

Chapter 22

Fluorescence Studies of Pyrene-Labeled, Water-Soluble Polymeric Surfactants

Michael C. Kramer, Jamie R. Steger, and Charles L. McCormick

Department of Polymer Science, University of Southern Mississippi,
Hattiesburg, MS 39406-0076

The synthesis of a fluorescently labeled water-soluble terpolymer based on acrylamide (AM) and the surface-active monomer sodium 11-Acrylamidoundecanoate (SA) is reported. Incorporation of 2-(1-pyrenylsulfonamido) ethyl acrylamide (APS) into the monomer feed yields a terpolymer with solution properties that are different from previously synthesized AM/SA copolymers. APS fluorescent label acts as a model hydrophobe; changes in pH and ionic strength that drive the viscosity response in AM/SA copolymers also affect the fluorescence emission properties of the APS label. Steady-state fluorescence emission studies reveal significant constriction of the polymer chain as pH decreases or electrolyte concentration increases. Fluorescence quenching studies suggest that the salt-induced chain collapse results in enhanced structuring of mixed aggregates formed by SA and APS units. Highly sensitive photophysical techniques confirm the pronounced pH and salt-responsiveness of AM/SA-based polymers observed in viscosity studies.

Previous work in our research laboratories has focused on the synthesis and characterization of fluorescently labeled water-soluble copolymers. The use of a covalently bound fluorescent moiety allows microscopic evaluation of the solution properties via steady state and transient fluorescence studies. Incorporation of a bulky hydrophobic group such as pyrene yields a polymer with associative properties via ground state inter- and intrapolymer hydrophobic interaction. Currently underway is an investigation of the solution properties of amphiphilic polyelectrolytes based on the surface-active monomer sodium 11-acrylamidoundecanoate (SA). The primary objective is the elucidation of ionic strength and pH effects on polymer conformation in aqueous media. Changes in these parameters affect the ability of these systems to sequester hydrophobic molecules. Assessment of salt- and pH-driven polymer

solution behavior leads to a better understanding of the phase-transfer properties exhibited by such materials.

Experimental

Materials. All reagents and solvents were purchased from Aldrich Chemical Co.. Acrylamide was recrystallized twice from acetone. Other materials were used as received. Water for synthesis and solution preparation was deionized and possessed a conductance $< 10^{-7}$ mho/cm.

Instrumentation. A Bruker AC-200 NMR spectrometer was used to determine ^1H and ^{13}C NMR spectra. Molecular weights were obtained with a Chromatix KMX-6 low angle laser light scattering photometer equipped with a 633 nm HeNe laser. Refractive index increments (dn/dc) were measured with a Chromatix KMX-16 laser differential refractometer. UV-VIS spectra were recorded with a Hewlett Packard 8452A diode array spectrophotometer. HPLC was carried out on a Hewlett Packard 1050 Series chromatograph fitted with a photodiode array UV detector and an Alltech Versapak C_{18} reversed-phase column. Viscosities were measured with a Contraves LS-30 rheometer at 25 °C and a shear rate of 6 sec^{-1} . Steady-state fluorescence spectra were measured on a Spex Fluorolog-2 fluorescence spectrometer equipped with a DM3000F data system. Excitation and emission slit widths of 1 mm and right angle geometry were employed.

Sodium 11-Acrylamidoundecanoate (SA). The methods of Gan (*1*) were employed in the synthesis of SA monomer. Purity was confirmed via NMR and melting point determination.

2-(1-Pyrenylsulfonamido) Ethylacrylamide. The procedure employed by Ezzell and McCormick (*2,3*) was followed to give pyrene monomer in good yield and purity according to NMR and HPLC.

Pyrene-labeled AM/SA Copolymer (AMSA/Py) (4) (Figure 1). A solution of sodium 11-acrylamidoundecanoate (1.8 g, 6.5 mmol) and acrylamide (9.0 g, 127 mmol) in 340 ml H_2O was added to the reaction flask immersed in a 50 °C water bath. After 2-(1-pyrenylsulfonamido) ethyl acrylamide (0.51 g, 1.3 mmol) that had been finely ground with a mortar and pestle was added to the flask, the solution was degassed with nitrogen for one hour, then sodium dodecyl sulfate (21.7 g, 75.2 mmol) added. At this point direct bubbling of nitrogen through the solution was stopped to prevent excessive foaming. After stirring the solution under nitrogen for 3 hours, most of the fluorescent comonomer had dissolved into SDS micelles. A degassed solution of potassium persulfate (50 mg, 0.19 mmol) in 5 ml H_2O was then

injected into the monomer/surfactant solution. After stirring under nitrogen for three hours, the viscous polymer solution was added to 1400 ml acetone to yield a white, rubbery precipitate. The polymer was washed with refluxing methanol for 16 hours in a Soxhlet extractor to remove residual monomer and surfactant. Good yield ($> 70\%$) is obtained.

Results and Discussion

Polymer Syntheses. Gan (5) first reported the copolymerization of acrylamide with sodium 11-acrylamidoundecanoate (SA) at a monomer feed level of ≥ 30 mole %. Reactivity ratio studies indicated a random incorporation of SA monomer, and the copolymer composition approximates that of the monomer feed. When a growing radical encounters a micelle containing polymerizable vinyl monomers, the localized monomer concentration is much higher than in the aqueous phase. Micellization also serves to orient the monomers. This is observed as a fast overall polymerization rate yielding high molecular weight polymer. When SA is homopolymerized, high conversion and molecular weight ($M_w > 10^6$ g/mol) is obtained in a short period of time (< 1 hr) (1). Even though a low feed level of SA is employed, high conversion within a short period of time is obtained.

Gan reported molecular weights in the $5 \times 10^5 - 2 \times 10^6$ g/mol range. The molecular weight of AM/SA copolymers is observed to decrease to the lower limit as AM content is increased from 0 to 70 mole % (5). AMSA/Py (Figure 1) has a molecular weight of 1.8×10^6 g/mol. Good monomer and surfactant purity may account for the higher molecular weight obtained relative to other AM/SA copolymers.

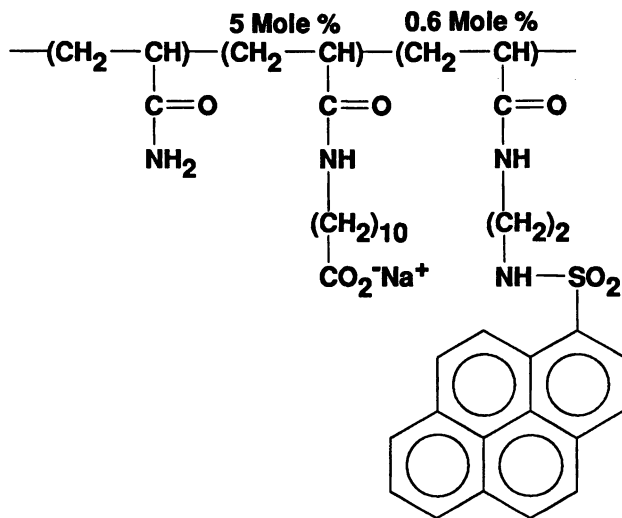


Figure 1. Pyrene-labeled AM/SA copolymer.

SA incorporation is assumed to equal monomer feed based on Gan's work and the high conversion obtained. When a copolymer of AM and SA with 90/10 AM/SA (mol/mol) in monomer feed was characterized by integration of ^1H NMR spectra, a copolymer composition of 10 ± 1 mole % was determined. If a similar weight percent incorporation of SA is assumed, 0.6 mole % incorporation for AMSA/Py is determined from UV-Vis studies ($\epsilon = 24,120 \text{ M}^{-1} \text{ cm}^{-1}$ at 352 nm (2,3,6)). The polymerization utilized 1 mole % fluorescent monomer in the monomer feed, but only 60 % of the monomer is incorporated. This is not an unusual result. Ezzell and McCormick (3) also reported low pyrene comonomer content in acrylamide copolymers synthesized both homogeneously and in the presence of added surfactant. Pyrene labels may be sufficiently hydrophobic such that the solubility in SDS micelles is limited, and incomplete dissolution into SDS micelles would result.

The use of an externally added surfactant to copolymerize a hydrophobic monomer with a hydrophilic monomer in aqueous solution can impart unique microstructural characteristics (7). A "blocky" microstructure may result from the inherent heterogeneity of the medium. When such a polymerization is carried out, the propagating radical in aqueous solution adds to the hydrophilic monomers in the aqueous phase. When the macroradical encounters a micelle, polymerization of all monomers within the micelle can occur if the micellar lifetime is sufficiently long. At high surfactant concentrations, this is a valid assumption, as k_p is quite high ($10^3 - 10^4 \text{ M}^{-1} \text{ sec}^{-1}$) for acrylamide and its related monomers (8). Since virtually all of the hydrophobic monomer, along with a fraction of the acrylamide (5,9) is partitioned into the micellar interior, a "block" of hydrophobic monomer is formed.

Viscosity and Fluorescence Studies. The block-like microstructure exhibited by these systems manifests itself in the rheological, as well as the photophysical response. Ezzell and McCormick (2,3,6) reported microstructural effects on the solution behavior of pyrene-labeled polyacrylamides synthesized by homogeneous and heterogeneous techniques. Pyrene labeled acrylamide copolymer synthesized in the presence of SDS exhibited a pronounced upwards curvature in the viscosity profile. This viscosity response is reflective of a low C^* , or critical overlap concentration. At this concentration, interchain hydrophobic association drives the chain overlap that results in polymer aggregation.

Viscosity studies provide a reasonable assessment of the bulk, macroscopic solution behavior, but a detailed analysis requires the use of a more sensitive characterization technique. Fluorescence emission studies are inherently sensitive to low chromophore concentrations and angstrom-level motions.

Our research group has investigated pyrene label excimer emission to elucidate solution behavior. As the degree of hydrophobic association increases, so does the interaction between two isolated pyrenes bound to polymer to form the sandwich-like conformation characteristic of an excimer. Interpolymer hydrophobic association is observed as an increase in excimer relative to that of "monomer" emission. A typical fluorescence spectrum of AMSA/Py is shown in Figure 2. The peak maxima at 380, 400, and 420 nm arise from the fluorescence emission of isolated pyrenes (monomer emission). The broad, structureless band centered

around 520 nm results from emission of excited dimeric pyrene (excimer). The intensity of excimer emission relative to that of the monomer emission (I_E/I_M) increases with polymer concentration. This response parallels the macroscopic viscosity behavior (4,6). I_E/I_M and apparent viscosity are plotted as a function of polymer concentration in Figure 3. I_E/I_M steadily increases with polymer concentration. The intermolecular hydrophobic associations that drive the viscosity profile also increase the population of excited pyrene dimers. Enhancements in intramolecular associations are also observed as an increase in I_E/I_M . As salt or acid is added to solution of SA copolymers, shielding or elimination of electrostatic repulsions between carboxylate groups of the SA unit leads to coil collapse that reduces the viscosity and I_E/I_M in these systems(4,10).

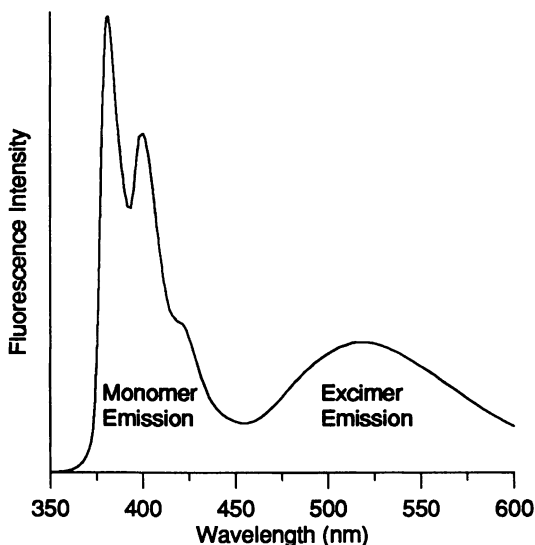


Figure 2. Fluorescence emission spectrum of pyrene-labeled polymer AMSA/Py. Polymer concentration: 0.052 g/dl in H_2O . Chromophore concentration: 3.4×10^{-5} M. Excitation wavelength: 340 nm.

Salt and pH Effects. The effects of salt and pH on the degree of excimer emission are shown in Figure 4. Viscosity studies have shown that pH decrease and salt addition lower the viscosity of AM/SA copolymers. Intrapolymer micellization of SA units is enhanced at the expense of charge-charge repulsions that interfere with association of the surfactant groups. Figure 5 illustrates this trend. As salt or acid are added, the shielding or elimination of electrostatic interactions collapses the coil. Pyrene groups within the polymer coil are closer to one another. The increase in localized chromophore concentration is observed as an increase in excimer emission relative to monomer emission.

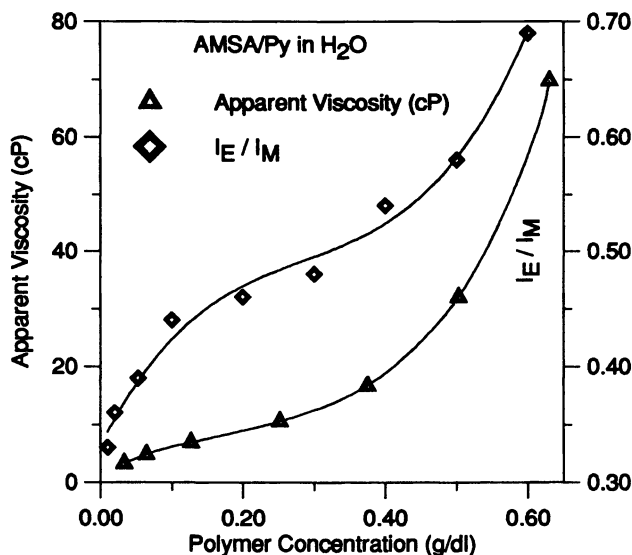


Figure 3. Apparent viscosity and excimer emission/monomer emission (I_E/I_M) as a function of AMSA/Py concentration in H_2O . (I_E/I_M = intensity at 519 nm / intensity at 400 nm).

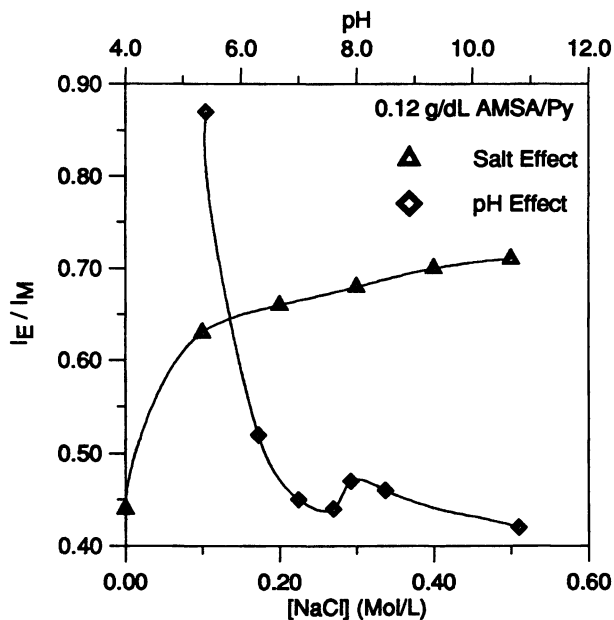


Figure 4. Salt and pH dependence of I_E/I_M . AMSA/Py polymer concentration: 0.12 g/dl.

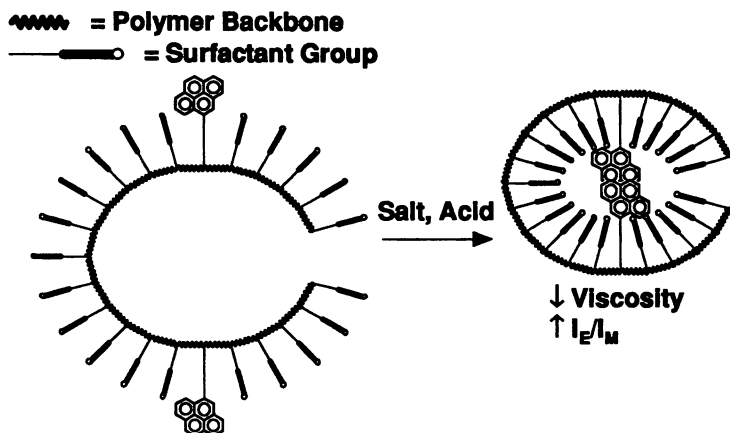


Figure 5. Proposed mechanism of salt/pH-triggered polymeric micelle structuring and photophysical enhancement.

Fluorescence Emission Quenching Studies. In order to probe the accessibility of the aqueous environment to the pyrenylsulfonamido chromophore, fluorescence quenching experiments were carried out using the amphiphilic quencher nitromethane (MeNO_2). First order quenching constants were calculated using the Stern-Volmer equation (1).

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \quad (1)$$

Equation 1 describes dynamic Stern-Volmer fluorescence quenching, where I_0 = emission intensity in the absence of quencher Q , I = emission intensity in the presence of Q , $[Q]$ = quencher concentration, and the Stern-Volmer quenching constant K_{SV} is expressed as:

$$K_{SV} = k_q\tau_0 \quad (2)$$

where k_q is the quenching rate constant usually expressed in $\text{M}^{-1} \text{sec}^{-1}$, and τ_0 is the lifetime of unquenched chromophore. If quenching occurs by a dynamic mechanism, a plot of I_0/I versus $[Q]$ yields a straight line with slope = K_{SV} .

Shown in Table I are the results of Stern-Volmer plots for nitromethane quenching of 0.12 g/dL AMSA/Py in deionized water and aqueous sodium chloride solutions. The linearity of the I_0/I versus $[Q]$ plots verifies the dynamic nature of fluorescence quenching. With the average APS fluorescence lifetime determined by Ezzell and McCormick (6) ($\tau_0 \cong 13 \times 10^{-9} \text{ sec}$), quenching rate constants can be calculated. The Stern-Volmer quenching constants and quenching rate constants approximate previously reported values (6,12). The magnitude of the quenching rate constants signifies a dynamic, diffusion-controlled process.

As electrolyte concentration increases, K_{SV} decreases. It should be noted that sodium chloride does quench APS fluorescence, but only slightly. Therefore, it is

valid to assume that any change in the nitromethane Stern-Volmer quenching constant with added sodium chloride arises from electrolyte-induced conformational changes and not from sodium chloride quenching. As salt is added, the coil collapses. Any changes in K_{sv} would indicate a change in the accessibility of the amphiphilic quencher to the pyrene label. The results from this study suggest that salt-triggered collapse creates a rigid environment that restricts quencher-label diffusion. K_{sv} decrease would then reflect an increase in the viscosity of the mixed microdomain formed by SA and pyrene label.

Table I. Nitromethane Quenching of Pyrenesulfonamide Fluorescence in 0.12 g/dl Aqueous AMSA/Py

[NaCl] (M)	K_{sv} (M ⁻¹)	k_q (M ⁻¹ sec ⁻¹)
0	35	2.7×10^9
0.1	32	2.5×10^9
0.3	25	1.9×10^9
0.5	20	1.5×10^9

Summary

A description of the photophysical response of a fluorescently labeled water-soluble polymer containing surface-active functional groups is presented. The enhancement in AMSA/Py excimer emission with increasing polymer concentration follows the association-induced viscosity response. pH and salt-induced collapse of the polymer coil also enhances excimer emission due to intramolecular label aggregation. Results from fluorescence quenching studies suggest salt-induced microdomain structuring to give SA/pyrene aggregates with increased rigidity.

Acknowledgments

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