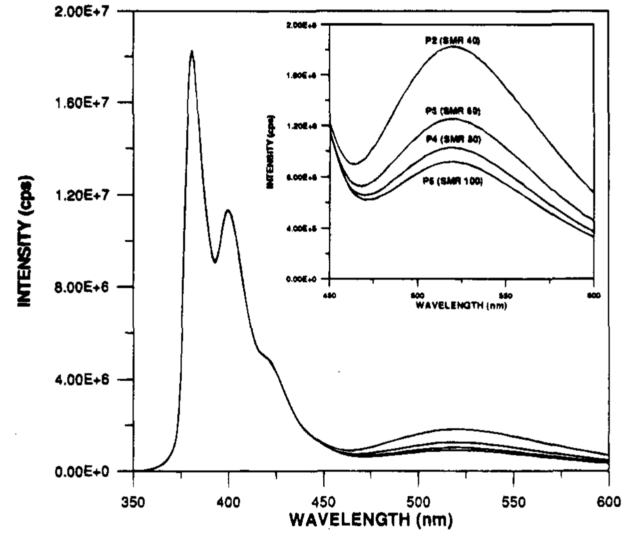

Figure 8. Fluorescence spectra of terpolymers **P2–P5** in 0.5 M NaCl. Inset: Excimer region from 450 to 600 nm for **P2** (SMR 40), **P3** (SMR 60), **P4** (SMR 80), and **P5** (SMR 100).

Excimer formation in labeled polymer systems may occur through either intermolecular or intramolecular fluorescent label interactions. Measurement of I_E/I_M as a function of terpolymer concentration in water at various pH values and in 0.5 M NaCl was also conducted to confirm the absence of interpolymer aggregates. I_E/I_M values were measured in deionized water at pH 3.0 and 7.0 and in 0.5 M NaCl (pH 7.0). Only modest increases were noted over a tenfold increase in concentration for **P2–P5** under these conditions. This contrasts with studies on uncharged AM/APS copolymers prepared by micellar[8,9] polymerization in which an increase in I_E/I_M with concentration due to intermolecular hydrophobic associations of the APS labels was observed.

At pH 7, nearly all the acrylic acid groups of **P2–P5** are in the carboxylate ion form. Under these conditions, the high density of charged groups reduces hydrophobic

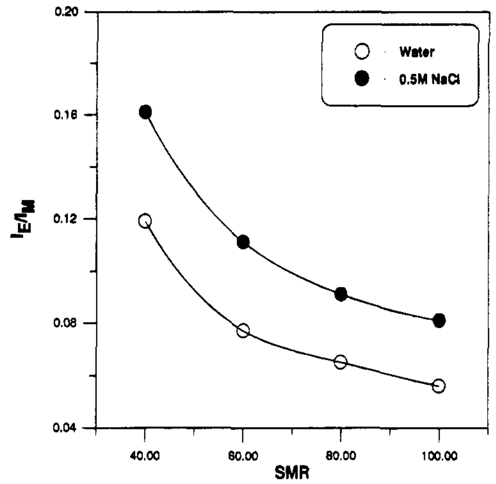

Figure 9. I_E/I_M as a function of the SMR (eq 1) used in polymerization for terpolymers **P2**–**P5** in water and 0.5 M NaCl.

intermolecular associations. Urry[16] has proposed a mechanism involving water structuring around charged and hydrophobic groups to explain conformational transitions in synthetic proteins. He suggests that water may not simultaneously participate in the hydration of hydrophobic and charged groups. In the present case, the water structure which drives interpolymer hydrophobic associations in the AM/APS copolymers appears to have been disrupted by the high local concentration of charged groups. Even with the addition of NaCl, which shields the carboxylate charges and enhances the structure of water, no significant increase in I_E/I_M is noted with terpolymer concentration.

Under acidic conditions, the properties of the terpolymers may be expected to be more like those of the nonionic AM/APS copolymer. However, previous studies of AM/AA copolymers[17,18] indicate that hydrogen bonding between AA and AM groups plays a crucial role in the solution properties of these systems over a wide range of pH. Light scattering studies by Kulicke and Horl[17] support increases in chain stiffness and excluded volume effects due to intramolecular hydrogen bonding between adjacent amide and carboxylate groups. These effects were dependent on composition, reaching a maximum at approximately 65 mol % acid groups. Also, Klenina and Lebedeva[18] reported that hydrogen-bonding effects in AM/AA copolymers are enhanced at low pH. They reported precipitation due to strong hydrogen bonding between acid and amide groups for copolymers containing more than 30 mol % AA in 0.05 M HCl. Accordingly, precipitation of **P1**–**P5** (cf. Figure 10) is observed at or below pH 2.8, depending on polymer concentration. Strong intracoil hydrogen bonding is likely responsible for the monotonic increase in I_E/I_M with terpolymer concentration at a pH of 3.0.

Intramolecular label interactions in these systems should be related to polymer conformation (long-range label interactions) or neighboring group interactions. Differentiation of these two types of interactions has been accomplished by monitoring changes in I_E/I_M in solvents or solutions where the macromolecular dimensions are significantly altered. The dependence of I_E/I_M on the thermodynamic quality of the solvent,[19] for example, is an indication of long-range label interactions. Steady-state emission spectra and I_E/I_M values of **P2**–**P5** in deionized water, in 0.5 M NaCl, and at various pH values are expected to yield valuable information about the extent of interactions present in aqueous terpolymer solutions.

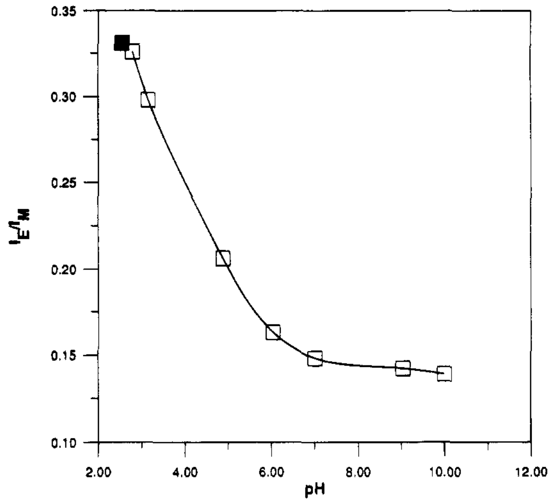

Figure 10. I_E/I_M as a function of solution pH for **P2** in deionized water at 0.05 g/dL. The filled symbol indicates the onset of phase separation.

Fluorescence spectra in deionized water at neutral pH (Figure 7) indicate significant values of I_E/I_M for terpolymers **P2**–**P5**. At a pH of 7 in deionized water, the degree of ionization of the carboxylate groups along the polymer backbone is high and the polymer chains are in an extended conformation. Intramolecular interactions of APS labels or "blocks" of labels from distant parts of the molecule are unfavorable due to the rigidity of the polymer backbone. However, addition of electrolytes to the system results in charge shielding, allowing a more random coil conformation. The emission spectra of **P2**–**P5** in deionized water (Figure 7) and in 0.5 M NaCl (Figure 8) reveal qualitative similarity, even though the polymer coils experience a large decrease in hydrodynamic volume in 0.5 M NaCl (as evidenced by large decreases in apparent viscosities shown in Figures 4 and 5). Figure 9 also illustrates the effects of NaCl addition. For terpolymers synthesized at each respective SMR, I_E/I_M is only slightly higher for the terpolymers in 0.5 M NaCl, even though the bulk viscosity is an order of magnitude lower. Apparently, the polymer can adopt multiple conformations in solution and APS interactions within the blocky microstructure (which were spatially inaccessible in deionized water) may occur in 0.5 M NaCl.

The fact that APS chromophore interactions occur on the local level even in 0.5 M NaCl is apparent when the I_E/I_M ratio for these systems is examined as a function of pH. An example of the pH-dependent behavior is shown in Figure 10 (0.05 g/dL). These data indicate a collapse of the polymer coil below pH 6 until phase separation occurs below pH 2.8 (solid symbol). This classical behavior for labeled polyacids as a function of pH[20,21] is indicative of long-range chromophore interactions in the compact polymer coil at low pH.

Since it is evident that excimer formation at neutral pH in these terpolymer systems arises from a unique microstructure, we may now compare the microstructure and relative label proximity within this series of terpolymers. Examination of the excimer peaks in the fluorescence spectra of **P2**–**P5** (insets, Figures 7 and 8) reveals that excimer formation scales with the micellar parameters in the polymerization feed. **P2** (SMR 40) has the largest excimer peak followed by **P3** (SMR 60), **P4** (SMR 80), and **P5** (SMR 100), respectively. The I_E/I_M vs SMR curves in Figure 9 show a striking resemblance to the curve for micellar occupancy number, *n*, versus SMR (Figure 3). I_E/I_M plots as a

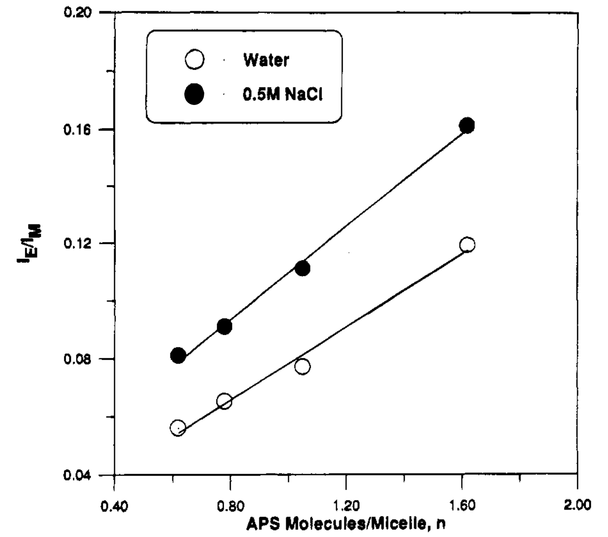

Figure 11. I_E/I_M versus n (eq 2) for **P2–P5** measured in deionized water and 0.5 M NaCl.

function n (for **P2–P5**) in deionized water (○) and in 0.5 M NaCl (●) are shown in Figure 11. The plot of I_E/I_M as a function n in Figure 11 indicates that label proximity is directly related to the initial number of hydrophobic monomers per micelle.

Reid and Soutar[22,23] have noted that a linear dependence of I_E/I_M with local chromophore composition can be predicted from kinetic models according to

$$I_E/I_M = k_c k_{em}[M] \tag{4}$$

where k_c is a composite of rate constants for internal conversion, intersystem crossing, fluorescence, and dimer deactivation processes and k_{em} is the rate constant for excimer formation. The term [M] is a concentration term indicative of the local chromophore concentration and the number of potential excimer sites in the polymer. Equation 4 is a general expression and should be applicable to a variety of systems. For example, in random copolymer systems utilizing labels such as vinylnaphthalene, [M] is a function of the number of adjacent chromophore pairs, and it is anticipated that I_E/I_M is a linear function of the number of potential excimer sites at constant temperature if the proper corrections for energy migration are included.[19,22,23] However, in the present system, a linear fit (Figure 11) is obtained without consideration of energy migration according to

$$I_E/I_M = Kn \tag{5}$$

where K is a constant encompassing the rate constants for the photoprocesses considered in eq 4 as well as those necessary to describe the kinetics for dimer formation and deactivation. As noted below, the photophysics of these terpolymer systems are complex and no relationship to a specific kinetic scheme can be made at this time. However, the use of a single term to describe the extent of excimer formation clearly indicates that the initial number of APS monomers per micelle, n, in micellar polymerizations can be used to control local chromophore microcomposition. Although a blocky microstructure is present, other forces play crucial roles in determining the associative behavior of **P2–P5** in solution. In the next section the nature and environment of these "microblocky" polymer segments is addressed.

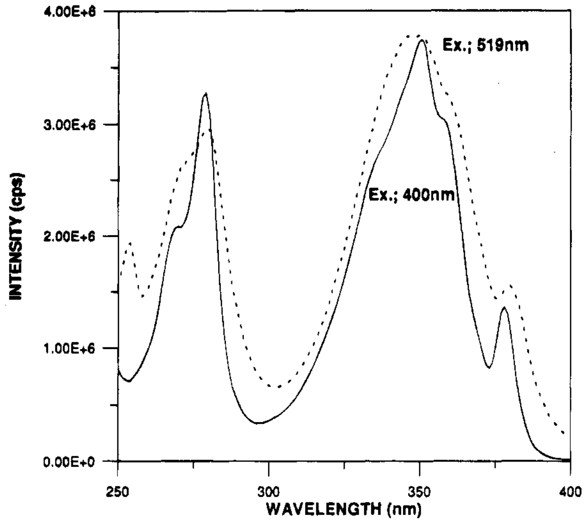

Figure 12. Fluorescence excitation spectra of **P2** (SMR 40) in water at 0.02 g/dL: top line (dotted), λ_{em} = 519 nm; bottom line (solid), λ_{em} = 400 nm.

Steady-State Fluorescence Excitation Studies. Steady-state fluorescence excitation spectra were obtained for terpolymers **P2–P5** in both deionized water and 0.5 M NaCl at neutral pH. Spectra were measured by exciting from 250 to 400 nm and measuring the emission at both 400 (monomer) and 519 nm. The excitation spectra for all terpolymers in water were essentially identical, as expected. Furthermore, addition of NaCl had little effect on the characteristics of the excitation spectrum regardless of the wavelength at which the emission was monitored. A representative example is shown for **P2** (SMR 40) in deionized water (Figure 12). A slight red shift is noted for the long wavelength emitting species (top line, dotted) as compared to that of the monomer (bottom line, solid). Further excitation studies[24] at a lower band-pass (<1 nm) utilizing a more sensitive instrument have indicated a red shift of 2–3 nm in these systems at pH values from 3 to 7. It was noted for AM/APS copolymer systems that the "excimer" excitation exhibited a 4 nm red shift,[9] indicating chromophore pairs existed in the group state. Perturbation of the ground-state energy levels yielded species of lower energy for the dimers in that system. Although the long wavelength emitting species in **P2–P5** appears to be similar in nature to that in the previous system,[9] the existence of dynamically formed excimers cannot be ruled out.

Also, we found it possible to excite the ground-state dimers exclusively by employing an excitation wavelength of 410 nm. The emission spectra from 450 to 650 nm showed a broad, symmetrical, but structureless peak centered around 510 nm. This result is consistent with previous studies[9] employing an excitation wavelength of 395 nm.

Time-Resolved Fluorescence Emission Studies. Time-resolved fluorescence emission studies for APS model compounds have been published previously.[9] In this work, fluorescence lifetimes of PSGL (Figure 1) in deionized water (pH 3 and 7) and 0.5 M NaCl (pH 7) were measured. PSGL (0.5 μmol/L) exhibited only monomer fluorescence at the specified conditions and the decays were exponential, yielding lifetimes of approximately 12–13 ns. These values are consistent with previous fluorescence decays of PSGL in aqueous solution,[9] indicating that the addition of NaCl or aqueous acid does not decrease the lifetime of the pyrenesulfonamide chromophore.

The emission decay curves at short (419 nm) and long wavelength (519 nm) were complex and not resolvable into a complementary set of sum (monomer − 419 nm) and difference (excimer − 519 nm) of exponential terms as is dictated by a classical excimer equilibrium kinetic analysis.[25] It is possible that multiple excimer geometries, energy migration within block sequences of APS groups to excimer-forming sites, a microheterogeneous environment for the emitting species, and even emission from excited groud-state dimers can contribute to the complex, multiexponential decay curves recorded for terpolymers **P2**−**P5**. Due to the number of processes which may be involved, no physical significance should be attached to individual components.

Also, we attempted to measure fluorescence decay times of the ground-state dimers by direct excitation at 410 nm. Under all solution conditions (pH, NaCl concentration, etc.), these decays were complex, and the sum of three to four exponentials was necessary to satisfactorily fit these decays. Due to their complexity, no interpretation of these decays is possible.

Conclusions

A series of AM/AA/APS terpolymers have been synthesized by micellar polymerization, varying the surfactant to APS ratio (SMR) in the polymerization. The terpolymers were found to be of essentially identical composition and molecular weight. Their solution properties were investigated by viscometry, light scattering, and fluorescence spectroscopy and compared with those of a control AM/AA copolymer.

Solution viscosity profiles, dynamic light scattering studies, and measurements of I_E/I_M as a function of terpolymer concentration indicate the absence of intermolecular hydrophobic association of the APS labels. The lack of intermolecular associations is an interesting result since interpolymer APS interactions were noted in uncharged AM/APS copolymers of similar label content. This is likely due to two effects. At high pH the presence of a large number of carboxylate groups may sufficiently perturb the water structure, removing the driving force responsible for intermolecular hydrophobic associations. At low pH it is likely that intramolecular hydrogen bonding is responsible for stabilizing intramolecular chromophore interactions to the exclusion of intermolecular interactions.

Since the above studies did not indicate interpolymer association, photophysical studies comparing terpolymers synthesized in the presence of varying amounts of surfactant could be interpreted by considering intramolecular interactions of the APS chromophores only. Measurement of I_E/I_M as a function of solution pH indicates that chromophore interactions are on the local level (i.e., through "near" neighbors) even in the presence of 0.5 M NaCl.

Values of I_E/I_M for the terpolymers scale with the initial SDS to APS molar ratio (SMR) in their respective polymerization feeds. The resulting terpolymers exhibit variably blocky microstructures evidence by decreasing I_E/I_M, and thus APS label proximity with increasing SMR. Also I_E/I_M scales linearly with n, the initial number of APS molecules per SDS micelle. While the issue of hydrophobic comonomer migration during polymerization was not specifically addressed, the linearity of I_E/I_M with n implies that if APS migration occurs on the time scale of free-radical propagation, it is uniform over the surfactant to APS ratios in this study.

Also steady-state excitation and time-resolved emission studies indicate the presence of ground-state dimers in aqueous solutions of the terpolymers.

This assessment of the terpolymer microstructures accessible through micellar polymerization is extremely important in light of complementary studies on AM/AA/(decylphenyl)acrylamide terpolymers.[12] The critical aggregation concentration and extent of hydrophobic associations are extremely dependent on the SMR in micellar polymerization in these studies. Also, these studies indicate that composition and charge density of hydrophobically modified AM/AA copolymers may play critical roles in determining associative properties in solution.

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