Aggregation Phenomena in Aqueous Solutions of Hydrophobically Modified Polyelectrolytes. A Probe Solubilization Study

Lev E. Bromberg*,† and David P. Barr‡

Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Bruker Instruments, Inc., 19 Fortune Drive, Manning Park, Billerica, Massachusetts 01821

Received December 18, 1998; Revised Manuscript Received March 23, 1999

ABSTRACT: Aggregation in the solutions of a hydrophobically modified polyelectrolyte, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-poly(acrylic acid) (Pluronic-PAA), has been studied as a function of temperature, pH, and molecular weight. Pluronic-PAA undergoes temperature- and pH-responsive aggregation in aqueous solutions, as shown by solubilization of fluorescent dyes and spin-labeled probes. Stable gel structures cross-linked by aggregates are formed above the critical micellization temperature (cmt). The aggregates provide a nonpolar microenvironment to pyrene that is insensitive to the ionization. The microenvironment of pyrene below the cmt at low pH is nonpolar, suggesting the presence of hydrophobic microdomains unrelated to the temperature-induced micellization. Full ionization of PAA leads to weakening of hydrophobic microdomains below the cmt. Above the cmt, 12-doxylstearic acid and spin-labeled steroid hormone can be solubilized into Pluronic-PAA aggregates. Solubilization results in a rise in the microviscosity of the environment of the spin probe.

Introduction

Hydrophobically modified polyelectrolytes (HMP) are a group of copolymers that are distinguished from nonionic copolymers by sensitivity of associative properties of their aqueous solutions to pH, ionic strength, and temperature.[1−3] On the other hand, associative interactions between hydrophobic groups in HMP differentiate them from polyelectrolytes in certain "anti-polyelectrolyte effects", such as an increase of solution viscosity upon addition of salt.[4−6] The presence of ionic, hydrogen-bonding, and hydrophobic groups in HMP makes them prone to all the interactions that also govern the properties of protein solutions, and thus we believe HMP mimic proteins in that regard. On the other hand, the ready availability of HMP has generated much interest in their applications ranging from oil production to cosmetic and pharmaceutical formulations.[1−19] Recently, a novel class of HMP has emerged whereby a poly(propylene oxide) (PPO) group acts as a temperature-sensitive hydrophobe.[17−34] Attachment of the PPO group onto a polyelectrolyte is a means of creating a dually responsive material by adding temperature sensitivity to an already pH- and ion-sensitive polymer. In the present work, we continue our studies of a distinct species of such dually responsive HMP whereby poly(acrylic acid) (PAA) segments are grafted onto Pluronic poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO−PPO−PEO) block copolymers via C−C bonding.[17−19,23−34] High molecular weights and extreme sensitivity to temperature characterize these PEO−PPO−PEO−PAA copolymers, so that above certain temperatures their semidilute aqueous solutions can form reversible gels with significant elastic moduli.[23,24] Yet, they combine the polyelectrolyte properties of PAA with the associative capabilities of Pluronics. Gelation is caused by appearance of micelle-like aggregates whereby collapsed chains of PPO form a core.[27] The cores may belong to one (intramolecular aggregation) or several (inermolecular aggregation) Pluronic-PAA chains. Aggregation in Pluronic-PAA aqueous solutions starts at very low polymer concentrations.[23] The intramolecular aggregation of Pluronics connected by PAA into ladderlike structures is a pertinent feature of Pluronic-PAA. Above well-defined micellization temperatures, intermolecular associations become dominant and lead to the formation of aggregates that serve as physical cross-links in the gelation process, as shown by light scattering,[25] NMR,[34] SEC,[25,26] SANS,[27] and DSC[25] techniques. Temperature-sensitive chemical shifts belonging to methyl groups in ^{13}C and ^{1}H NMR provide direct evidence for the aggregation of PPO blocks to be a cause of micelle appearance.[34] While aggregates allow for the solubilization of proteins and hydrophobic drugs,[28,30] the abundance of carboxyl groups makes the PEO−PPO−PEO−PAA copolymer mucoadhesive, properties useful in drug delivery.[19,33]

In the present study, we applied the technique of solubilization of hydrophobic compounds[35,36] to ascertain temperature-, pH-, and concentration-dependent formation of micelle-like structures in the PEO−PPO−PEO−PAA copolymers. Spectroscopic techniques are well established for investigating a range of physical properties of micellar solutions.[35,37,38] The objective of the study was twofold: (i) to reflect on the properties of the copolymer itself by varying parameters of the probe environment, such as temperature, pH, polymer concentration, and molecular weight, and (ii) to contribute to the understanding of the location of the probes in the copolymer aggregates. The dye and pyrene probe fluorescence and surface tension measurement characterize the onset of intermolecular aggregation, and the location of the probes is studied by the fine structure of the pyrene spectra (I_3/I_1) as well as by temperature-sensitive ESR spectra of the spin probes.

* To whom all correspondence should be addressed at 15 Sherwood Road, Swampscott, MA 01907. E-mail cpbrolev@aol.com.
† Massachusetts Institute of Technology.
‡ Bruker Instruments, Inc.

10.1021/ma981946k CCC: $18.00
Published on Web 04/22/1999

Experimental Section

Materials. Pluronic F127 NF was obtained from BASF Corp. (Parsippany, NJ) and used without further treatment. It has a formula $EO_{100}PO_{65}EO_{100}$, nominal molecular weight 12 600, molecular weight of PPO segment 3780, 70 wt % of EO, and cloud point in 1% aqueous solutions above 100 °C. Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-poly(acrylic acid) (CAS #186810-81-1) was synthesized by dispersion/emulsion polymerization of acrylic acid along with simultaneous grafting of poly(acrylic acid) onto Pluronic backbone as described in detail elsewhere.[29] We will use the abbreviation Pluronic-PAA for the polymer. The polymer was fractionated in aqueous buffers at 15 °C by size exclusion chromatography as previously described.[26] The polydispersity of each fraction did not exceed 2.1. Unless noted otherwise, molar masses of the fraction used in this work most were 3.15 $\times$ 10^6 (M_n), 3.61 $\times$ 10^6 (M_w), and 3.99 $\times$ 10^6 (M_z). The polymer consisted of 43% Pluronic F127 and 57% poly(acrylic acid) as measured by FTIR and NMR.[25,26,29] An identical synthetic procedure (but without Pluronic) was used to synthesize poly(acrylic acid) (PAA). Phenoxazon-9 (Nile Red), pyrene (99%, optical grade), doxyl-12-stearic acid, and 3-doxyl-17β-hydroxy-5α-androstane were all obtained from Aldrich and were used as received, except for pyrene which was repeatedly recrystallized from absolute ethanol following sublimation. All other chemicals, gases, and organic solvents of the highest purity available were obtained from commercial sources.

Procedures. To prepare solutions of Pluronic-PAA, the polymer samples were dispersed in distilled water and gently stirred at 4 °C for 48 h. The pH was adjusted with 5 M NaOH as needed. The solutions were filtered through Acrodisc nylon filters (Gelman Sciences) with pore diameters of 0.8 μm and stored at 4 °C. Polymer concentration in the solutions was controlled within $\pm$0.005%. Potentiometric titration was performed with 0.1 M NaOH using a Metrohm model 736 titration system equipped with a titration manipulator and a thermostat. The initial PAA and Pluronic-PAA concentration in solution was set at 70 mM acrylic acid units.[26] The degree of ionization (α) was determined by potentiometric titration as described elsewhere.[26]

The Wilhelmy plate method (Sigma 701 automatic tensiometer, KSV Instruments, Ltd.) was employed for measuring the surface tension of the copolymer solutions. Temperature control within 0.05 °C was achieved using a refrigerated bath/circulator. Octanol-to-water partition coefficients (log *P*) of Nile Red and 3-doxyl-17β-hydroxy-5α-androstane were calculated using a Molecular Modeling Pro (revision 2.14) program.

Fluorescence Measurements. Fluorescence spectra were recorded using a 10 mm path length quartz cell in a thermostated cuvette holder using a Shimadzu model RF-5301 PC spectrofluorophotometer with a UV/vis polarizer under controlled temperature conditions (slit widths 5.0, 3.0, or 1.5 nm) at right-angle geometry. Nile Red, a hydrophobic dye widely utilized as a microprobe for the cell lipophilicity[39−41] and in polymer and protein solution studies,[42−44] was applied in the study of critical micellization temperatures. The dye solubilization experiments were performed by adding a small amount of solid Nile Red to polymer solutions, resulting in $\sim$15 μM effective dye concentration. The suspension was briefly sonicated and then allowed to equilibrate at a given temperature for 48 h. The suspension was then centrifuged at 6000*g* for 10 min at the same temperature, and fluorescence spectra were measured. Three scans were averaged, and the spectra were corrected for scattering in blank polymer solutions. The intensity of the excitation (λ_{em} = 650 nm) and emission (λ_{ex} = 570 nm)[42] spectra strongly depended on temperature (Figure 1).

Properties of the aggregates formed in the polymer solutions were assessed by steady-state pyrene fluorescence studies. A stock solution of 1 mM pyrene in absolute methanol was prepared, from which 1−3 μL was added to 3.0 mL of an aerated aqueous sample. The sample was then allowed to equilibrate for 48 h at a given temperature, and excitation (λ_{em} = 390 nm) and emission (λ_{ex} = 335 nm)[45] spectra were recorded. The ratio of the intensities of the third (384 nm) to the first (373 nm) vibronic peak (I_3/I_1)[46,47] in the emission spectra as well as the ratio of the intensities at 334 and 337 nm in the excitation spectra[48] of the monomer pyrene was used to estimate the polarity of the pyrene microenvironment.[49,50] Each spectrum was obtained by averaging three scans, corrected for scatter using equivalent blank polymer solution, and I_3/I_1 values were averaged over three different estimates. The reproducibility of the results was better than 5%. Typical spectra are shown in Figure 2. The spectra were normalized using I_3 (emission), I_{334} (excitation, 45 °C), or I_{337} (excitation, 10 °C) bands' intensities.

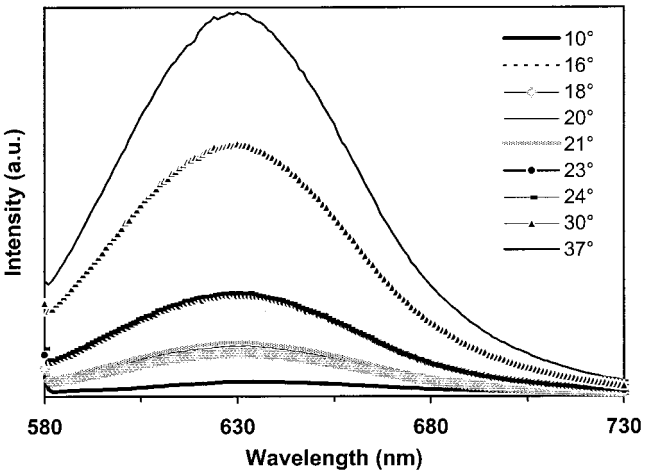

Figure 1. Emission spectra of Nile Red in 1 w/v % Pluronic-PAA aqueous solution (pH 7.0) as a function of temperature. Excitation was at 650 nm. An effective dye concentration $\sim$15 μM was used. Molecular weights of the polymer were 3.15 $\times$ 10^6 (M_n), 3.61 $\times$ 10^6 (M_w), and 3.99 $\times$ 10^6 (M_z).

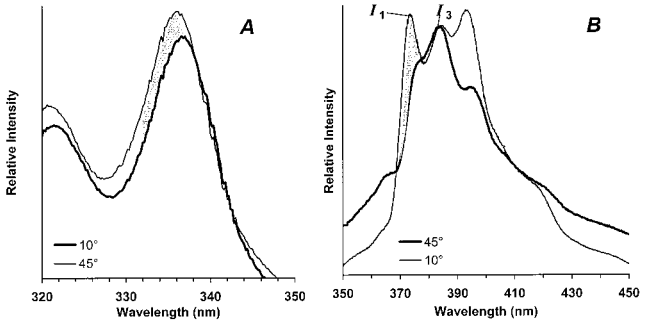

Figure 2. Excitation (A) and emission (B) spectra of pyrene in 1 w/v % Pluronic-PAA aqueous solution (pH 7.0) as a function of temperature. In (A), λ_{em} = 390 nm; in (B), λ_{ex} = 335. In (B), I_1 and I_3 stand for the first and the third vibronic peak intensities. Areas of the spectra used to calculate I_3/I_1 as well as the ratio of the intensities at 334 and 337 nm are shown in gray. Concentration of pyrene was 0.6 μM. The spectra were normalized using I_3 (emission), I_{334} (excitation, 45 °C), or I_{337} (excitation, 10 °C) bands' intensities. For other details, see Figure 1.

ESR Spectroscopy. Loading of polymer solutions with spin probes doxyl-12-stearic acid or 3-doxyl-17β-hydroxy-5α-androstane was achieved by adding 30 μL of a corresponding methanolic probe solution to 3.0 mL of a 1 w/v % polymer solution. The mixtures were equilibrated at 4 °C for 72 h. Effective concentrations allowing for optimum spectral resolution were found experimentally for each probe and are given throughout. The presence of about 1 v/v % or less of methanol in aqueous solutions of Pluronic copolymers has been shown to have no appreciable effect on the solutions' thermodynamics.[35]

ESR spectra were measured using a Bruker EMX 6/1 spectrometer operating at 9.65 GHz. A Bruker ER 4131 variable temperature unit equipped with an ER 166GCTMVT-

S-Q flat cell and dewar assembly was used to control the sample temperature. Spectra were analyzed using the WIN-EPR data processing program. The spectrometer settings for the ESR experiments were as follows: microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 2 G; center field, 3439 G; sweep width, 100 G; time constant, 82 ms; conversion time, 82 ms; sweep time, 84 s. The rotational correlation time was estimated from the equation[51−53]

$$\tau_c = 6.51 \times 10^{-10} \Delta H_0 \left[\left(\frac{h_0}{h_{-1}} \right)^{0.5} + \left(\frac{h_0}{h_{+1}} \right)^{0.5} - 2 \right]$$

where ΔH_0 is the line width of the mid-field line (in gauss), and h_{-1}, h_0, and h_{+1} are the peak-to-peak heights of the low-, mid-, and high-field lines, respectively. We assume that the constant 6.51×10^{-10} can be used, to a good approximation, for our spin probes.[54]

The order parameter was calculated as

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - (A_{xx} + A_{yy})/2}$$

where A_{xx}, A_{yy}, and A_{zz} are the principal components of the **A** tensor in the absence of molecular motion and $A_{\parallel}$ and $A_{\perp}$ are derived from the experimental spectra using maximum and minimum approximation.[55] Order parameters were calculated using values $A_{xx} = 6$ G, $A_{yy} = 6$ G, and $A_{zz} = 32$ G.[56]

Results and Discussion

Fluorescence Probe Study. Pronounced temperature-dependent solubility changes were observed for each concentration of Pluronic-PAA. The existence of temperatures corresponding to massive formation of micelle-like aggregates (providing hydrophobic environment to water-insoluble molecules) is well documented in the case of Pluronic solutions.[35,36,57] There, the length and overall content of the poly(propylene oxide) (PPO) segments govern the "solubilization power" of the Pluronic, i.e., the number of the probe molecules per PPO chain as well as the amount of the probe per micelle. It might be expected that the mechanism of solubilization in the Pluronic-PAA solutions would be phenomenologically similar to the one in Pluronic solutions, as the onset of gelation in Pluronic-PAA (caused by aggregation) approaches the critical micellization temperature (cmt) of the parent Pluronic.[25] On the other hand, a 10^3-fold higher molecular weight as well as the presence of ionized segments grafted onto Pluronic may impart peculiar behavior to the Pluronic-PAA.

Both Nile Red and pyrene used as probes in the present study are hydrophobic compounds marginally soluble in water (log P is 5.18 and 2.2 for pyrene[58] and Nile Red, respectively). Temperature dependencies of the relative emission intensity of Nile Red as well as the I_3/I_1 and I_{334}/I_{337} values can be used for the cmt determination in Pluronic-PAA solutions (Figure 3).

The cmt values found by both methods at 22−23 °C were 2−3 deg above the onset of aggregation in analogous Pluronic-PAA solutions observed at ∼20 °C in SEC, DSC, NMR, and rheological experiments.[24−26,34] A temperature range of about 5 °C where the I_3/I_1 change takes place was also observed in the Pluronic solutions and can probably be attributed to the polydispersity of the polymers under investigation.[50]

Using the dye solubilization technique, we determined cmt values as functions of the Pluronic-PAA concentration and molecular weight at fixed pH 7.0 (Figure 4).

It is interesting to observe that a strong dependence of cmt on the molecular weight held at concentrations

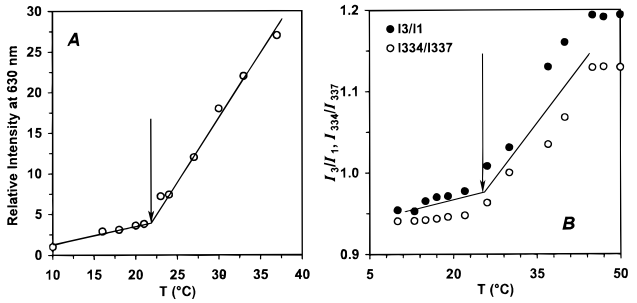

Figure 3. Temperature dependencies of relative emission intensity of Nile Red at 630 nm (A) and I_3/I_1 and I_{334}/I_{337} of pyrene (B). Arrows show the critical micellization temperature (cmt). For other details, see Figures 1 and 2.

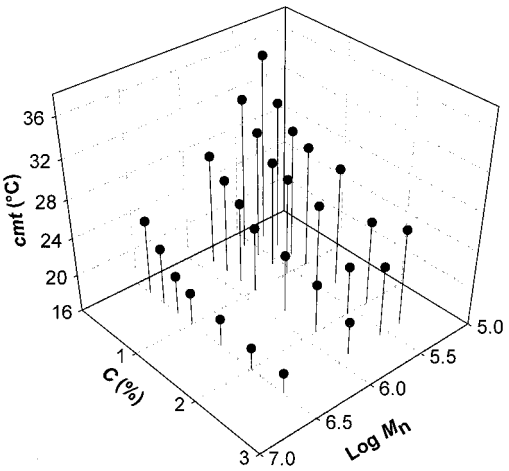

Figure 4. Cmt values as functions of the Pluronic-PAA concentration and molecular weight at pH 7.0 as determined from Nile Red emission spectra.

below 1.5 w/v %. A dependence of the overlap concentration on molecular weight characteristic of the rodlike polyelectrolytes in the low salt limit was recently observed in Pluronic-PAA solutions.[23] At temperatures above the cmt the solutions became more viscous, at $C > 0.5\%$ evolving into strong viscoelastic gels. As in the case of micellization of Pluronics,[59] linear relations between 1/cmt and log C were observed:

$$1/\mathrm{cmt} = \sigma \log C + I$$

where σ (slope) and I (intercept) are constants.

The parameters $\sigma = (7-9) \times 10^{-5}$ and $I = (3.2-3.4) \times 10^{-3}$ found from the Nile Red solubilization data (Figure 4) are in excellent agreement with $\sigma = 7.63 \times 10^{-5}$ and $I = 3.37 \times 10^{-3}$ for the parent Pluronic F127.[59] These parameters also correspond to σ and I obtained from the T_{gel} value defined as an onset of the storage modulus (G') increase in Pluronic-PAA solutions.[25] As the I_3/I_1 value is a useful indicator of the local dielectric constant surrounding the probe,[60] it was employed to characterize the pyrene microenvironment in the aggregates. The I_3/I_1−pH−temperature plot obtained for 1 w/v % Pluronic-PAA solutions (fraction with $M_n = 3.15 \times 10^6$ was used in all subsequent experiments) is shown in Figure 5.

Below pH 7, the I_3/I_1 grew from 0.95 to 1.13 when temperature increased from 15 to 37 °C. Above pH 7, the I_3/I_1 was generally lower than at more acidic pH but declined with pH more considerably at lower temperatures. Remarkably, the I_3/I_1 observed at all pH and temperatures in the range 0.745−1.13 never reached

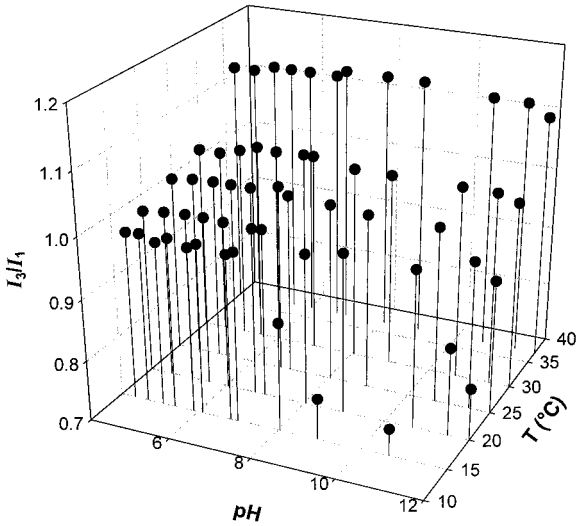

Figure 5. 3D I_3/I_1−pH−temperature plot obtained for 1 w/v % Pluronic-PAA solutions ($M_n = 3.15 \times 10^6$) from pyrene emission spectra.

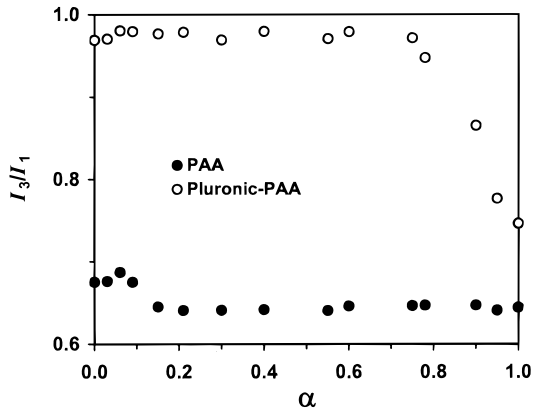

Figure 6. Variation in I_3/I_1 ratio in the emission spectra of pyrene loaded into 1 w/v % PAA (1) or Pluronic-PAA (2) as a function of the degree of ionization (α) of the polymers.

the value of ca. 0.63 characteristic for pyrene in water[46] and generally was above the I_3/I_1 in the bulk PPO of molecular weight close to the PPO block in Pluronic-PAA. Nivaggioli et al.[50] measured I_3/I_1 in the bulk PPO 4000 to vary from 0.67 to 0.76 at temperatures from 15 to 37 °C. The relatively high I_3/I_1 values suggest that the Pluronic-PAA provides a nonpolar environment for the pyrene, notwithstanding the presence of the hydrophilic PEO and PAA segments. The presence of hydrophobic microdomains in the Pluronic-PAA solutions can be assumed, even at temperatures below the cmt. McCormick and co-workers[47,61] postulated the existence of such microdomains in aqueous solutions of terpolymers of hydrophobically modified acrylamide and acrylic acid. In our case, hydrophobic microdomains below the cmt can originate from regions of sterically constrained Pluronic connected to PAA or another Pluronic molecule via multiple C−C bonds.[25]

To explain the pronounced pH dependence of the I_3/I_1 at lower temperatures (Figure 5), ionization of carboxyl groups belonging to PAA segments must be considered. Figure 6 shows the effect of ionization on the I_3/I_1 in 1 w/v % solutions of Pluronic-PAA and PAA synthesized by dispersion polymerization without Pluronic. Dramatic differences in behavior of Pluronic-PAA and PAA are observed at 15 °C (that is, below the cmt of the Pluronic-PAA).

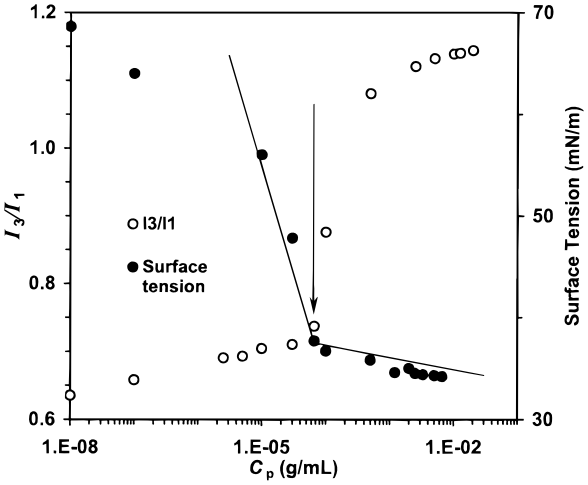

Figure 7. Surface tension and I_3/I_1 as functions of Pluronic-PAA concentration (C_p) at pH 7.0 and 37 °C. Pyrene concentration was 0.6 μM throughout. Arrow indicates the critical micelle concentration (cmc).

Unmodified PAA showed I_3/I_1 close to 0.69 at $\alpha < 0.1$, and as ionization progressed, the I_3/I_1 remained close to that in water. These results are analogous to those observed with un-cross-linked PAA[62,63] and PAA gels.[46] However, the Pluronic-PAA solution had an I_3/I_1 of about 0.96 up to $\alpha \approx 0.8$, at which point the I_3/I_1 started to decay down to 0.75 at full ionization. Thus, the polarity of the local microenvironment of pyrene at $\alpha < 0.8$ resembled that in sodium dodecyl sulfate micelles[64] or in PAA gel modified with 20 mol % of *n*-octyl acrylate hydrophobe.[46] Aqueous suspensions of un-ionized Pluronic-PAA appeared as compact aggregates stable on shaking for at least 3 days, while at $\alpha > 0.1$ clear solutions were observed. Thus, the average polarity inside the Pluronic-PAA aggregates could not be invoked to explain high I_3/I_1 values throughout the ionization range. Rather, the pyrene must have been incorporated into the nonpolar cores of the micelles that still existed even at complete ionization of the Pluronic-PAA. In analogous experiments with Pluronic solutions, Nivaggioli et al.[50] showed that, for a given Pluronic copolymer, the micropolarity probed by pyrene is not affected by the bulk polymer concentration once micelles are formed. The pyrene is located inside the hydrophobic core of the Pluronic micelles and is not in contact with water, as evidenced by the insensitivity of the I_3/I_1 to the change of the solvent.[50] Recently, we have shown that the pyrene solubilized into Pluronic-PAA is essentially insensitive to the presence of an ionic quencher, Tl^+, above the cmt.[31] In the presence of micelles, the pyrene must be buried inside the PPO cores.

These results were further substantiated by observation of the concentration effects on the surface tension and micropolarity in Pluronic-PAA solutions. Surface tension and I_3/I_1 data in the absence of added salt for the Pluronic-PAA at 37 °C are presented in Figure 7 plotted vs copolymer concentration (C_p). A change in slope was observed in the surface tension curve at a specific copolymer concentration, after which the surface tension values decreased more gradually.

This change, indicating the onset of aggregation of Pluronic-PAA molecules, coincided with the increase in the I_3/I_1 values, thereby confirming pyrene solubilization into micelles.[65] The characteristic sigmoidal shape of the I_3/I_1 vs C_p isotherm allows for estimate of the partition coefficient of pyrene between micelles and aqueous

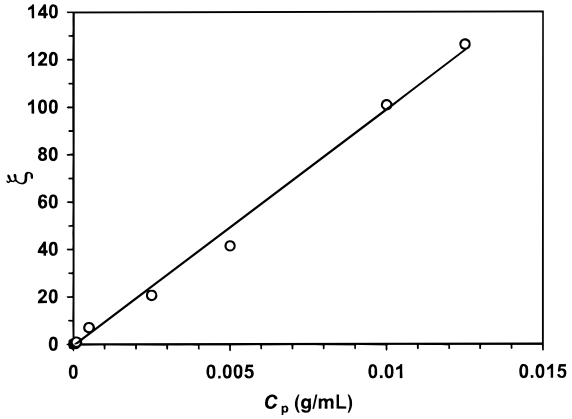

Figure 8. Partitioning of pyrene between micellar and aqueous phases as a function of polymer concentration at pH 7.0 and 37 °C. See text for details.

phase by an approach forwarded by Wilhelm et al.[66] and used by many others.[48,67−69] Namely, assuming that pyrene simply partitions between the aqueous phase and PPO core of the micelles with an equilibrium partition coefficient K, the data in Figure 7 can be fitted to an isotherm:

$$K = \xi \frac{\rho}{C_p f} \tag{1}$$

where $\xi = [(I_3/I_1)_w - (I_3/I_1)]/[(I_3/I_1) - (I_3/I_1)_m]$; indexes w and m stand for the aqueous and micellar phases, respectively, ρ is the density of the inner core of the aggregates, and f is the weight fraction of the PPO segment in the copolymers.

A plot of ξ versus C_p appeared to be a straight line ($R^2 > 0.99$) with a slope proportional to K (Figure 8).

Approximating ρ by the density of the bulk PPO (1.0 g/mL) and taking $f = 0.118$,[25] the slope in Figure 8 yields $K = 8.4 \times 10^4$, in excellent agreement with $K \approx 10^5$ found for pyrene in Pluronic solutions by Hurter and Hatton.[70] The partition coefficient of pyrene between the polystyrene-*b*-poly(acrylic acid) block copolymer micelles and water phase was also reported to be on the order of 10^5.[71]

Spin Probe Study. While water-insoluble probes, such as Nile Red and pyrene, appear to be embedded into the hydrophobic cores of the Pluronic-PAA aggregates, amphiphilic probes might reveal subtle differences between the probe environment along the radii of the aggregates. The 12-doxylstearic acid spin probe was used to monitor changes in the anisotropic ESR spectra with temperature (Figure 9).

The composite structure of the spectra measured at pH 7.0 hinted at the presence of a slower and a faster component. To describe the relative changes in the line intensities, parameters pertaining to the slow motional (I_s) and motionally narrowed (I_f) components of the low-field line were used, analogously to the technique described by Vesterinen et al.[72] (Figure 10).

The slow component could be attributed to the dissociated, anionic form of the probe, whereas the fast spectrum has been ascribed to the undissociated (and therefore less polar) form of the acid.[73−75] The latter species tends to associate with the PPO segments in the core of the Pluronic micelles.[73] To avoid structural complexity of the ESR spectra of the *x*-doxylstearic acids in the Pluronic solutions, Caragheorgheopol et al.[73] solubilized the spin probes in 0.1 N NaOH aqueous

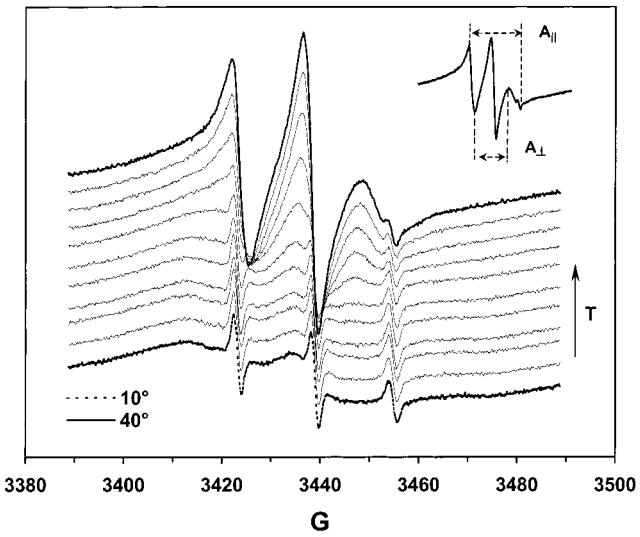

Figure 9. ESR spectra of the doxyl-12-stearic acid in 1 w/v % copolymer solution (pH 7.0) in the temperature range 10−40 °C. Effective spin probe concentration was 26 mM. Spectra were measured every 3 °C. Insert shows $A_{||}$ and $A_\perp$ values in the spectrum taken at 37 °C.

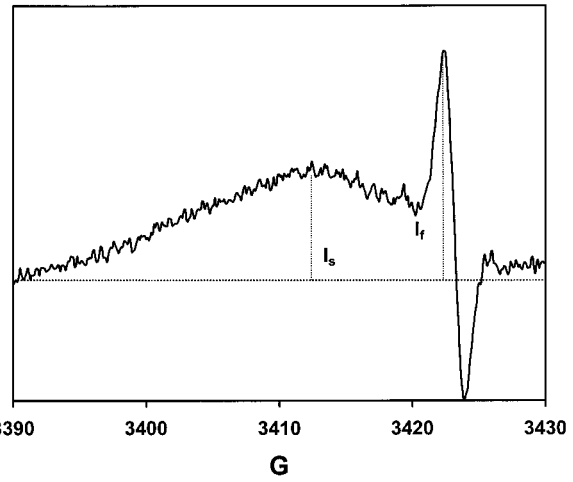

Figure 10. Enlarged low-field line of the ESR spectrum of the doxyl-12-stearic acid in 1 w/v % copolymer solution (pH 7.0) at 13 °C. Parameters I_s and I_f are defined that describe the slow and fast motional components of the spectra, respectively.[72]

solutions where the fast component of the spectra was nonexistent. In our case, however, such a procedure is impossible without weakening of the micellization.[26] Nevertheless, the composite spectra are quite revealing when the normalized, relative peak intensities are considered (Figure 11). As can be seen, the relative intensity of the slow modes of the anionic probe increased somewhat above 25 °C.

These slight changes are indicative of solubilization of even ionized species into PPO aggregates formed at elevated temperatures. Increase of the relative intensities of the fast modes was quite dramatic above 27 °C, reflecting incorporation of the undissociated species into polymer micelles. The nitrogen hyperfine coupling constant of the radical (a_N) can be used as a further clue into the location of the spin probe (Figure 12).

Decrease of the a_N above 25 °C shows that upon micellization even the anionic, highly mobile species is drawn into a nonpolar environment.[72] The a_N values at elevated temperatures approached that of the 5-doxy-

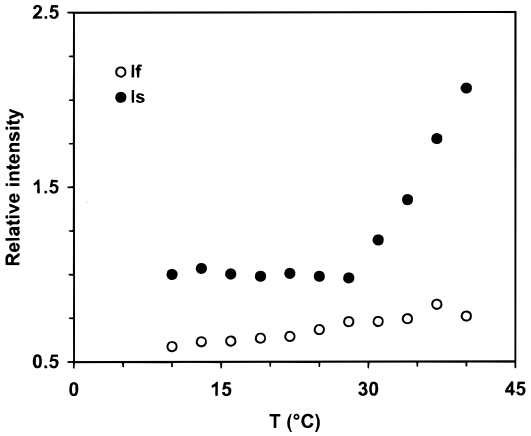

Figure 11. Relative intensities of the slow and fast motional spectral components vs temperature in the ESR spectra of the doxyl-12-stearic acid in 1 w/v % copolymer solution. The peak intensities are normalized using I_f at 10 °C as a reference.

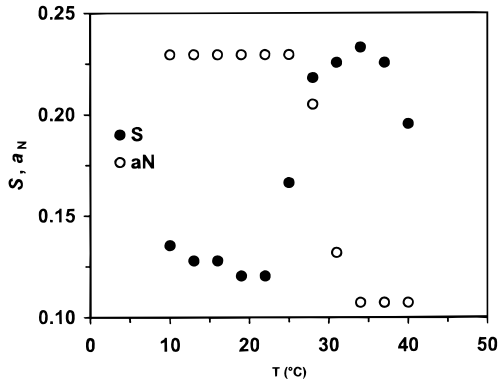

Figure 12. Effect of temperature on the order parameter (S) and ^{14}N hyperfine coupling constant (a_N) in the ESR spectra of the doxyl-12-stearic acid in 1 w/v % copolymer solution.

lstearic acid in pure PPO (a_N = 14.3 G).[73] This is a remarkable result considering that there must be a considerable energy barrier toward diffusion of the doxylstearic acid through the micelle−solution interface due to the repulsion between the ionized spin probe and dissociated carboxyl groups of the PAA in the corona of the micellar aggregates.[27] Interestingly, an opposite trend (i.e., an increase of the a_N with temperature) was observed in the spectra of 5-doxylstearic acid in 10 w/w % aqueous alkaline solution of Pluronic P85.[73] An explanation of this variance may lie in the differences of mobility of the 5- and 12-doxylstearic acids, as shown by Kutsumizu and Schlick.[76] Namely, the 5-doxylstearic acid would be located mostly near the polar interface between aggregate and aqueous environment, whereas more hydrophobic 12-doxylstearic acid tends to be located in the nonpolar interior of the aggregate.[76,77] More restricted mobility near the interface results in the increase of a_N of 5-doxylstearic acid with a rise in the interfacial surface upon micellization. Contrariwise, a much higher mobility within the nonpolar core of the micellar aggregates where 12-doxylstearic acid is drawn produces the decrease of a_N when massive formation of micelles occurs on heating. The above differences are highlighted by the increase of the order parameter above 22−25 °C (Figure 12) in our system and the decline of the S values at elevated temperatures in the spectra of 5-doxylstearic acid in alkaline solution of Pluronic.[73]

The effect of the probe radial location (relative to the center of the micellar aggregate) is further seen in

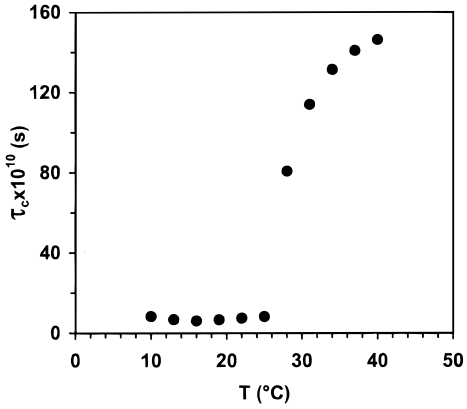

Figure 13. Effect of temperature on the rotational correlation time (τ_c) in the ESR spectra of the doxyl-12-stearic acid in 1 w/v % copolymer solution.

temperature effects on the rotational correlation time (Figure 13).

Solubilization of the 12-doxylstearic acid into the bulk of micelles resulted in a dramatic raise of τ_c above 20 °C, indicating up to a 17-fold increase in microviscosity (η) of the probe's environment, as follows from the Debye−Stokes−Einstein equation:

$$\tau_c = 4\pi\eta r^3/3kT$$

where r is the radius of the tumbling entity (spin probe), and k and T are the Boltzmann constant and absolute temperature, respectively.

A similar growth of microviscosity was observed upon solubilization of spin probes, such as 2,2,6,6-tetramethylpiperidine-*N*-oxide (TEMPO), into solutions of dodecyl sulfate surfactants above the surfactant's cmc.[54] The τ_c increase becomes more pronounced upon addition of PEO, which enhances the microviscosity of the formed aggregates.

Interestingly, a decrease of τ_c in the spectra of 4-dodecanoyl-2,2,6,6-tetramethyl-1-piperidinyloxy (DD-TEMPO) suspended in 1% aqueous solutions of Pluronic-PAA copolymers (identical to the ones used in the present study) was recently observed when the solutions were heated from 23 to 37 °C.[34] In contrast to the 12-doxylstearic acid, the DD-TEMPO spin probe associates closer to the hydrated polar regions bordering the nonpolar PPO aggregates.[73] Thus, when the temperature decreases and these regions lose hydration water, the τ_c decreases to the values found in PPO. Reduction of τ_c with temperature was also observed with 4-[*N*,*N*-dimethyl-*N*-(methylene)$_n$ammonio]-2,2,6,6-tetramethylpiperidine-1-oxyl iodide (n = 11 or 16) spin probes in Pluronic solutions.[73] The latter probes reside on the hydrated PEO/PPO interface of the micelles.

It has been mentioned in the Introduction that the Pluronic-PAA copolymers under study undergo reversible sol−gel transitions at temperatures suitable for applications in the human body. Therefore, solutions of these copolymers can be used as in situ gelling agents for drug delivery applications[19] and particularly in the delivery of steroid hormones.[30,78,79] Consequently, it was of interest to study the behavior of a spin-labeled steroid hormone, 3-doxyl-17β-hydroxy-5α-androstane (DA), in aqueous solutions of PEO−PPO−PEO−PAA. Figure 14 shows ESR spectra of the DA in 1 w/v % PEO−PPO−PEO−PAA solution (pH 7.0).

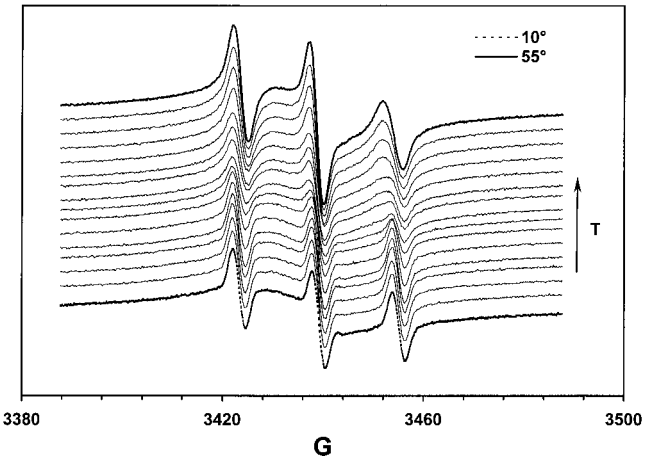

Figure 14. ESR spectra of the 3-doxyl-17β-hydroxy-5α-androstane in 1 w/v % copolymer solution (pH 7.0) in the temperature range 10−55 °C. Effective spin probe concentration was 0.32 mM. Spectra were measured every 3 °C.

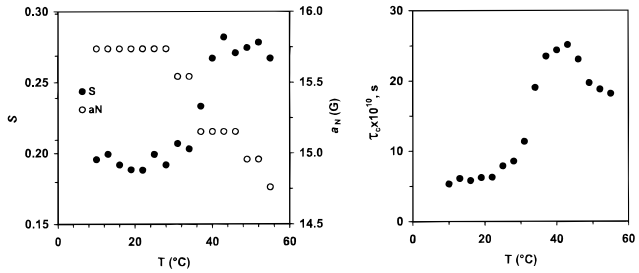

Figure 15. Effect of temperature on the order parameter (S), ^{14}N hyperfine coupling constant (a_N), and the rotational correlation time (τ_c) in the ESR spectra of the 3-doxyl-17β-hydroxy-5α-androstane in 1 w/v % copolymer solution.

As can be seen, the intensity of the narrow component of the spectra increased above certain temperatures, which corresponded to the massive micellization in the polymer solution. Spectra of the DA probe revealed trends (increase of S and τ_c and decrease of a_N above micellization temperatures) similar (but somewhat less dramatic) to the ones observed with the 12-doxylstearic acid spin probe (Figure 15). The DA probe is very hydrophobic (log P = 5.6), and thus one expects solubilization behavior similar to that of pyrene or nondissociated species of the doxylstearic acid.

Altogether, the above results provide spectroscopic evidence for the presence of a constrained shell of hydrophobic (PPO) segments near the ionizable (PAA) segments, a coexistence analogous to the one found in self-assembling ionomer solutions of poly(ethylene-*co*-methacrylic acid).[76,77]

Concluding Remarks

We have studied aggregation in the solutions of hydrophobically modified polyelectrolyte, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide)-*g*-poly(acrylic acid) (Pluronic-PAA), by solubilization of either fluorescent or spin-labeled probes into the aggregates. The dye solubilization technique yielded the critical micellization temperature (cmt) of the copolymers, which depended strongly on the Pluronic-PAA molecular weight. The Pluronic-PAA aggregation can be visualized as a process of formation of intermolecular micelles where cores composed of aggregated PPO chains are bridged by covalently attached PAA and PEO−PAA segments (Figure 16).

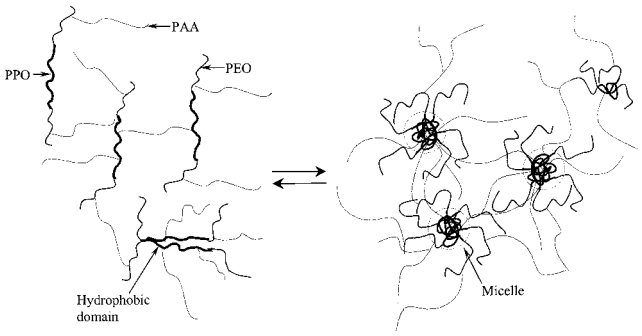

Figure 16. Schematic representation of associations and aggregation in aqueous solutions of Pluronic-PAA.

Despite the grafted poly(acrylic acid), solubilization of Nile Red and pyrene into Pluronic-PAA aggregates above the cmt, just like in the Pluronic micelles, leads to partitioning of the probes into the PPO cores. The probes then become buried inside aggregates, which make them insensitive to the aqueous environment. Remarkably, the microenvironment of pyrene below the cmt is nonpolar, suggesting the presence of hydrophobic microdomains unrelated to the temperature-induced micellization. At low pH, these microdomains are further stabilized by hydrogen bonding. At T < cmt, substantial ionization of the Pluronic-PAA (and thus increase in repulsive interactions between carboxyls) is required to lower the effect of these microdomains. Above the cmt, gel structures cross-linked by micelles are so stable that the polarity of the pyrene microenvironment is virtually insensitive to the ionization. Solubilization of partially dissociated (and thus amphiphilic) 12-doxylstearic acid into Pluronic-PAA aggregates was followed by ESR. The undissociated, less polar species is apparently incorporated into the PPO core of the aggregates. Decrease of the nitrogen hyperfine coupling constant of the radical above the cmt shows that upon micellization even the dissociated, anionic probe is drawn into a nonpolar environment, despite a considerable energy barrier toward diffusion of the doxylstearic acid through the micelle−solution interface. The spin-labeled hormone, 3-doxyl-17β-hydroxy-5α-androstane, also gets captured into the Pluronic-PAA aggregates. The ability of the Pluronic-PAA aggregates to hold hydrophobic compounds, including drugs, can thus be controlled by temperature- and pH-responsive associations.

Intra- and intermolecular associations in Pluronic-PAA solutions resemble those in solutions of other polyelectrolytes containing hydrophobic groups, such as hydrophobically modified alkali-swellable emulsion (HASE) polymers, developed by Jenkins and co-workers,[80] which find applications in paints and coatings formulations. However, the unique feature of the graft-comb Pluronic-PAA copolymers introduced by us[17−19,23−34] is that the only hydrophobic segment present there is poly(propylene oxide), the aqueous solubility of which is temperature-dependent.[81] Hence, unlike HASE polymers, associations in Pluronic-PAA solutions are extremely temperature-sensitive.[24] Yet another kind of temperature-sensitive graft-copolymers of Pluronics and poly(acrylic acid) was described by Hoffman et al.[20,21] There, Pluronics that have an appropriate lower critical solution temperature (LCST) are grafted onto a long-chain poly(acrylic acid). At temperatures above the LCST, interchain associations of Pluronics in these copolymers lead to viscosification of the solution and

turbidity, characteristic of phase separation. The Pluronic-PAA solutions described herein exhibit no macroscopic phase separation, despite the abundance of aggregates.[27,29] Aggregation in Pluronic-PAA solutions is unrelated to the LCST of the Pluronic used.[17,25]

Acknowledgment. The authors are grateful to Dr. Paul D. T. Huibers and Prof. T. Alan Hatton, who were the first to point out the existence of hydrophobic microdomains in the Pluronic-PAA solutions.

References and Notes

(1) *Water Soluble Polymers*; Glass, J. E., Ed.; Advances in Chemistry 213; American Chemical Society: Washington, DC, 1986. *Polymers as Rheology Modifiers*; Schulz, D. N., Glass, J. E., Eds.; ACS Symposium Series 462; American Chemical Society: Washington, DC, 1991.

(2) Bock, J.; Siano, D. B.; Valint, P. L.; Pace, S. J. In *Polymers In Aqueous Media*; Glass, J. E., Ed.; Advances in Chemistry 223; American Chemical Society: Washington, DC, 1989; p 411. Siano, D. B.; Bock, J.; Myer, P. Valint, P. L. *Ibid*., p 425. Winnik, M. A.; Yekta, A. *Curr. Opin. Colloid Interface Sci*. **1997**, *2*, 424.

(3) McCormick, C. L. In *Water-Soluble Polymers. Synthesis, Solution Properties, and Applications*; Shalaby, S. W., McCormick, C. L., Butler, G. B., Eds.; ACS Symposium Series 467; American Chemical Society: Washington, DC, 1991; Chapter 1. Middleton, J. C.; Cummins, D. F.; McCormick, C. L. *Ibid*., Chapter 22.

(4) Magny, B.; Iliopoulos, I.; Audebert, R. In *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., Davis, R., Schulz, D. N., Eds.; Springer: Berlin, 1994; Chapter 4.

(5) Magny, B.; Lafuma, F.; Iliopoulos, I. *Polymer* **1992**, *33*, 3151.

(6) Wang, T. K.; Iliopoulos, I.; Audebert, R. In *Water-Soluble Polymers. Synthesis, Solution Properties, and Applications;* Shalaby, S. W., McCormick, C. L., Butler, G. B., Eds.; ACS Symposium Series 467; American Chemical Society: Washington, DC, 1991; Chapter 14.

(7) Hourdet, D.; L'allouret, F.; Audebert, R. *Polymer* **1994**, *35*, 2624.

(8) L'allouret, F.; Hourdet, D.; Audebert, R. *Colloid Polym. Sci*. **1995**, *273*, 1163.

(9) L'allouret, F.; Maroy, P.; Hourdet, D.; Audebert, R. *Rev. Inst. Fr. Pet.* **1997**, *52*, 117.

(10) Bock, J.; Varadaraj, R.; Schulz, D. N.; Maurer, J. J. In *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., Davis, R., Schulz, D. N., Eds.; Springer: Berlin, 1994; Chapter 3.

(11) Bokias, G.; Hourdet, D.; Iliopoulos, I.; Staikos, G.; Audebert, R. *Macromolecules* **1997**, *30*, 8293.

(12) Wang, T. K.; Iliopoulos, I.; Audebert, R. *Polym. Bull*. **1988**, *20*, 577.

(13) Petit, F.; Audebert, R.; Iliopoulos, I. *Colloid Polym. Sci*. **1995**, *273*, 777.

(14) Bokias, G.; Hourdet, D.; Iliopoulos, I.; Staikos, G.; Audebert, R. *Macromolecules* **1997**, *30*, 8293.

(15) Tribet, C.; Porcar, I.; Bonnefont, P. A.; Audebert, R. *J. Phys. Chem. B* **1988**, *102*, 1327.

(16) Hourdet, D.; L'alloret, F.; Durand, A.; Lafuma, F.; Audebert, R.; Cotton, J.-P. *Macromolecules* **1998**, *31*, 5323.

(17) Bromberg, L.; Lupton, E. C.; Schiller, M. E.; Timm, M. J.; McKinney, G. W.; Orkisz, M.; Hand, B. Int. Patent Appl. WO 97/00275, 1997.

(18) Ron, E. S.; Bromberg, L.; Orkisz, M.; Kearney, M.; Luczak, S.; Timm, M. J.; Wrobel, S. J.. Int. Patent Appl. WO 98/06438, 1998.

(19) Bromberg, L. E.; Ron, E. S. *Adv. Drug Delivery Rev*. **1998**, *31*, 197.

(20) Hoffman, A. S.; Chen, G. Int. Pat. Appl. WO 95/24430, 1995.

(21) Chen, G.; Hoffman, A. S.; Kabra, B.; Randeri, K. In *Poly(ethylene glycol) Chemistry and Biological Applications*; ACS Symposium Series 680; Milton Harris, J., Zalipsky, S., Eds.; American Chemical Society: Washington, DC, 1997; Chapter 27.

(22) Maroy, P.; Hourdet, D.; L'Alloret, F.; Audebert, R. Eur. Pat. Appl. 0583814A1, 1994.

(23) Bromberg, L. *Langmuir* **1998**, *14*, 5806.

(24) Bromberg, L. *Macromolecules* **1998**, *31*, 6148.

(25) Bromberg, L. *J. Phys. Chem. B* **1998**, *102*, 1956.

(26) Bromberg, L. *J. Phys. Chem. B* **1998**, *102*, 10741.

(27) Huibers, P. D. T.; Bromberg, L. E.; Robinson, B. H.; Hatton, T. A. M*acromolecules*, in press.

(28) Bromberg, L.; Orkisz, M.; Roos, E.; Ron, E. S.; Schiller, M. *J. Controlled Release* **1997**, *48*, 305.

(29) Bromberg, L. *Ind. Eng. Chem. Res*. **1998**, *37*, 4267.

(30) Bromberg, L. E.; Mendum, T. H. E.; Orkisz, M. J.; Ron, E. S.; Lupton, E. S. *Proc. Polym. Mater. Sci. Eng*. **1997**, *76*, 273.

(31) Bromberg, L. *Langmuir*, in press.

(32) Orkisz, M. J.; Bromberg, L.; Pike, R.; Lupton, E. C.; Ron, E. S. *Proc. Polym. Mater. Sci. Eng*. **1997**, *76*, 276.

(33) Bromberg, L. E.; Orkisz, M. J.; Ron, E. S. *Polym. Prepr*. **1997**, *38*, 626.

(34) Bromberg, L. E.; Goldfeld, M. G. *Polym. Prepr*. **1998**, *39*, 681.

(35) Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. *Macromolecules* **1994**, *27*, 2414.

(36) Nivaggioli, T.; Alexandridis, P.; Hatton, T. A.; Yekta, A.; Winnik, M. A. *Langmuir* **1995**, *11*, 730.

(37) Hunter, R. J. *Foundations of Colloid Science*; Oxford University Press: New York, 1987; Vol. 1, p 600.

(38) Grieser, F.; Drummond, C. J. *J. Phys. Chem*. **1988**, *92*, 5580.

(39) Fowler, S. D.; Greenspan, P. *J. Histochem. Cytochem*. **1985**, *33*, 833.

(40) Greenspan, P.; Mayer, E. P.; Fowler, S. D. *J. Cell Biol*. **1985**, *100*, 965.

(41) Knobler, H.; Fainaru, M. *J. Chromatogr*. **1987**, *421*, 136.

(42) Watkins, D. M.; Sayed-Sweet, Y.; Klimash, J. W.; Turro, N. J.; Tomalia, D. A. *Langmuir* **1997**, *13*, 3136.

(43) Deye, J. F.; Berger, T. A.; Anderson. A. G. *Anal. Chem*. **1990**, *62*, 615.

(44) Sackett, D. L.; Wolff, J. *Anal. Biochem*. **1987**, *167*, 228.

(45) Marinov, G.; Michels, B.; Zana, R. *Langmuir* **1998**, *14*, 2639.

(46) Philippova, O. E.; Hourdet, D.; Audebert, R.; Khokhlov, A. R. *Macromolecules* **1997**, *30*, 8278.

(47) Branham, K. D.; Snowden, H. S.; McCormick, C. L. *Macromolecules* **1996**, *29*, 254.

(48) Lee, K. Y.; Jo, W. H.; Kwon, I. C.; Kim, Y.-H.; Jeong, S. Y. *Macromolecules* **1998**, *31*, 378.

(49) Dong, D. C.; Winnik, M. A. *Can. J. Chem*. **1984**, *62*, 2560.

(50) Nivaggioli, T.; Alexandridis, P.; Hatton, T. A.; Yekta, A.; Winnik, M. A. *Langmuir* **1995**, *11*, 730.

(51) Kevelson, D. *J. Chem. Phys*. **1960**, *33*, 1094.

(52) Schreier, S.; Ernandes, J. R.; Cuccovia, I.; Chaimovich, H. *J. Magn. Reson*. **1978**, *30*, 283.

(53) Caragheorgheopol, A.; Caldararu, H.; Dragutan, I.; Joela, H.; Brown, W. *Langmuir* **1997**, *13*, 6912.

(54) Wang, Y.; Lu, D.; Yan, H.; Thomas, R. K. *J. Phys. Chem. B* **1997**, *101*, 3953.

(55) Griffith, O. H.; Jost, P. C. In *Spin Labeling. Theory and Applications*; Berliner, L. J., Ed.; Academic Press: New York, 1976; Chapter 12.

(56) Smith, I. C. P.; Butler, K. W. In *Spin Labeling. Theory and Applications*; Berliner, L. J., Ed.; Academic Press: New York, 1976; Chapter 11.

(57) Hurter, P. N.; Alexandridis, P.; Hatton, T. A. In *Solubilization in Amphiphilic Copolymer Solutions*; Christian, S. D., Scamehorn, J. F., Eds.; Marcel Dekker: New York, 1995; Chapter 6.

(58) Kamlet, M. J.; Doherty, R. M.; Carr, P. W.; Mackay, D.; Abraham, M. H.; Taft, R. *Environ. Sci. Technol*. **1988**, *22*, 503.

(59) Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. *J. Am. Oil Chem. Soc*. **1995**, *72*, 823.

(60) Kalyanasundarum, K. *Photochemistry in Microheterogeneous Systems*; Academic Press: Orlando, FL, 1987.

(61) Varadaraj, R.; Branham, K. D.; McCormick, C. L.; Bock, J. In *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., Davies, R. M., Schulz, D. N., Thies, C., Eds.; Springer-Verlag: Berlin, 1994; Chapter 2.

(62) Maltesh, C.; Somasundaran, P. *Colloids Surf*. **1992**, *69*, 167.

(63) Magny, B.; Iliopoulos, I.; Zana, R.; Audebert, R. *Langmuir* **1994**, *10*, 3180.

(64) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc*. **1977**, *99*, 2039.

(65) Kabanov, A. V.; Nazarova, I. R.; Astafieva, I. V.; Batrakova, E. V.; Alakhov, V. Yu.; Yaroslavov, A. A.; Kabanov, V. A. *Macromolecules* **1995**, *28*, 2303.
(66) Wilhelm, M.; Zhao, C.; Wang, Y.; Winnik, M. A.; Mura, J.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 10330.
(67) Nakashima, K.; Winnik, M. A.; Dai, K. H.; Kramer, E. J.; Washiyama, J. *Macromolecules* **1992**, *25*, 6866.
(68) Prochazka, K.; Martin, T. J.; Munk, P.; Webber, S. E. *Macromolecules* **1996**, *29*, 6518.
(69) Zhao, J.; Allen, C.; Eisenberg, A. *Macromolecules* **1997**, *30*, 7143.
(70) Hurter, P. N.; Hatton, T. A. *Langmuir* **1992**, *8*, 1292.
(71) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339.
(72) Vesterinen, E.; Dobrodumov, A.; Tenhu, H. *Macromolecules* **1997**, *30*, 1311.
(73) Caragheorgheopol, A.; Caldararu, H.; Dragutan, I.; Joela, H.; Brown, W. *Langmuir* **1997**, *13*, 6912.
(74) Haering, G.; Luisi, P. L.; Hauser, H. *J. Phys. Chem*. **1988**, *92*, 3574.
(75) Caldararu, H.; Caragheorgheopol, A.; Vasilesku, M.; Dragutan, I.; Lemmetyinen, H. *J. Phys. Chem*. **1994**, *98*, 5320.
(76) Kutsumizu, S.; Schlick, S. *Macromolecules* **1997**, *30*, 2329.
(77) Kutsumizu, S.; Schlick, S. *Macromolecules* **1997**, *30*, 2320.
(78) Ron, E. S.; Bromberg, L.; Luszak, S.; Kearney, M.; Deaver, D. R.; Schiller, M. *Proc. Int. Symp. Control. Relat. Bioact. Mater*. **1997**, *24*, 407.
(79) Ron, E. S.; Bromberg, L.; Luszak, S.; Kearney, M.; Deaver, D. R.; Schiller, M. *Proc. 8th Int. Symp. Recent Adv. Drug Delivery Systems*; Salt Lake City, 1997; p 186.
(80) Jenkins, R. D.; Silebi, C. A.; El-Aasser, M. S. *Polym. Mater. Sci. Eng*. **1989**, *61*, 629. Jenkins, R. D.; DeLong, L. M.; Bassett, D. R. In *Hydrophilic Polymers: Performance with Environmental Acceptance*; Glass, J. E., Ed.; American Chemical Society: Washington, DC, 1996; p 425. Tirtaatmadja, V.; Tam, K. C.; Jenkins, R. D. *Macromolecules* **1997**, *30*, 3271. Tirtaatmadja, V.; Tam, K. C.; Jenkins, R. D. *Macromolecules* **1997**, *30*, 1426. Kumacheva, E.; Rharbi, Y.; Winnik, M. A.; Guo, L.; Tam, K. C.; Jenkins, R. D. *Langmuir* **1997**, *13*, 182. Horiuchi, K.; Rharbi, Y.; Spiro, J. G.; Yekta, A.; Winnik, M. A.; Jenkins, R. D.; Bassett, D. R. *Langmuir* **1999**, *15*, 1644. English, R. J.; Gulati, H. G.; Jenkins, R. D.; Khan, S. A. *J. Rheol*. **1997**, *41*, 427.
(81) Bromberg, L. *Polymer* **1998**, *39*, 5663.

MA981946K