*Macromolecules* **1999,** *32,* 4317−4326

# Hydrophobically Modified Poly(sodium 2-acrylamido-2-methylpropanesulfonate)s Bearing Octadecyl Groups: A Fluorescence Study of Their Solution Properties in Water

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*Received March 3, 1999; Revised Manuscript Received April 28, 1999*

ABSTRACT: Fluorescently labeled amphiphilic polyelectrolytes have been prepared by free-radical copolymerization in dimethylformamide using azobis(isobutyronitrile) as the initiator of sodium 2-acrylamido-2-methylpropanesulfonate (AMPS) and (1) *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (PyODA) in 98:2 and 95:5 molar ratios, (2) *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (NpODA) in 98:2 and 95:5 molar ratios, and (3) a mixture of the two labeled monomers in a molar ratio of 95:4:1 (AMPS: NpODA:PyODA). The solution properties of the copolymers in water and in salt solutions have been studied by viscometry, $^1$H NMR spectroscopy, and fluorescence spectroscopy. Evidence from nonradiative energy transfer between excited naphthalene (Np*) and pyrene (Py) in aqueous solutions of the doubly labeled polymers points to the formation of polymeric micelles. The inefficiency of nonradiative energy transfer between Np* and Py in mixed solutions of the singly labeled polymers indicates that the micelles are mostly unimolecular. The quenching of fluorescence of polymer-linked pyrene by nitromethane and thallium nitrate has been used to assess the accessibility of the chromophore to neutral molecules and to cationic species, respectively, in water and in solutions of increasing ionic strength. The results are discussed in light of previous studies of the properties in solution of neutral polymers carrying the same hydrophobic substituents as PAMPS−Py(Np)ODA and of amphiphilic PAMPS substituted with various other hydrophobic groups.

## Introduction

Water-soluble hydrophobically modified polymers act as effective rheology controllers in many important commercial products, such as water-borne paints and coating fluids, cosmetics,[1] or foodstuff.[2] They have found use as well in drug delivery,[3] oil recovery, and water treatment. The functions of these polymers are controlled by a number of factors, including the chemical structure and electrostatic charge of the monomer units and the overall composition and architecture of the macromolecule. Amphiphilic derivatives of polyelectrolytes are of particular practical importance, since they tend to be of higher solubility in water, compared to the case of neutral polymers, and the properties of their solutions may be affected by changes in pH and/or ionic strength. It is interesting to note that in these polymers the hydrophobic interactions, which favor intra- or interpolymeric association, are in direct competition with electrostatic interactions, which force the polymer chain to expand via charge repulsion between monomer units. The control of their chemistry poses an intriguing challenge to the polymer chemist.

Strauss and co-workers[4] reported the first preparation and characterization of hydrophobically modified polyelectrolytes, such as poly(2-vinylpyridine) partially quaternized with *n*-dodecyl bromide and alternating copolymers of maleic acid and alkyl vinyl ethers. These copolymers became known as "polysoaps" since their solution properties mimic those of ordinary soaps.[5] The field of amphiphilic polyelectrolytes underwent a rebirth in the early 1980s, when several research groups undertook the synthesis of modified polyelectrolytes with controlled architecture, to understand how molecular interactions steer the macroscopic properties of hydrophobically modified polyelectrolytes.[6−9] The importance of this work can easily be appreciated if one considers, for example, the macroscopic consequences of inter- and intrapolymeric association. If the association of hydrophobic groups involves several polyelectrolyte chains, macroscopic phase separation tends to occur. However, if the association takes place only among substituents linked to the same polymer, leading to the formation of "unimer micelles", the solution remains clear. The balance between the two types of interactions can, in principle, be controlled by varying the polymer concentration or by the addition of salts or surfactants: in highly dilute solution intrapolymeric association is expected to predominate, while at higher concentration interpolymeric interactions should become preponderant.

Recent work by Morishima demonstrates that the preference for inter- vs intrapolymeric association also depends critically on the chemical structure of the polymer.[7] For example, random copolymers of sodium 2-acrylamido-2-methylpropanesulfonate (AMPS) and a methacrylamide carrying bulky N-substituents, such as cyclododecyl, adamantyl, or naphthylmethyl groups, form unimer micelles in aqueous solution, regardless of polymer concentration.[10,11] A similar observation was reported by McCormick et al., in the case of hydrophobically modified polyacrylamides.[12] In these systems, randomly modified polymers formed unimers, whereas those carrying "blocky sequences" of hydrophobic sub-

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10.1021/ma990328g CCC: $18.00 © 1999 American Chemical Society
Published on Web 06/11/1999

stituents tended to undergo interpolymeric association, even though the overall content of hydrophobic groups was the same. Experimental evidence for the formation of unimer micelles was obtained from dynamic light scattering measurements, small-angle X-ray scattering data, and fluorescence spectroscopy.[13] In the later case, the experiments were carried out with polymers carrying a small number of dyes, such as pyrene, naphthalene, or 8-anilino-1-naphthylsulfonic acid, in addition to nonfluorescing hydrophobic groups. The chromophores reported on the interactions by changes in their photophysical properties, such as nonradiative energy transfer efficiency,[14] excimer formation,[15] and fluorescence depolarization.

In the cases discussed above, the labeled polyelectrolytes were prepared by statistical copolymerization of sodium 2-acrylamido-2-methylpropanesulfonate, a monomer carrying a hydrophobic substituent, and a monomer carrying the fluorescent dye. Thus, it can be expected that the probe and the hydrophobic substituents are linked to distant monomer units along the macromolecule. In studies of hydrophobically modified poly(*N*-isopropylacrylamides), Ringsdorf et al.[16] demonstrated that the solution properties of copolymers decorated randomly with chromophores and hydrophobic groups were quite different from those of the corresponding copolymers bearing the dye and the hydrophobic group attached to the same monomer unit. Both types of copolymers form interpolymeric micelles in cold water, but evidence from NRET experiments[17,18] and quenching studies[19] indicated that the polymer containing chromophore and hydrophobic group in the same monomer unit adopt a more compact conformation and exchange of polymer chains between micelles is all but precluded.

To assess the effect of molecular architecture on the solution properties of hydrophobically modified polyelectrolytes, we set about to prepare a series of labeled sodium poly(sodium 2-acrylamido-2-methylpropanesulfonate)s (PAMPS), in which the chromophore and the hydrophobic group are linked to the same monomer unit. Five fluorescently labeled amphiphilic PAMPS were prepared by copolymerization of AMPS, *N*-[4-(1-pyrenyl)butyl]-*N*−*n*-octadecylacrylamide, and/or *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (Figure 1). The synthesis, characterization, and solution properties of these copolymers are reported here. Special emphasis will be placed on results obtained from fluorescence techniques, supported by viscosity measurements and $^1$H NMR data. Evidence is given for the formation of unimers in aqueous solutions of the copolymers, even when the level of hydrophobic group incorporation is as low as 2 mol %. Fluorescence quenching studies were performed using nitromethane as a nonionic quencher and the thallium ion as a cationic quencher to assess the degree of protection experienced by the chromophores in aqueous solutions of various ionic strengths.

## Experimental Section

**Materials.** Water was deionized with a Barnstead NANOpure water purification system. Tetrahydrofuran (THF) was distilled from sodium under nitrogen. Dimethylformamide was distilled under reduced pressure. 4-(1-Pyrenyl)butyric acid, octadecylamine, carbonyldiimidazole, thallium nitrate, nitromethane, 2-acrylamido-2-methylpropanesulfonic acid (AMPS), and *N*,*N*-azobis(isobutyronitrile) (AIBN) were obtained from Aldrich Chemical Co. Inc. Nitromethane was dried over $CaCl_2$ and distilled before use. Thin-layer chromatography (TLC) was performed with silica plates (Merck) eluted with $CH_2Cl_2$/MeOH (9/1 v/v). *N*-[4-(1-Pyrenyl)butyl]-*N*-*n*-octadecylacrylamide[16] and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide[18] were prepared as described previously. Poly(sodium 2-acrylamido-2-methylpropanesulfonate) (PAMPS) and poly(sodium 2-acrylamido-2-methylpropanesulfonate)-*co*-[*N*-(1-pyrenemethyl)-methacrylamide (PAMPS−Py1) were prepared as described previously.[20]

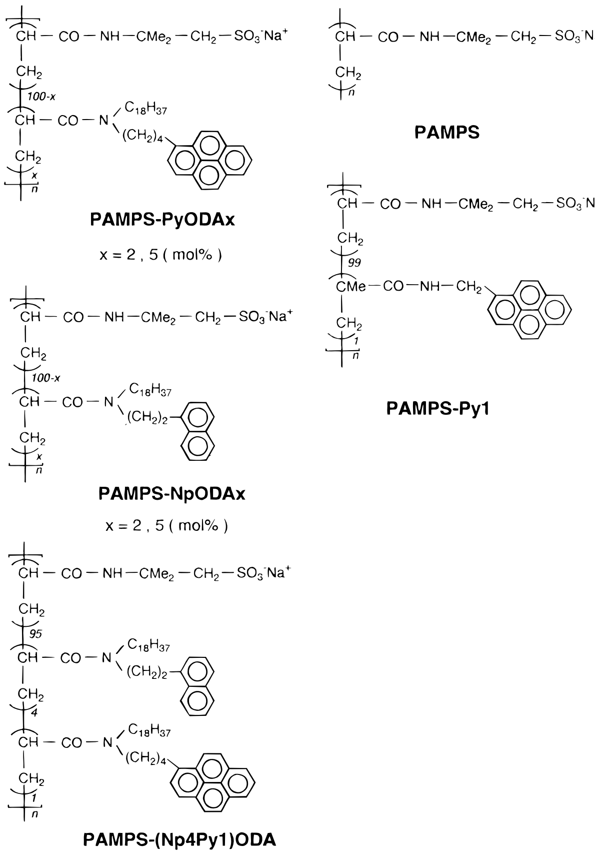

**Figure 1.** Structure of the polymers used in this study.

**Instrumentation.** Proton NMR spectra were recorded on Bruker 200 and 500 MHz spectrometers. Infrared spectra were recorded on a BioRad FTS-40 spectrometer. UV spectra were measured with a Hewlett-Packard 8452A photodiode array spectrometer. Gel permeation chromatography (GPC) measurements were performed with a Waters 590 programmable HPLC system (eluent 0.1 M $NaNO_3$, flow rate of 0.7 mL/min, Ultrahydrogel columns (Waters)) equipped with a Waters 486 UV detector and a Waters 410 differential refractometer. Standard pullulan samples, obtained from Showa Denko K.K., were used for calibration. Viscometry measurements were carried out with an Ubbehlode viscometer kept at 30 °C. Spectrapore membranes (12 000−14 000 MW cutoff) obtained from the Spectrum Co. were used for dialysis.

**Polymerizations. Sodium 2-Acrylamido-2-methylpropanesulfonate−*N*-[4-(1-Pyrenyl)butyl]-*N*-*n*-octadecylacrylamide Copolymer (2 mol % Py) (PAMPS−PyODA2).** A solution of 2-acrylamido-2-methylpropanesulfonic acid (4.9 mmol) and sodium carbonate (2.5 mmol) in DMF (5.0 mL) was stirred at 0 °C for 1 h. The solution was warmed to room temperature, and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (0.1 mmol) was added to the resulting solution. The reaction mixture was degassed with nitrogen for 15 min. It was heated to 65 °C. AIBN (3.4 mg, 0.021 mmol) was added to initiate the polymerization. The mixture was kept at 65 °C for 30 h, and then it was cooled in dry ice/MeOH. The polymer was isolated by precipitation into diethyl ether (600 mL). It was purified further by three consecutive precipitations from MeOH into diethyl ether. It was dissolved in water and subjected to dialysis against water for 2 days. The polymer

**Table 1. Physical Properties and Composition of the Polymers**

| | monomer feed (mmol)[a] | | | composition (mol %) | | | |
|---|---|---|---|---|---|---|---|
| polymer | AMPS | NpODA | PyODA | Py | Np | $M_n$[d] | $M_w$ ($M_w/M_n$)[d] |
| PAMPS−PyODA2 | 4.9 | 0.10 | 0.10 | 1.9,[b] 2.1[c] | 2.2,[b] 2.7[c] | 27 000 | 65 000 (2.4) |
| PAMPS−PyODA5 | 4.7 | 0.26 | 0.25 | 4.6,[b] 4.5[c] | 4.8,[b] 5.3[c] | 25 000 | 58 000 (2.3) |
| PAMPS−NpODA2 | 4.9 | 0.23 | 0.07 | 0.8[b] | 3.5[b] | 32 000 | 100 000 (3.1) |
| PAMPS−NpODA5 | 4.7 | | | | | 26 000 | 70 000 (2.7) |
| PAMPS−(Np4Py1)ODA | 4.7 | | | | | 24 000 | 58 000 (2.4) |
| PAMPS | | | | | | 71 000 | 175 000 (2.5) |
| PAMPS−Py1 | | | | | | 66 000 | 156 000 (2.3) |

[a] In all cases, [AIBN] = 0.02 mmol. [b] From UV absorbance spectrum. [c] From $^1$H NMR spectrum (solvent: $CD_3OD$). [d] From GPC data, calibrated against pullulans.

was isolated by lyophilization (1.00 g, 93% conversion). The polymer was characterized and used without further purification.

**Sodium 2-Acrylamido-2-methylpropanesulfonate−*N*-[4-(1-Pyrenyl)butyl]-*N*-*n*-octadecylacrylamide Copolymer (5 mol % Py, PAMPS−PyODA5).** The polymer was prepared following the procedure described above, starting with AMPS (4.7 mmol) and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (0.25 mmol) (see Table 1).

**Sodium 2-Acrylamido-2-methylpropanesulfonate−*N*-[2-(1-Naphthyl)ethyl]-*N*-*n*-octadecylacrylamide Copolymers (PAMPS−NpODA2, PAMPS−NpODA5).** They were prepared following the same procedure, starting from AMPS and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide in varying amounts (see Table 1).

**Doubly Labeled Polymer (PAMPS−(Np4Py1)ODA).** It was prepared following the same procedure, starting form AMPS, *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide, and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (see Table 1).

**Fluorescence Measurements.** Fluorescence spectra were recorded on a SPEX Industries Fluorolog 212 spectrometer equipped with a GRAMS/32 data analysis system. The slits were set at 1.0 mm (emission) and 1.0 mm (excitation). The excitation wavelength was 344 nm for pyrene and 290 nm for naphthalene. Samples for spectroscopic analysis were prepared from stock solutions (0.5 g $L^{-1}$). Ionic strength was adjusted by addition of NaCl, except in the case of the quenching experiments with thallium nitrate, where sodium acetate was employed. For quenching experiments with thallium ion, aliquots of aqueous solutions of thallium nitrate were added to solutions of the polymers, keeping the polymer concentration constant (PAMPS−PyODA2, 0.05 g $L^{-1}$; PAMPS−PyODA5, 0.02 g $L^{-1}$). Nitromethane aqueous solutions were obtained by dilution of a saturated nitromethane aqueous solution. The nitromethane concentration was determined from absorption spectra (water, $\epsilon_{336} = 1.56$ mol$^{-1}$ cm$^{-1}$ L, $\epsilon_{260} = 13.0$ mol$^{-1}$ cm$^{-1}$ L). The polymer concentrations were kept constant (PAMPS−PyODA2, 0.05 g $L^{-1}$; PAMPS−PyODA5, 0.02 g $L^{-1}$). The ionic strength was adjusted by addition of sodium chloride. All samples were kept at room temperature for 24 h prior to measurements.

**Determination of the Spectroscopic Parameters.** The pyrene excimer to monomer intensity ratios $[I_E/I_M]^{Py}$ were calculated by taking the ratio of the intensity (peak height) at 480 nm to the intensity at 378 nm. In the case of naphthalene, the excimer to monomer intensity ratios $[I_E/I_M]^{Np}$ were determined by taking the ratio of the intensity (peak height) at 378 nm to the intensity at 343 nm. The extent of pyrene emission due to NRET from naphthalene was determined by taking the ratios $I_{Py}/I_{Np}$ of the intensity at 378 nm to the intensity at 340 nm after correcting for emission due to direct pyrene excitation. The corrections were performed by subtracting from each spectrum the emission spectrum of PAMPS−PyODA2 ($\lambda_{exc} = 290$ nm) of identical pyrene concentration.

Fluorescence quenching can occur through a dynamic mechanism, in which case the quencher has to diffuse toward the excited chromophore.[14] Alternatively, quenching can be due to formation of complexes between the fluorophore and the quencher prior to excitation of the former, which leads to static quenching.[14,21] In microheterogeous systems "static" quenching can occur when the fluorophore and quencher are in close proximity. In the case of dynamic quenching, the ratio of the fluorescence intensity in the absence ($I_0$) and in the presence ($I$) of quencher is related to the quencher concentration [Q] by eq 1, where $K_{SV}$ is the Stern−Volmer constant, $k_q$ is the bimolecular quenching constant, and $\tau_0$ is the lifetime of the fluorophore in the absence of quencher:

$$I_0/I = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \qquad (1)$$

In microheterogeous systems such as hydrophobically modified polymers in water, it is possible that only a fraction of the fluorophores is accessible to quenchers. The fraction can be estimated by applying a modified Stern−Volmer model derived for systems in which there are heterogeneities in quencher and chromophore concentrations and diffusion coefficients.[22] This model assumes that only a fraction, *f*, of the fluorophores is readily quenchable, while a fraction, (1 − *f*), is protected against quenching. The fraction accessible and the Stern−Volmer quenching constant $K_{SVa}$ can be obtained from eq 2:

$$I_0/I = [1 - (fK_{SVa}[Q])/(1 + K_{SVa}[Q])]^{-1} \qquad (2)$$

## Results

**Preparation and Characterization of the Polymers.** Poly(sodium 2-acrylamido-2-methylpropanesulfonate) and its *N*-octadecyl copolymers were prepared by free-radical polymerization in dimethylformamide under conditions leading to a high conversion rate. The polymers were purified by repeated precipitations of methanolic solutions into diethyl ether. Diethyl ether is a poor solvent for PAMPS but a good solvent for the hydrophobic monomers. Residual sodium 2-acrylamido-2-methylpropanesulfonate was removed by exhaustive dialysis against water. The purity of the polymers was assessed by GPC, using UV absorbance and refractive index detectors in tandem, to ascertain the absence of low-molecular weight UV-absorbing species. Four singly labeled octadecyl copolymers and one doubly labeled octadecyl copolymer were synthesized: PAMPS−PyODA2, PAMPS−PyODA5, PAMPS−NpODA2, PAMPS−NpODA5, and PAMPS−(Np4Py1)ODA (Figure 1). The digits in the polymer designations refer to the molar percent of chromophore incorporated. The unmodified polyelectrolyte, PAMPS, was prepared under the same conditions.

The chemical composition of the copolymers was determined from their $^1$H NMR spectra and from their UV absorption spectra recorded in $CD_3OD$ and $CH_3OH$, respectively (Table 1). Analysis by $^1$H NMR gave the ratios of AMPS units to *n*-octadecyl group, calculated from the area of the broad singlet at 2.21 ppm, attributed to the resonance of main-chain methine protons,[23,13] and the area of the triplet centered at 0.88

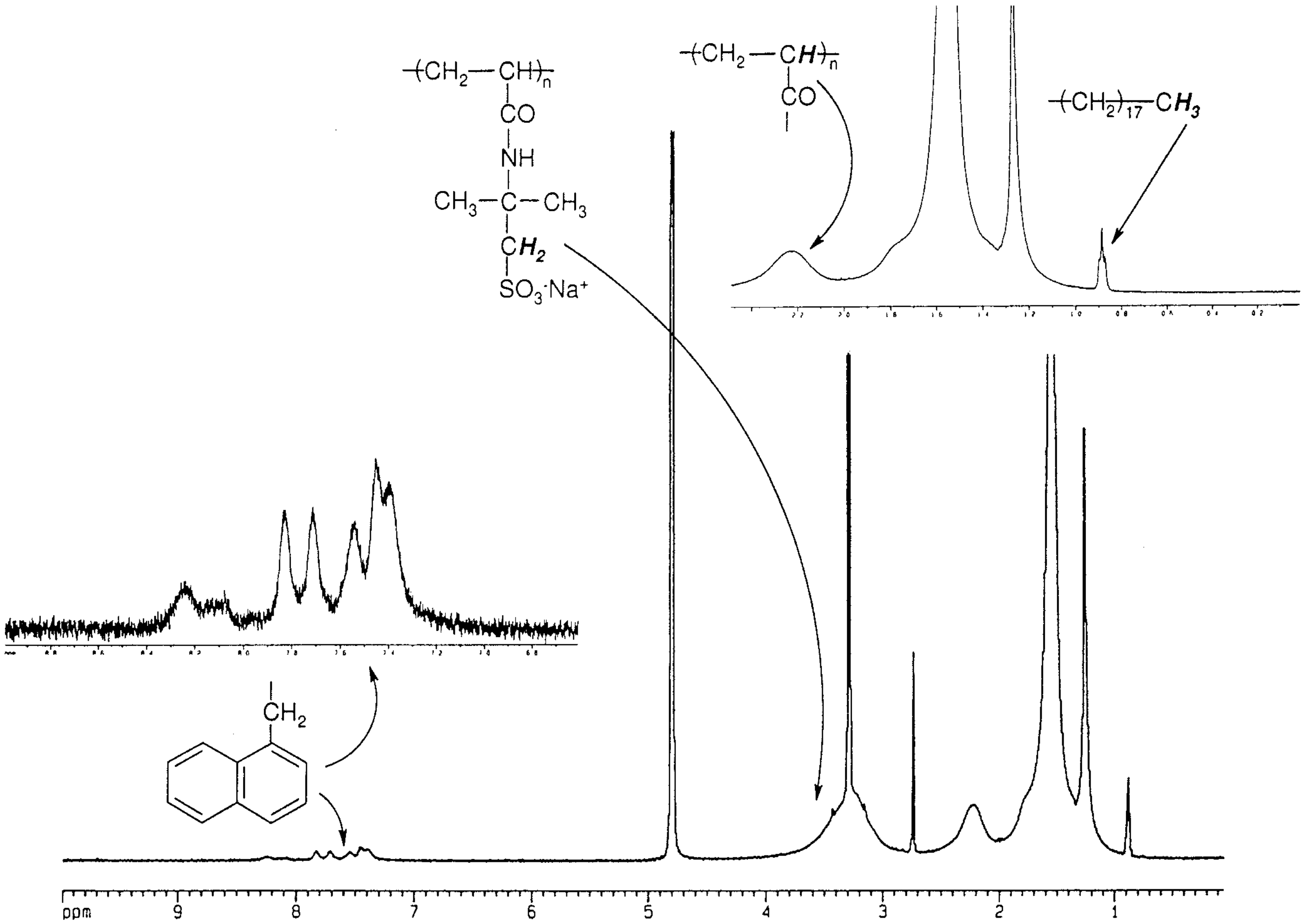

**Figure 2.** $^1$H NMR spectrum of PAMPS−NpODA5 in deuterated methanol.

ppm, assigned to the resonance of the terminal methyl protons of the octadecyl chains (Figure 2). In the case of the doubly labeled polymer, composition determination by $^1$H NMR yields the amount of AMPS units to the sum of the two octadecyl-containing monomers (4.3 mol %). Compositions were calculated also from the UV absorbance of polymer solutions in methanol, assuming that the extinction coefficients of naphthalene (6700 cm$^{-1}$ mol$^{-1}$ L at 282 nm) and pyrene (32 800 cm$^{-1}$ mol$^{-1}$ L at 342 nm) in the polymers are identical to those of the corresponding monomers. In the case of the doubly labeled polymer the naphthalene content was determined from the solution absorbance at 282 nm, after subtraction of the pyrene contribution to the absorbance at this wavelength. Satisfactory agreement (within 10%) between the two techniques was achieved in all cases. The composition also corresponds closely to the monomer feed ratios.

Molecular weights of the copolymers were estimated by GPC measurements run in aqueous sodium nitrate solution and calibrated against pullulan standards. The molecular weight values determined for the unmodified polylelectrolyte and PAMPS−Py1 are in the same range as previously described ($M_n \sim 70\,000$);[24] however, we note that the average molecular weights ($M_n$) calculated for the hydrophobically modified polymers are significantly lower ($M_n \sim 25\,000-30\,000$). The large difference in the $M_n$ values of PAMS and the hydrophobically modified polymers should not be a surprise. Even though the polymerizations were conducted under strictly identical conditions, the modified polymers, which adopt a more compact globular form, are expected to elute at shorter retention times, compared to their unmodified analogues. To clarify this point, we measured the intrinsic viscosity of methanolic solutions of the copolymers and of PAMPS. We were unable to detect any significant difference among the values recorded for solutions of the various polymers. This circumstantial evidence lends support to the assumption that the polymers described here have very similar molecular weights. Experiments addressing this point are in progress.

**$^1$H NMR Spectroscopy.** While the $^1$H NMR spectra of all the copolymers in methanol-$d_4$, a good solvent for PAMPS, are well-resolved (see Figure 2 for example), spectra run under identical instrumental conditions with solutions of the polymers in $D_2O$ exhibit significant broadening of certain signals. Figure 3 presents the $^1$H NMR spectrum of PAMPS−NpODA5 in $D_2O$. The resonance of the terminal methyl protons of the octadecyl chain appears as a broad singlet centered at $\delta$ 0.8 ppm, rather than a well-resolved triplet, as is observed in the spectrum of the polymer dissolved in methanol (Figure 2). The signal due to the naphthyl protons resonance ($\delta$ 7.4−8.5 ppm) also exhibits significant loss in resolution and in intensity. This line broadening indicates that the motion of the naphthalene groups and of the octadecyl chains is highly restricted in the hydrophobic microdomains formed within the unimers.[13] In contrast, we note that the signals attributed to the resonances of main-chain protons and the protons of the AMPS side chains retain their sharpness. The signals at 1.6 ppm, attributed to the geminal methyl groups,

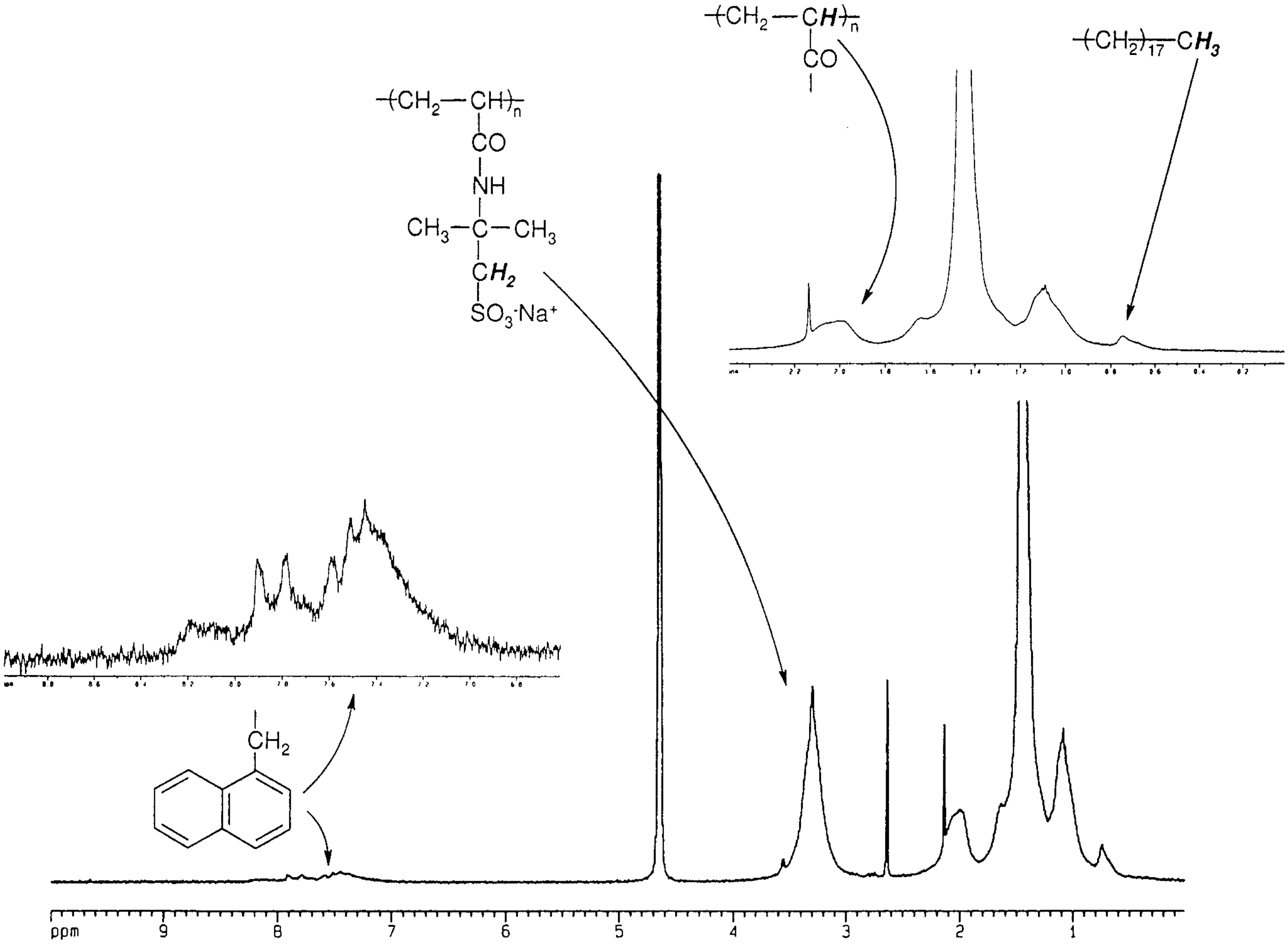

**Figure 3.** $^1$H NMR spectrum of PAMPS−NpODA5 in deuterated water.

and the signal at 3.3 ppm, attributed to the methylene protons α to the sulfonate group, have almost the same line width in spectra recorded in water and in methanol. Also, the signals due to the main-chain methylene protons (1.7 ppm, shoulder) and of the main-chain methine protons (2.2 ppm) retain their sharpness in water. Thus, while the hydrophobic groups experience severe motion restriction, as a result of hydrophobic association, the local motion of the AMPS units, which make up on average 95% of the monomer units in a chain, retain local freedom of motion.

**Viscosity Studies.** Reduced viscosities of copolymer solutions in water and in 0.1 M NaCl were measured as a function of copolymer concentration. Huggins plots obtained for PAMPS−PyODA5 and PAMPS−PyODA2 are presented in Figure 4. The reduced viscosities of the salt-free solutions increase with decreasing polymer concentration. The sharpness of the increase upon dilution reflects the extent of chain expansion due to the increase in segmental electrostatic repulsion. This behavior is characteristic of strong polyelectrolytes. The reduced viscosities of the copolymers salt solutions are reduced considerably, compared to those of salt-free solutions, and the corresponding Huggins plot shows a positive slope. This trend reveals the salt-induced chain contraction attributed to the shielding of charge repulsion between sulfonate groups along the chain. While solutions of PAMPS−PyODA5 and PAMPS−PyODA2 exhibit the same overall behavior, it is worth noting that in all cases the values recorded for solutions of the least substituted copolymer are significantly higher than those of PAMPS−PyODA5. This difference may be taken as an indication of the relative compactness of the unimers: as the level of hydrophobic modification increases, the micelles tend to contract, an effect triggered by the hydrophobic association of the pyrene−octadecyl groups. These effects are displayed also by the spectroscopy of the fluorescent dyes attached to the polymers, as described in the following sections.

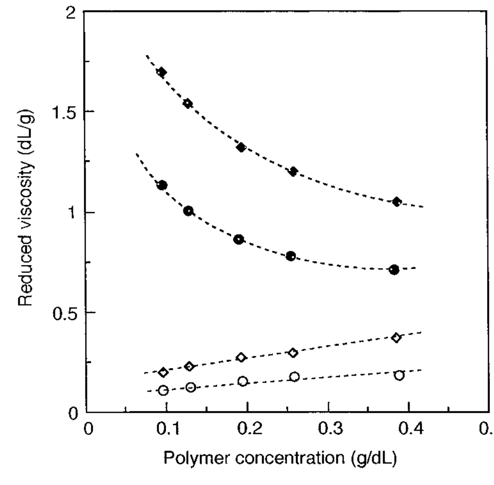

**Figure 4.** Huggins plots for solutions of PAMPS−PyODA5 and PAMPS−PyODA2 in water (full symbols) and in 0.1 M NaCl (open symbols); temperature, 30 °C: (●) PAMPS−PyODA5, (◆) PAMPS−PyODA2.

**Photophysical Properties of the Copolymers in Solution. Pyrene-Labeled Copolymers.** Steady-state

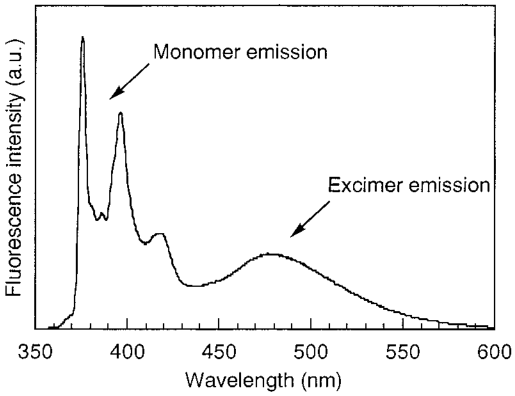

**Figure 5.** Fluorescence spectrum of PAMPS−PyODA5 in methanol; $\lambda_{exc} = 344$ nm, polymer concentration 0.1 g $L^{-1}$.

emission spectra were measured for solutions of both polymers in methanol and water and in aqueous NaCl solutions of increasing ionic strength. In water, the copolymers show a strong, broad, featureless emission centered at 480 nm (intensity $I_E$), in addition to the characteristic well-resolved emission, due to locally isolated excited pyrenes, with the (0,0) band located at 378 nm (monomer emission, intensity $I_M$). The broad emission originates from pyrene excimers. Identical excitation spectra were obtained for emissions monitored at 378 and 480 nm, and their maxima correspond to the UV absorption data. Therefore, both monomer and excimer emissions originate from excited isolated pyrene chromophores. The fact that pyrene excimer emission is strong implies that the pyrene groups are in close spatial proximity. This may be due either to a nonstatistical distribution of the pyrene groups along the polymer chain or to the occurrence of hydrophobic microdomains that bring together pyrene groups attached to monomer units well separated along the polymer chain. It seems unlikely that the pyrene-containing units are clustered in blocks since the polymerization was conducted under conditions shown previously to lead to hydrophobically modified PAMPS having random distributions of the monomer units along the chain.[25] Moreover, the spectra of the same polymers in methanol, a good solvent for PAMPS (Figure 5), present a weaker excimer emission, contrary to what would be expected to occur if pyrene groups were attached to neighboring monomer units.

Spectra of PAMPS−PyODA2 and PAMPS−PyODA5 were recorded for solutions of concentration ranging from 0.005 to 0.05 g $L^{-1}$.The ratio $I_E/I_M$ was insensitive to changes in polymer concentration ($[I_E/I_M]^{Py} = 0.35 \pm 0.02$, PAMPS−PyODA5; $[I_E/I_M]^{Py} = 0.089 \pm 0.001$, PAMPS−PyODA2). This observation suggests that the hydrophobic microdomains occur within a single polymer chain. Further evidence for the formation of unimers arises from experiments based on nonradiative energy-transfer experiments described below.

**Naphthalene-Labeled Copolymers.** Steady-state fluorescence spectra of PAMPS−NpODA2 and PAMPS−NpODA5 were recorded for solutions of the polymers in water and in methanol. In methanol, the polymers exhibited a structured spectrum with a maximum at 340 nm, characteristic of locally isolated excited naphthalenes. No excimer emission was observed. In contrast, polymer solutions in water present spectra with a significant contribution from naphthalene excimer emission ($\lambda_{max} = 400$ nm). This emission persists in solutions of very low concentration ($[I_E/I_M]^{Np} = 0.29 \pm 0.01$, PAMPS−NpODA5, water, 0.05 g $L^{-1}$). Thus, the spectroscopy of the naphthalene-labeled polymers also suggests the formation of unimers in aqueous solutions.

**Mixed Solutions of Pyrene-Labeled and Naphthalene-Labeled Copolymers.** Naphthalene and pyrene are known to interact as energy donor (Np) and energy acceptor (Py) via nonradiative energy transfer (NRET). For this process to occur the two chromophores have to be in close spatial proximity. In the case of the naphthalene/pyrene pair the characteristic distance, $R_0$, is approximately 29 Å.[26] Thus, only chromophores located within the same hydrophobic microdomain are expected to interact. In systems where NRET takes place, excitation at 290 nm will result in a complex emission consisting of emission from Np* (310−400 nm) and of an emission from Py* excited by NRET from Np*. Since pyrene itself has a weak absorbance at 290 nm, excitation at 290 nm also results in a weak emission from directly excited pyrene. This contribution to the spectrum was taken into account where required. Spectra of aqueous solutions of mixtures of PAMPS−PyODA5 and PAMPS−NpODA5 (total polymer concentration: 0.05 g $L^{-1}$) feature a strong emission due to naphthalene and a weak contribution from pyrene assigned to the emission of chromophores directly excited at 290 nm. The naphthalene emission intensity is identical to that determined from spectra recorded from solutions of PAMPS−NpODA5 with the same naphthalene concentration. Thus, NRET between naphthalene and pyrene did not occur, confirming the existence of unimolecular micelles, which present no tendency to intermix.[27]

**Doubly Labeled Copolymer.** This terpolymer, PAMPS−(Np4Py1)ODA, carries both pyrene and naphthalene attached at random along the polymer chain. As in the case of the singly labeled copolymers, the chromophore-containing monomer units also carry an octadecyl chain. The octadecyl group molar content was set to be 5 mol %, identical to that of singly labeled samples, while the molar ratio of naphthyl to pyrenyl groups was chosen as 4:1, to facilitate the occurrence of NRET. Emission spectra, upon excitation at 290 nm, of PAMPS−(Np4Py1)ODA solutions in methanol and in water are presented in Figure 6. The spectrum obtained from methanolic solution (Figure 6, top) consists of a contribution from Np* and a weak contribution from Py*. The latter emission is due in part to pyrene excited by NRET from Np* and in part from directly excited pyrene. The emission spectrum of a solution of PAMPS−PyODA2 of identical pyrene concentration, also excited at 290 nm, is shown as well, giving a measure of the expected intensity of the direct pyrene emission in these conditions. The emission spectrum of PAMPS−(Np4Py1)-ODA in water (Figure 6, bottom) presents a much stronger contribution from pyrene, while the emission due to Np* is weaker. In aqueous solutions of the polymer, the two chromophores are brought into close proximity, allowing significant NRET to occur. By analogy with the case of the aqueous solutions of singly labeled polymer solutions, it is expected that the doubly labeled polymer also forms unimolecular micelles.

**Spectroscopy of the Copolymers in Aqueous Salt Solutions.** The emission spectra of the singly and doubly labeled copolymers were measured in aqueous solutions of increasing salt concentration. Most noticeable are the salt-induced increase of excimer emission,

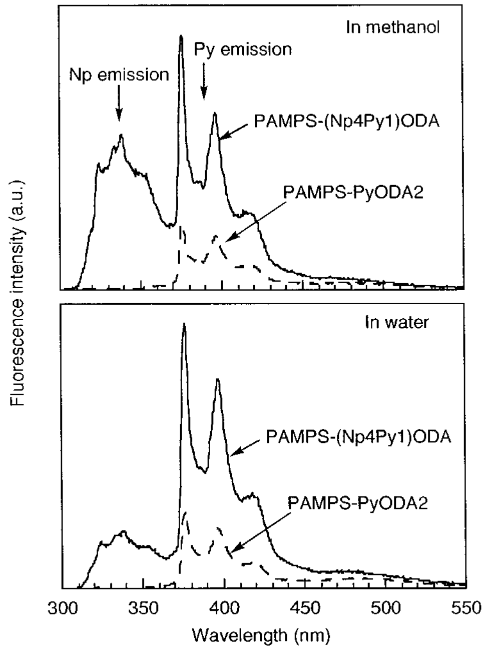

**Figure 6.** Fluorescence spectra of PAMPS−(Np4Py1)ODA and PAMPS−PyODA2 in methanol (top) and in water (bottom): dotted line, $\lambda_{exc}$ = 344 nm; full line, $\lambda_{exc}$ = 290 nm. Polymer concentration = 0.1 g L$^{-1}$.

relative to monomer emission in the spectra of singly labeled polymers and, in the case the doubly labeled copolymer, the salt-induced enhancement of NRET between Np* and Py. These effects are illustrated in Figure 7a, which presents emission spectra of PAMPS−PyODA5 in aqueous solutions of ionic strength 0−0.1 M, and Figure 7b, which displays the emission ($\lambda_{exc}$ 290 nm) of PAMPS−(Np4Py1)ODA in solutions of ionic strengths 0 and 0.1 M. Also presented (insets) are the changes of the ratios $I_E/I_M$ and $I_{Py}/I_{Np}$ in solutions of the two polyelectrolytes of increasing ionic strength. The ratios increase sharply in the low ionic strength domain (0−0.2 M). The ratios continue to increase slightly in solutions of higher ionic strength. The spectroscopy of the pyrene-labeled copolymers undergoes additional minor, but revealing, changes easily detectable by steady-state fluorescence. First, in the emission (Figure 7a) the excimer band undergoes a slight blue shift, from 484.5 nm [NaCl] = 0 to 482 nm [NaCl] = 1.0 M. Second, excitation spectra measured from solutions of high salt concentration for the excimer and the monomer emissions are different (Figure 8). They present the same overall shape, but the former is red-shifted by ca. 2 nm. Also, the peak-to-valley ratio computed for the excimer excitation spectrum (1.92) is smaller than that obtained from the monomer excitation spectrum (2.40).[15] These differences, diagnostic of the occurrence of pyrene ground-state aggregates, were not displayed by the excitation spectra recorded from salt-free solutions. Taken together, these features point to a significant contraction of the unimers in solutions of high-salt concentration, corroborating the conclusions drawn from intrinsic viscosity determinations.

**Fluorescence Quenching Experiments.** Two quenchers of pyrene fluorescence were employed: nitromethane ($CH_3NO_2$), a neutral species, and thallium nitrate, a cationic quencher. Both compounds are soluble in water, with nitromethane possessing some affinity for hydrophobic environments. Nitromethane quenches pyrene fluorescence at a diffusion-controlled rate by an electron-transfer mechanism. The thallium ion affects pyrene emission via a heavy atom effect that requires short-range interaction between the two species.[28] The results of the quenching studies are reported in terms of the Stern−Volmer model (see Experimental Section). Linear Stern−Volmer plots were obtained for nitromethane quenching of pyrene excimer and monomer emissions in solutions of PAMPS−PyODA5 and PAMPS−PyODA2 in water and in 0.1 M aqueous NaCl. Stern−Volmer constants (Table 2) are smaller than the

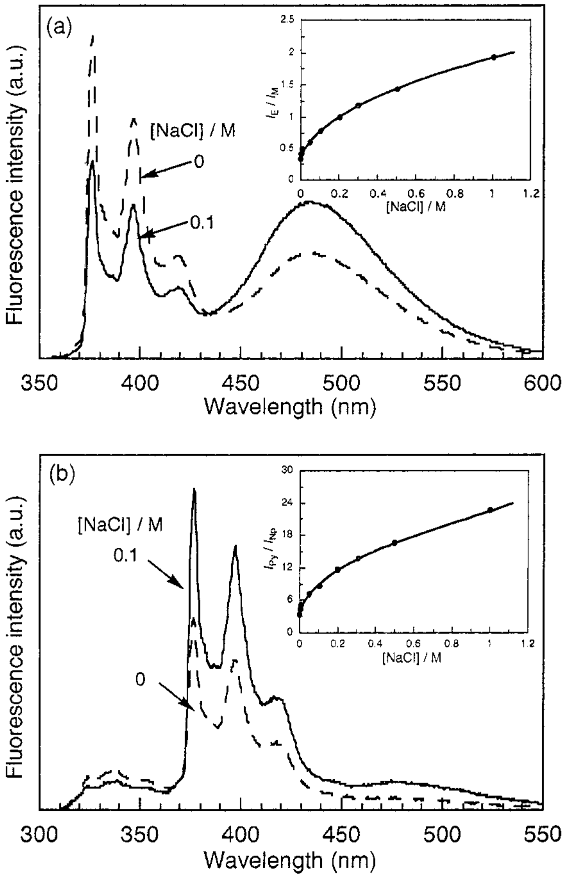

**Figure 7.** Fluorescence spectra of hydrophobically modified PAMPS in solutions in water and in aqueous NaCl (0.1 M): (a) PAMPS−PyODA5, $\lambda_{exc}$ = 344 nm, polymer concentration 0.02 g L$^{-1}$; (b) PAMPS−(Np4Py1)ODA, $\lambda_{exc}$ = 290 nm, polymer concentration 0.05 g L$^{-1}$. Insets: (a) changes in the ratio $I_E/I_M$ for PAMPS−PyODA5 as a function of NaCl concentration; (b) changes in the ratio $I_{Py}/I_{Np}$ for PAMPS−(Py1Np4)ODA as a function of NaCl concentration.

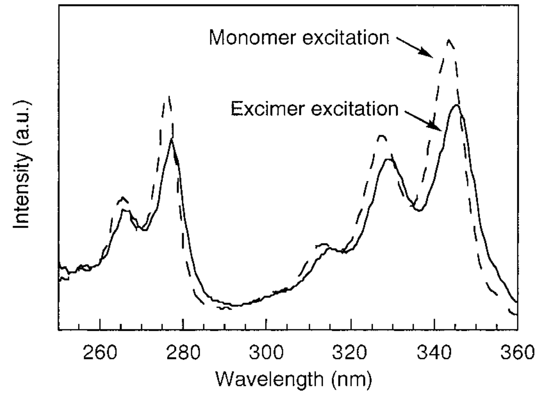

**Figure 8.** Excitation spectra of PAMPS−PyODA5 in aqueous NaCl (0.1 M): full line, $\lambda_{em}$ = 480 nm (excimer); broken line, $\lambda_{em}$ = 378 nm (monomer).

**Table 2. Quenching Data for Nitromethane and PAMPS−PyODA2[a] and PAMPS−PyODA5[b] in Water and in Aqueous NaCl (0.1 M)**

| polymer | | $K_{SV}$ (mol$^{-1}$ L) water | $K_{SV}$ (mol$^{-1}$ L) 0.1 M NaCl |
|---|---|---|---|
| PAMPS−PyODA5 | monomer | 362 ± 3 | 286 ± 4 |
| | excimer | 202 ± 2 | 123 ± 1 |
| PAMPS−PyODA2 | monomer | 429 ± 2 | 402 ± 1 |
| | excimer | 260 ± 2 | 167 ± 1 |

[a] Polymer concentration: 0.05 g L$^{-1}$. [b] Polymer concentration: 0.02 g L$^{-1}$.

rate constant for pyrene in water ($K_{SV}$ = 1040 ± 100 M$^{-1}$),[19] indicating that the chromophores are protected against quencher molecules by the polymer chains. Corresponding Stern−Volmer constants take a lower value in PAMPS−PyODA5, compared to PAMPS−PyODA2, and in both cases, the values are higher for quenching in water, compared to quenching in salt solution. Since NaCl is not a quencher of pyrene fluorescence, the effects must arise from electrolyte-induced conformational or environmental changes sensed by the probe. The fact that no downward curvature was observed in the quenching plots indicates that there is no "saturation of binding sites" for the quencher. The low quenching efficiency in salt solutions reflects an increased protection of the chromophores as a result of the contraction of polymer chain and, presumably, tightening of the hydrophobic domains. Since the decrease of the emission intensity in steady-state experiments can be due to dynamic and static quenching,[14,21] the change in lifetimes of pyrene in the presence of quencher has to be analyzed.

Thallium nitrate quenching experiments were carried out next with the two pyrene−octadecyl substituted polyelectrolytes, PAMPS−PyODA5 and PAMPS−PyODA2, as well as the pyrene-labeled polyelectrolyte, PAMPS−Py1 (see Figure 1), which has approximately the same content of pyrene but does not carry additional hydrophobic groups. Slightly different polymer concentrations were selected in each case in order to keep the pyrene concentration ([Py] = 4.4 × 10$^{-6}$ mol L$^{-1}$) the same in all measurements (see Table 2).[29] Stern−Volmer plots for the three polymers in water and in 0.05 M NaOAc solutions are shown in Figure 9a,b. Efficient quenching occurred in salt-free solutions, but the Stern−Volmer plots deviate from linearity. The plot related to Tl$^+$ quenching of PAMPS−Py1 presents an upward curvature, indicating a contribution of static quenching mechanism.[7] This polymer assumes an extended conformation in water. Therefore, the chromophores are of easy access to the cationic quencher which binds to the polyanion via electrostatic interaction. In contrast, the plots related to the pyrene−octadecyl polymers show a mild, but clear, downward curvature: the ratio $I_0/I$ increases rapidly at low quencher concentration [Tl$^+$] < 4 × 10$^{-5}$ M but only slightly at higher quencher concentrations. Such deviation from ideal Stern−Volmer dynamic quenching signals that a fraction of the chromophores is not accessible to the quencher. The fraction, *f*, of accessible chromophores can be estimated by applying a modified Stern−Volmer model derived for systems in which there are heterogeneities in quencher and chromophore concentrations and diffusion coefficients (see Experimental Section). This analysis was applied to analyze the data presented in Figure 9a. The fraction of pyrene groups accessible remains high ($f$ > 0.94, see Table 3). Values determined for the least substituted polymer are slightly larger than those obtained for PAMPS−PyODA5, which may indicate that the hydrophobic microdomains formed by the former polymer are slightly more hydrated.

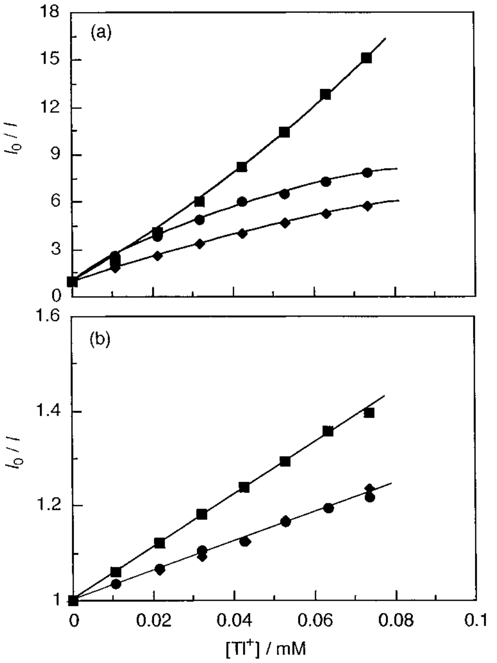

**Figure 9.** Ratio $I_0/I$ of pyrene monomer emission intensity in the absence and in the presence of thallium ion in solutions of pyrene-labeled PAMPS: (a) water; (b) 0.05 M sodium acetate (NaOAc); (●) PAMPS−PyODA5 (0.02 g L$^{-1}$); (◆) PAMPS−PyODA2 (0.05 g L$^{-1}$); (■) PAMPS−Py1 (0.1 g L$^{-1}$).

The thallium ion quenching efficiency is greatly reduced by salt addition (Figure 9b). This effect could arise from enhanced compartmentalization of the chromophores in the hydrophobic microdomains, as it is expected that added salt enhances intrapolymer micellization, as discussed previously. The reduction in Tl$^+$ quenching efficiency is more likely due to dilution of atmospheric Tl$^+$ counterions (maximum concentration: 8 × 10$^{-5}$ M) in the presence of added NaOAc (concentration: 10$^{-1}$ M). Stern−Volmer plots recorded for thallium quenching of pyrene emission from solutions of PAMPS−PyODA5 of increasing ionic strength illustrate the effect (Figure 10).

## Conclusions

Viscometry, $^1$H NMR spectroscopy, and various fluorescence techniques have been employed to examine the associative behavior of hydrophobically modified sodium poly(sodium 2-acrylamido-2-methylpropanesulfonate)s carrying a few mole percent of pyrenylbutyl−*n*-octadecyl groups distributed at random along the chain. The polyelectrolytes form predominantly unimolecular micelles in water and in salt solutions. The micelles consist of hydrophobic cores that capture the pyrene groups and protect them against quenching by neutral molecules and cationic species. The polyelectrolyte main chain surrounds these hydrophobic microdomains in a "flowerlike" conformation, which accommodates electrostatic repulsion between neighboring monomer units (Figure 11).[30] It is interesting to compare the assembly of the PyODA-substituted polyelectrolytes to that of a closely related neutral water-soluble hydrophobically modified polymer, consisting of a poly(*N*-isopropylacrylamide)

**Table 3. Quenching Data for Thallium Nitrate and Pyrene-labeled PAMPS in Water and Aqueous NaOAc**

| polymer | | [NaOAc] = 0 | | [NaOAc] = $10^{-3}$ M | [NaOAc] = $10^{-1}$ M |
|---|---|---|---|---|---|
| | | $K_{SV}$ ($10^{-3}$ $M^{-1}$) | f | $K_{SV}$ ($10^{-3}$ $M^{-1}$) | $K_{SV}$ ($10^{-3}$ $M^{-1}$) |
| PAMPS−PyODA5[a] | monomer | 173 ± 2 | 0.94 ± 0.01 | 36.1 ± 0.3 | 1.7 ± 0.1 |
| | excimer | 162 ± 1 | 0.94 ± 0.01 | 33.0 ± 0.2 | 2.2 ± 0.1 |
| PAMPS−PyODA2[b] | monomer | 87 ± 2 | 0.96 ± 0.01 | 39.6 ± 0.2 | 2.1 ± 0.1 |
| | excimer | 66 ± 1 | 0.96 ± 0.01 | 33.0 ± 0.3 | 2.3 ± 0.1 |

[a] Polymer concentration: 0.02 g $L^{-1}$. [b] Polymer concentration: 0.05 g $L^{-1}$.

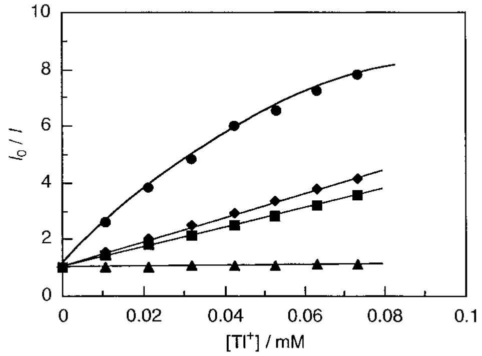

**Figure 10.** Ratio $I_0/I$ of pyrene monomer emission intensity in the absence ($I_0$) and in the presence ($I$) of thallium ion in aqueous solutions of PAMPS−PyODA5 (0.02 g $L^{-1}$): (●) water; (◆) [NaOAc] = $10^{-4}$ M; (■) [NaOAc] = $10^{-3}$ M; (▲) [NaOAc] = $10^{-1}$ M.

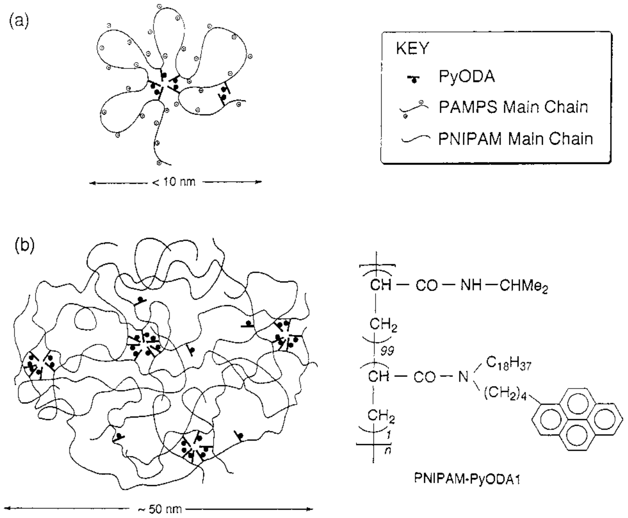

**Figure 11.** Schematic representation of the micellar assemblies formed in water by PAMPS−PyODA5 (a)[7] and by a pyrene−octadecyl-labeled poly(*N*-isopropylacrylamide) (PNIPAM−PyODA1) (b).[16]

main chain, carrying a few mole percent of the same pyrene-labeled octadecyl substituent (PNIPAM−PyODA1,[16−18] Figure 11). This polymer in water forms *interpolymeric* micellar structures, even in extremely dilute solutions. The hydrophobic clusters provide a similar protection of the pyrene groups against quenching by nitromethane.[19] The polymer main chain surrounding the hydrophobic microdomains interacts with water via hydrogen bonds.

Morishima and co-workers[6,11,13] have investigated in great detail the effect of the hydrophobic group content on the conformation and solution properties of amphiphilic sodium poly(sodium 2-acrylamido-2-methylpropanesulfonate)s. In most cases they observed that unimolecular micelles form only when the hydrophobic group content exceeds approximately 20 mol %. Their results, however, are limited to alkyl groups of 12 carbons, such as *n*-docecyl, cyclododecyl, and adamantyl moieties on the one hand and the cholesteryl group on the other. Our results with PAMPS−PyODA cannot be compared directly to these data, given the substantial difference between the hydrophobicity of dodecyl groups, a cholesteryl moiety, and an octadecyl chain. Work is in progress to assess the control of the aggregation of octadecyl-substituted PAMPS by "dialing in" the level of octadecyl group incorporation.

**Acknowledgment.** The work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to F.M.W. and by a fellowship to M.M. from the Ministry of Education, Science, Sports, and Culture, Japan.

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(27) Measurements of NRET were conducted also in polymer solutions of higher concentration (1.0 g $L^{-1}$). The spectra were recorded in the front-face geometry to circumvent artifacts due the inner-filter effects caused by the high-absorbing solutions. Even under these conditions we were unable to detect any significant level of NRET between Np and Py linked to different polymer chains.

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(29) As a consequence of the slightly different polyelectrolyte concentrations in the three solutions, the contribution of the polyelectrolyte to the ionic strength is different in each case: PAMPS−PyODA5, $2.1 \times 10^{-4}$ M; PAMPS−PyODA2, $0.7 \times 10^{-4}$ M; PAMPS−Py1, $4.2 \times 10^{-4}$ M.

(30) The size of the micelle indicated in this figure was obtained from dynamic light scattering measurements to be described in a forthcoming publication.

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