Time-Resolved Fluorescence Depolarization Studies of Naphthalene-Labeled Diblock Copolymer Micelles in Aqueous Media

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ABSTRACT: A–B diblock copolymers of polystyrene-*block*-poly(*tert*-butyl methacrylate) were prepared by anionic polymerization and labeled with one naphthalene either at the polystyrene block end (end-tagged) or at the polystyrene/poly(*tert*-butyl methacrylate) block boundary (mid-tagged); subsequently, the poly(*tert*-butyl methacrylate) block was 100% hyrolyzed to a poly(methacrylic acid) block. Micelles were prepared by dissolving the copolymer in a 80/20 vol % mixture of 1,4-dioxane/water and then dialyzing the solution into increasing water rich mixtures to remove all the 1,4-dioxane. The effect of pH on the micelle corona and core was investigated for four naphthalene-tagged micellar systems: (1) pure micelles composed of end-tagged copolymers, (2) mixed micelles composed of end-tagged and untagged copolymers, (3) pure micelles composed of mid-tagged copolymers, and (4) mixed micelles composed of mid-tagged and untagged copolymers. Quasi-elastic light scattering was used to measure the hydrodynamic diameter of the micelles as a function of pH, and the results indicate that, at low pH, the corona, which is composed of the poly(methacrylic acid block), is collapsed due to poor solubility, while at high pH the corona expands dramatically due to polyelectrolyte effects resulting from deprotonation. In conjunction with the light scattering study, steady-state fluorescence, time-resolved fluorescence, and time-resolved fluorescence depolarization properties were investigated over the same pH range. For the end-tagged copolymer micelles, one expects the probe to reside in the core, and this is reflected by the small changes of fluorescence properties as a function of pH. Significant naphthalene–naphthalene energy migration in the core of end-tagged copolymer micelles for all pH's studied was observed, unlike the situation in organic solvent for the analogous poly(methyl methacrylate) corona. This implies a more compact core in aqueous solution. For the mid-tagged copolymer micelles, one expects the probe to reside in the core/corona interface, and in the fluorescence data taken at different pH's indicate that the naphthalene probe resides in a considerably less restricted environment than for the end-tagged copolymer micelles, where the probe resides in the core. The general results of fluorescence studies indicate that the core is compact and relatively homogeneous and that the core/corona interface is not a distinct boundary but a more heterogeneous environment.

Introduction

Since early observations of block copolymer micelles, many basic micellar properties have been studied by using such techniques as light scattering, ultracentrifugation, viscometry, osmometry, size-exclusion chromatography, electron microscopy, and fluorescence.[1-7] Because fluorescence techniques are powerful and sensitive tools in studying polymer dynamics[8] and heterogeneous media,[7] we have recently focused on the applications of steady-state and time-dependent fluorometry and fluorescence depolarization to study micelle formation, structure, and behavior.[3-6] Time-dependent measurements with nanosecond and picosecond resolution are particularly valuable in the investigation of micellar properties at the molecular level.[9-11]

Polymer micelles are formed spontaneously when block copolymers are dissolved in a selective solvent mixture of a thermodynamically good solvent for the block that forms the corona and simultaneously a thermodynamically bad solvent for the block which forms the core.[1] The micellization of block copolymers is a reversible equilibrium process between micelles that are nearly monodisperse in mass and size, and nonmicellized polymers called unimers. This equilibrium is a closed association process[12] and is described by $nU \Leftrightarrow M$ where n is the association number and U and M represent unimer and micelle, respectively. The association number is the average number of copolymer molecules that form a single micelle and is dependent upon copolymer composition, system temperature, and solvent selectivity.

Previously, we reported polystyrene-*block*-poly(methyl methacrylate) micelle behaviors in organic media.[6] We found that the fluorescence properties change dramatically as a function of the collapse of the micelle core. In this paper, we employed similar fluorescence techniques to investigate the pH effects on the behaviors of four naphthalene-labeled copolymer micellar systems in aqueous media: (1) pure micelles composed of end-tagged copolymers, (2) mixed micelles composed of end-tagged and untagged copolymers, (3) pure micelles composed of mid-tagged copolymers, and (4) mixed micelles composed of a mid-tagged and untagged copolymers. The polystyrene-*block*-poly(methacrylic acid) copolymers are labeled with an average of one naphthalene either at the polystyrene end, N1SA-4, or at the block boundary, SN1A-1. The strategic labeling scheme and the number of micellar systems studied permit some interesting experiments which extend our further understanding of these types of diblock copolymer micelles in aqueous media.

Experimental Section

Copolymers. The diblock copolymers were prepared by anionic polymerization in tetrahydrofuran at −78 °C using cumylpotassium as an initiator. Dried and purified styrene was added to the initiator solution, resulting in the preparation of living polystyrene blocks. A single, 1,1-diphenylethylene molecule (DPE) was attached to the polystyryl anion to modify its reactivity, and *tert*-butyl methacrylate at −78 °C was slowly added to form the second block which was then terminated by degassed methanol. The copolymer was filtered, precipitated in a water–

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Chart I. Polystyrene-*block*-poly(*tert*-butyl methacrylate)

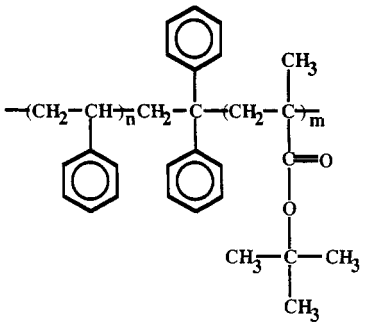

Table I. Characterization Data for Diblock Copolymers

copolymer[a]	$10^{-3}M_w$[b]	$10^{-3}M_wS$[b]	M_n/M_w[b]	mol % S[c]
N1SA-4	67.2	34.0	1.07	58.5
SA-10	57.8	30.1	1.10	60.0
SN1A-1	54.4	22.8	1.15	48.0
SA-24	47.7	22.0	1.06	50.0

[a] SA-10 and SA-24 are the unlabeled copolymers used for preparing mixed micelles (see text). [b] M_w and M_n refer to the parent *tert*-butyl polymer and M_{wS} refers to the molecular weight of polystyrene block, all measured by SEC. [c] Molar % of polystyrene measured by NMR.

methanol mixture, dried, and further characterized. These procedures are described in more detail elsewhere.[13] The structure of the polymer is given in Chart I.

Polystyrene-*block*-poly(*tert*-butyl methacrylate) was labeled with an average of one 2-vinylnaphthalene moiety either at the beginning of the polymerization for labeling at the polystyrene end or before the addition of DPE for labeling at the block boundary. The naphthalene group distribution is expected to obey a Poisson distribution.[14]

The poly(*tert*-butyl methacrylate) block was hydrolyzed for 5 h at 85 °C in mixture of 6 N aqueous HCl and 1,4-dioxane. The number of moles of HCl was typically about 2 times greater than that of the resulting methacrylic acid units. The excess water was removed by drying with anhydrous Na_2SO_4. The resultant poly(methacrylic acid) block copolymer was filtered and precipitated in cold hexane and then redissolved in 1,4-dioxane with subsequent freeze-drying of the solution. The degree of hydrolysis was estimated by NMR in a deuterated 1,4-dioxane/methanol mixture using tetramethylsiloxane as a reference standard. The typical degree of hydrolysis is 0.98.

The molar mass and molar mass distribution of the copolymers were determined by size-exclusion chromatography (SEC) in THF. The lengths of both blocks were determined by SEC from a comparison of M_w of the polystyrene block with that of the final copolymer. The relative content of styrene and methacrylate was obtained from NMR. The molecular characteristics of the samples are given in Table I.

Micelle Preparation. The method of micelle preparation is critical because improper techniques can lead to undesirable aggregations. Polymer micelles are prepared by dissolving the solid polymer in an 80/20 vol % mixture of 1,4-dioxane/water and vortexing for 30 min. Mixed micelles are prepared using the same method except an 80/20 wt % mixture of untagged/tagged solid polymer was dissolved. The concentration of copolymer in the solution was approximately 3–4 mg/mL for all micelle samples. The resulting micelle solution was typically transparent with a slightly bluish tint. Micelles are present in this mixture as formed, and while we have no direct evidence for the presence of unimers, micelles in this mixture may be capable of exchanging unimers.[15] This solution was then dialyzed to remove all traces of dioxane from the outer solution. Once the micelles are in 100% water, we do not expect unimers to exchange due to the energetic cost of pulling polystyrene blocks out of the glassy core.[15,16] Dialysis was performed by placing the micelle solution in a Spectra-por molecular porous membrane tubing (molecular weight cutoff = 6000–8000) and dialyzing stepwise against outer solutions of increasing water concentration (10% by volume) for at least 3 h per step. For the last step, the 90% water solution was dialyzed into a 100% water solution for several days; the dialysis solution was replaced once a day. The micelles were then dialyzed against the appropriate aqueous pH buffer at a constant ionic strength of 0.1 M. Once formed, the micelles are stable for many weeks at all pH's, except at pH 3.5. The pH 3.5 solutions tend to precipitate when filtering or transferring to different containers. However, these solutions are sufficiently stable for experimentation and can be vigorously shaken without precipitation.

Solvents. Spectral grade 1,4-dioxane was used as purchased (Aldrich). Deionized water was filtered through a 0.2-μm cellulose nitrate filter and was used in the preparation of all aqueous solutions. All unbuffered solutions used in this experiment were adjusted to approximately 0.1 M ionic strength with hydrated lithium chloride (99.9+% pure, Aldrich).

Buffered Solutions. Buffered solutions were used in this study to control the degree of ionization of the methacrylic acid groups, resulting in quite reproducible data. The pH 7 (Na_2HPO_4 and KH_2PO_4) and pH 11 (Na_3PO_4 and $NaHCO_3$) buffers were purchased as dried powders from Aldrich and prepared as directed. The ionic strength of these buffers was adjusted by dilution with deionized, filtered water (without LiCl) to provide a buffered solution with a 0.1 M ionic strength. The pH 3.5 buffer was prepared by dissolving 6 g of glacial acetic acid (Mallinckrodt) into 1 L of filtered deionized water. The pH's were measured with a Sargent-Welch 2050 pH meter.

Fluorescence Spectroscopy. Steady-state fluorescence spectra were recorded on a Photon Technology International LS-100 luminescence spectrophotometer described elsewhere.[6] Naphthalene-tagged copolymers were excited at 293 nm and scanned over the range 310–510 nm. The naphthalene group absorbs strongly at 293 nm, and this corresponds to the excitation wavelength used in the time-dependent fluorescence decay measurements.

Lifetime measurements were performed by the method of time-correlated single-photon counting, as described elsewhere.[3] Excitation was with a mode-locked laser with a doubled wavelength at 293 nm, and the decay of the naphthalene monomer fluorescence was monitored at 340 nm. The multichannel buffer has 8191 channels, and the time resolution is ca. 27 ps per channel for lifetime measurements and ca. 6.7 ps per channel for depolarization measurements. Fluorescence was detected through a Glan-Thompson polarizer located between the sample and monochromator. Lifetime measurements were made with the polarizer at the magic angle (54.7°) to ensure the proper ratio of parallel-to-perpendicular intensity was observed, while the polarizer was rotated between horizontal and vertical for depolarization measurements. Other experimental details and mathematical treatment of lifetime data have been described previously.[3,4]

The time-resolved anisotropy ($r(t)$) was computed by a channel-by-channel calculation using the fluorescence decays collected at vertical and horizontal polarizations from the following:

$$r(t) = [I_V(t) - GI_H(t)]/[I_V(t) + 2GI_H(t)] \quad (1)$$

where $I_V(t)$ and $I_H(t)$ are the fluorescence decays collected at vertical and horizontal polarization, respectively, and G is the instrumental anisotropy. The G factor corrects for the polarization dependence of the monochromator.[17] The G factor was determined experimentally using a dilute solution of naphthalene in cyclohexane (which acts as a freely rotating probe) at an optical density $\simeq$0.05, and also by measuring the intensity of scattered light under the appropriate conditions.[4] The experimentally determined G factor is 0.710 ± 0.005.

Quasi-Elastic Light Scattering (QELS). The apparent hydrodynamic diameter (D_H) of both micelles and unimers was measured with a Brookhaven BI 2030 apparatus with a 72-channel correlator. The scattering angle was 90° and the temperature was 25 °C. A He–Ne laser operating at 632.8 nm was used as a light source. The mathematical treatment of data is reported elsewhere.[3,4]

NMR. NMR measurements were performed using a General Electric Q E 300 (300 MHz) spectrometer.[3] The nonhydrolyzed polymers were dissolved in $CDCl_3$, and the hydrolyzed polymers were dissolved in mixtures of deuterated 1,4-dioxane and methanol.

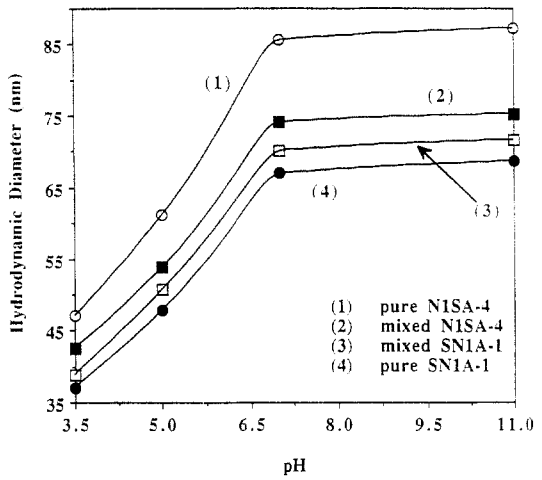

Figure 1. Apparent hydrodynamic diameter for the four aqueous micellar systems as a function of pH: pure N1SA-4 micelles, mixed N1SA-4 micelles, mixed SN1A-1 micelles and pure SN1A-1 micelles (curves 1–4, respectively). The concentration of copolymer in all solutions was in the range 2–3 mg/mL.

Table II. Micelle Properties

copolymer	pH	D_H, nm	r_∞	N_{ag}[c]
N1SA-4[a]	3.5	47.0	0.100	262
	5	61.1		
	7	85.6	0.0937	
	11	87.3	0.100	
N1SA-4[b]	3.5	42.5	0.130	143
	5	53.8		
	7	74.1	0.120	
	11	75.2	0.130	
SN1A-1[a]	3.5	37.1	0.140	70
	5	47.8		
	7	66.9	0.129	
	11	68.6	0.125	
SN1A-1[b]	3.5	38.9	0.134	84
	5	50.7		
	7	70.1	0.128	
	11	71.6	0.117	

[a] Completely tagged micelles. [b] Mixed micelles (20 wt % tagged, 80 wt % untagged). [c] N_{ag}, the aggregation number, are taken from ref 15 and are based on comparable micellar systems, in which micelles are in a solvent mixture (not at pH 3.5) of 80/20 volume % of 1,4-dioxane/water.

Results and Discussion

Characterization of Micelle Solutions by QELS. Since the potential for undesirable aggregations always exists, QELS was used to ensure that each micelle solution is monodisperse. QELS is a very sensitive technique for detecting aggregates[3] which are typically polydisperse compared to micelles. Micelles are quite monodisperse, and we prepared micelle solutions with a polydispersity on the order of 0.01–0.1.[18] All data presented in this paper are measured from solutions free of aggregation, as determined by QELS.

In aqueous media, the polystyrene (PS) block forms the core, and the polymethacrylic acid (PMA) block forms the corona of the micelle. At low pH, the PMA block is collapsed onto the core due to low solubility, and at high pH, the PMA block is stretched out as a polyelectrolyte because of deprotonation of the acid groups. Using QELS, we measured the hydrodynamic diameter, D_H, of micelles as a function of pH. The data are plotted in Figure 1 and tabulated in Table II. We studied four cases: pure and mixed micelles comprising PS end-tagged copolymers and pure and mixed micelles comprising mid-tagged copolymers. Pure micelles are made with 100 wt % labeled polymers such as N1SA-4 (end-tagged) and SN1A-1 (mid-tagged). Mixed micelles, however, are composed of 80 wt % unlabeled polymers and 20 wt % labeled polymers, for example, N1SA-4 (20 wt %)/SA-10 (80 wt %) (end-tagged) and SN1A-1 (20 wt %)/SA-24 (80 wt %) (mid-tagged). The cases of mixed micelles were investigated for the purpose of elucidating additional details about naphthalene–naphthalene energy migration and excimer formation (see later discussion). For each case, the D_H is pH dependent. All the curves in Figure 1 are the same shape, which means that these various types of micelles behave similarly as a function of pH. As mentioned above, pH $\geq$ 7 the PMA block is largely deprotonated such that the corona is stretched, making the micelle approximately twice as large as that at pH 3.5, where the corona is expected to be collapsed (see Figure 1 and Table II).[19] Above pH 7, D_H is nearly constant, indicating that the PMA block does not further deprotonate at pH's higher than 7.

There is a strong correlation between D_H and the aggregation number (see Table II). The increase in D_H in going from pH 3.5 to 11 is fairly consistent in all cases (from 31.5 to 40.3 nm), and the molecular weight of the polyacid segments are similar (DP_{PMA} from 190 to 235). There is not a perfect correlation between ΔD_H and DP_{PMA}, which is most likely the effect of the combined experimental error in the measurements.

As mentioned above in the "Micelle Preparation" section, pH 3.5 micelle solutions are metastable. Precipitation sometimes occurs if the solution is filtered or transferred to another container. In the case of filtration, the forced flow through small pores provides enough strain to induce aggregation. When the micelle solution is pipetted dropwise into another container, precipitation is most likely if the solutions are allowed to strike the container wall as droplets. If the solution is transferred flowing down the wall of the container, the micelle solution usually does not precipitate. However, the pH 3.5 micelle solution is stable when subjected to vortexing. All the data collected at pH 3.5 were taken from solutions without precipitation. Since the pH 3.5 micelle solutions were metastable, we did not attempt to make micelle solutions with pH below 3.5. All the other micelle solutions, however, are stable and do not precipitate for many weeks, if at all.

Steady-State Fluorescence. Because PMA conformation changes with pH, varying the pH is ideal for studying the effects of corona dynamics on the core and the core/corona interfacial region of the micelles. N1SA-4 was labeled at the end of the PS block in order to probe the core environment. For N1SA-4 mixed and pure micelles, changing the pH has insignificant effects on the steady-state fluorescence properties (spectra are not shown but are essentially identical to Figure 2a). The emission spectra shown in Figure 2 were obtained by exciting the samples at 293 nm and scanning from 310 to 510 nm. Emission maxima at 340 nm for naphthalene monomer were observed, but no distinguishable excimer emission was evident. Excimers are formed when the naphthalene groups are at the proper distance apart (~3 Å)[20] and if there is sufficient rotational freedom of pendant fluorophores and mobility of polymer chains[21] so that the fluorophores can achieve the cofacial "sandwich" geometry. Since excimer formation is not observed, the core environment is presumed to be fairly compact and restricted; furthermore, the absence of dynamic and "contact pair" excimers supports the assertion that there is less than one naphthalene per chain because when each chain is tagged with four naphthalenes, the micelles in aqueous media exhibit some excimer formation from contact pairs.[3] These results are consistent with the naphthalene probe being confined to the core of the micelles and being independent

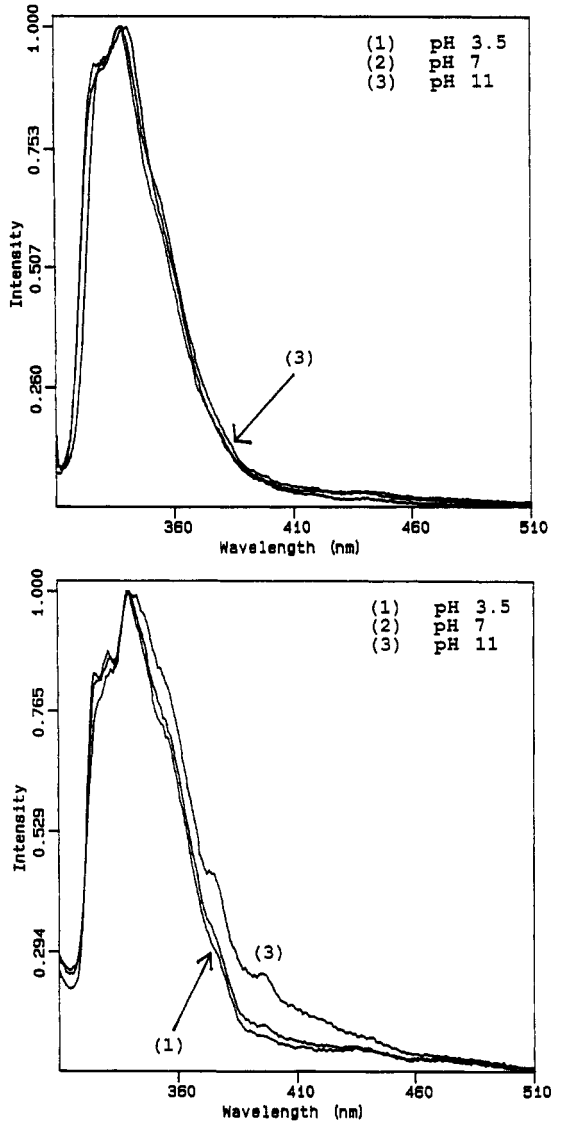

Figure 2. Steady state fluorescence spectra for pure SN1A-1 micelles (a, top) and mixed SN1A-1 micelles (b, bottom) at pH 3.5, 7, and 11 (curves 1–3, respectively). Excitation at 293 nm and emission at 310–510 nm.

of the dynamics of the corona. These conclusions are further supported by the depolarization data (see later discussion) as well as with those of an analogous study on an end-tagged copolymer micelle (N–S–MM) in organic media (1,4-dioxane/methanol mixtures) from a previous publication.[6] Previous results show that before micelle formation significant excimer formation occurred but decreased greatly after micellization.

In contrast to N1SA-4, the middle-tagged copolymer micelles, SN1A-1, exhibit significant differences when comparing the mixed and pure cases. For the case of pure SN1A-1 micelles, there are only minor changes in steady-state fluorescence spectra as a function of pH (Figure 2a). Again, we do not observe excimer formation, and the data may be interpreted similarly as for the cases of N1SA-4 with one exception—the copolymer is labeled at the block boundary for the purpose of probing the interfacial region. On the basis of our previous studies of middle-tagged diblock copolymer micelles in organic solvent,[6] we expected the dynamics of the corona would influence this interfacial region. This is not the case according to the spectra in Figure 2a. We believe that this is a case where the fluorescence probes do interfere with the system investigated as will be discussed later. The spectra shown in Figure 2a indicate that the core/corona interfacial region is more rigid than we anticipated. The rigidity may be due to overcrowding of the bulky naphthalene moieties. Apparently, based on the steady-state results, the rigidity is not affected by corona dynamics.

For the case of SN1A-1 mixed micelles, we did observe spectral changes as the pH was varied (see Figure 2b). Excimer emission (λ_{em} = 420 nm) increased with pH, which we presume to be the result of increasing mobility of the probe to rotate to the appropriate cofacial geometry as the corona is stretched (see depolarization results discussed later). By contrast, SN1A-1 pure micelles exhibit almost no spectral change as a function of pH (see Figure 2a). Mixed micelles are composed of 80/20 wt % of unlabeled/labeled copolymers. The increase in probe mobility is supported by depolarization data (see later discussion). Since probe interference is minimized in the case of SN1A-1 mixed micelles, these data are more reliable for characterizing the "true" interfacial region. The increase in probe mobility for mixed micelles may imply that the interfacial region is more heterogeneous. A "fluid" interfacial region at the block boundary between the core and corona was also observed for this type of copolymer micelle in organic media.[6] The steady-state fluorescence results generally suggest that there is not a sharp boundary between polymer types in the interfacial region. This generalization is supported by time-resolved fluorescence and depolarization data.

Time-Resolved Fluorescence. The time-resolved fluorescence data shown in Figure 3 are taken with excitation at 293 nm and emission at 340 nm for monomer emission. For the cases of N1SA-4 pure (see Figure 3a) and mixed (see Figure 3b) micelles, we observe almost no changes in the fluorescence decays as a function of pH, although QELS data show that, at pH 7 and 11, the hydrodynamic diameters of the micelles are approximately double that of pH 3.5. Again, we believe that the probe is confined to the compact and highly collapsed core, and it is reasonable that the chain dynamics of the corona do not perturb the core environment. This is consistent with the steady-state results. Characteristically, the decays of N1SA-4 mixed micelles are different from the pure micelles. The decays of N1SA-4 mixed micelles show a more rapidly decaying component at early times, although the decays at long times are very similar (compare Figure 3a and 3b; see Table III).

The monomer decay curves of SN1A-1 change only slightly with pH (see Figure 3c) and are similar to those of N1SA-4 pure micelles (see Figure 3a). This implies that the interfacial region is packed with bulky naphthalene moieties, making the region rigid and unaffected by corona dynamics. Again, this is consistent with steady-state results.

Figure 3d shows the time-resolved fluorescence decay curves of SN1A-1 mixed micelles at various pH's. In contrast to the decay curves of N1SA-4 mixed and pure micelles, the decay curves of SN1A-1 mixed micelles change significantly with pH. We anticipated that the dynamics of the corona would perturb the interfacial region. This seems to be especially true in the mixed micelle case. At pH 11, the PMA block composing the corona is sufficiently deprotonated to act as a polyelectrolyte attached to the PS core. The "polyelectrolyte effect" stretches the corona. By comparison, at pH 3.5 the corona is collapsed around the core and the collapsed corona might be expected to restrict molecular motion at the interfacial region. This implies that at pH 11, the probe is in a less restricted environment and that in the case of pH 3.5, the probe is more restricted. The less restricted environment at pH

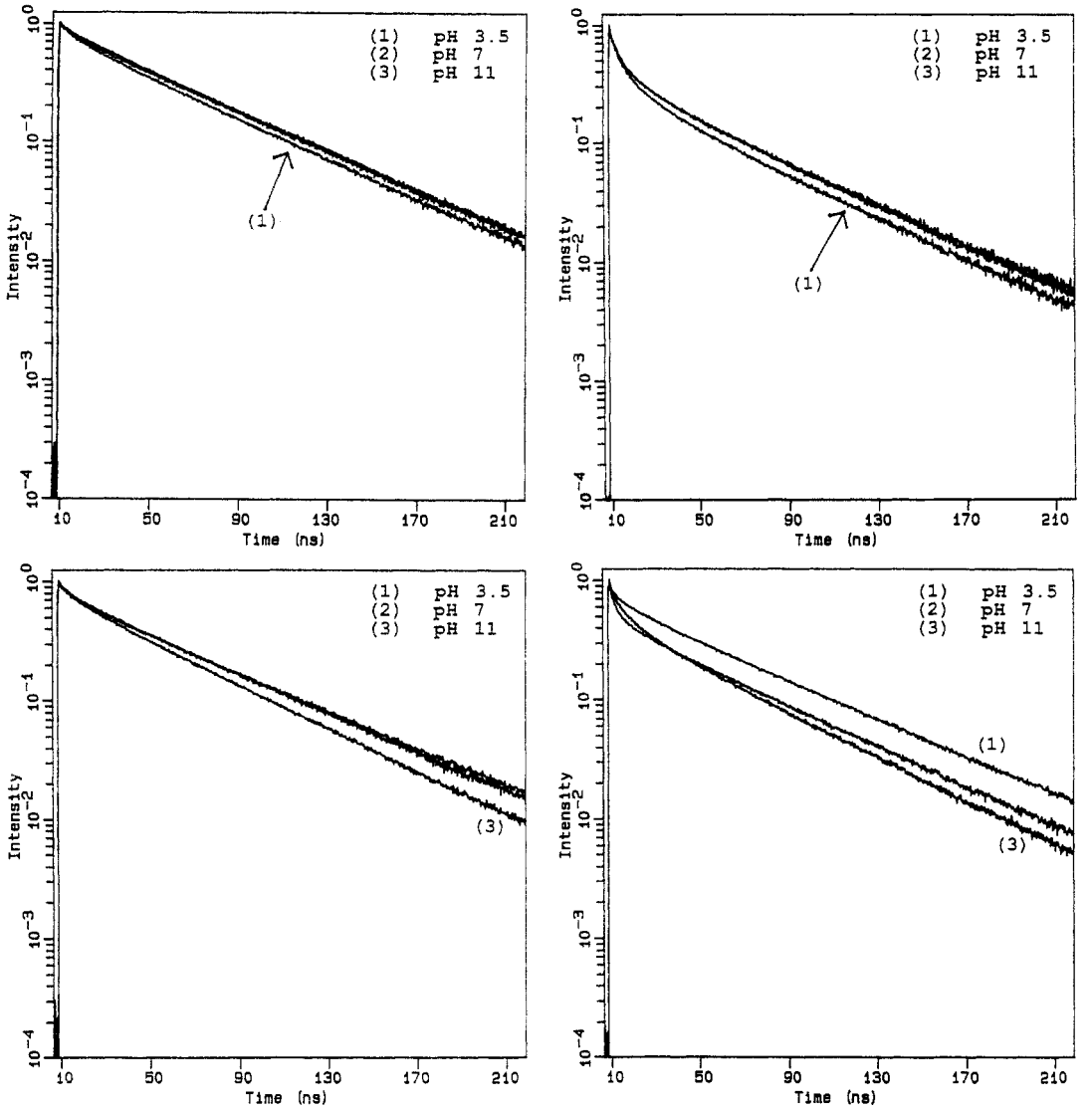

Figure 3. Time-resolved monomer fluorescence decays for pure N1SA-4 micelles (a, top left), mixed N1SA-4 micelles (b, top right), pure SN1A-1 micelles (c, bottom left), and mixed SN1A-1 micelles (d, bottom right) at pH 3.5, 7, and 11 (curves 1–3, respectively). Excitation at 293 nm and emission at 340 nm.

Table III. Time-Resolved Fluorescence Deconvolution Parameters

micelles	pH	τ_1[c]	a_1	τ_2[c]	a_2	τ_3[c]	a_3	τ_4[c]	a_4	τ_{av}[c]
N1SA-4[a]	3.5	0.808	0.104	5.70	0.143	23.7	0.162	53.7	0.591	36.5
	7	2.34	0.156	18.5	0.121	53.5	0.723			41.3
	11	1.30	0.150	17.2	0.14	53.6	0.709			40.6
N1SA-4[b]	3.5	0.713	0.288	4.14	0.341	16.1	0.156	50.0	0.215	14.9
	7	0.568	0.225	3.62	0.356	15.3	0.158	51.5	0.262	17.3
	11	0.499	0.251	3.43	0.345	16.4	0.148	50.8	0.257	16.8
SN1A-1[a]	3.5	0.234	0.087	2.91	0.153	18.7	0.147	55.9	0.613	37.5
	7	0.433	0.092	2.64	0.120	15.9	0.114	53.3	0.674	38.1
	11	1.67	0.161	13.3	0.189	53.2	0.651			37.4
SN1A-1[b]	3.5	0.371	0.220	2.48	0.137	17.6	0.120	55.4	0.486	29.6
	7	0.435	0.272	2.57	0.304	14.2	0.100	51.7	0.324	19.1
	11	0.125	0.325	2.09	0.197	11.7	0.201	47.1	0.277	15.8

[a] Completely tagged micelles. [b] Mixed micelles (20 wt % tagged, 80 wt % untagged). [c] Lifetime in ns.

11 promotes probe rotation and segmental motion which may enhance excimer formation; this is evident in the steady-state data. The decay of SN1A-1 mixed micelles at pH 3.5 (see Figure 3d, curve 1) is similar to the N1SA-4 mixed micelle where the probe resides in a region of high microviscosity. But the decay at pH 11 (see Figure 3d, curve 3) is significantly shorter than that at pH 3.5 (see Figure 3d, curve 1); this difference can be attributed to the change of environment of the probe as well as some quenching by excimer formation (the decay at pH 11 is the shortest; see Table III).

Time-Resolved Fluorescence Depolarization. The technique of time-resolved fluorescence depolarization is sensitive to probe motions of the individual naphthalene moiety and provides a unique insight into the local probe environment which is critical in characterizing the strategically labeled regions of the micelles. Probe motions are highly influenced by the motion of the pendant group as well as segmental motions because the probe environments of the micelles are relatively restricted. Depolarization data will be plotted as anisotropy vs time (see eq 1). Mixed micelles consisting of 80/20 wt % unlabeled/

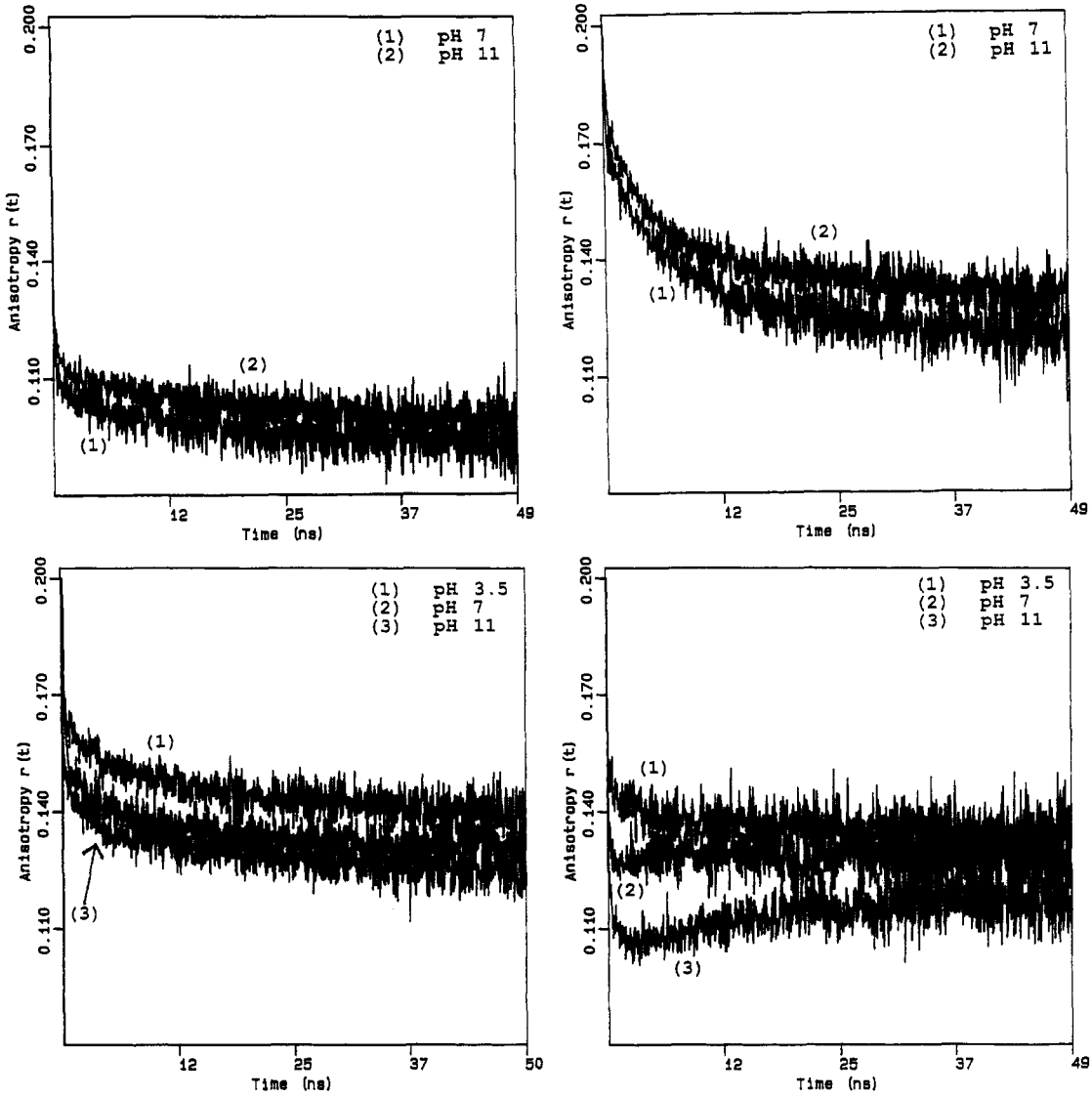

Figure 4. Anisotropy decays for pure N1SA-4 micelles (a, top left) and mixed N1SA-4 micelles (b, top right) at pH 7 and 11 (curves 1 and 2, respectively) and for pure SN1A-1 micelles (c, bottom left) and mixed SN1A-1 micelles (d, bottom right) at pH 3.5, 7, and 11 (curves 1–3, respectively). Excitation at 293 nm and emission at 340 nm.

labeled copolymers were prepared in order to minimize naphthalene–naphthalene energy migration during the depolarization experiments. Pure micelle systems were measured for comparison. For all depolarization measurements, we have reproducibly determined the G factor to a precision of ±0.005; this implies that even small changes in residual anisotropy, r_∞ (anisotropy at infinite time), are observable and can be interpretable. The initial anisotropy, r_0 (anisotropy at time zero), however, is more uncertain. As discussed previously,[6] the uncertainty in r_0 is related to a number of factors including the inherently complex photophysics of naphthalene, the experimental setup itself, laser instability during the 2-h data collection time for each anisotropy measurement, the wavelength dependence of the microchannel plate,[17] and even minute initial time shifts of the order 7 ps. In this study, we will focus on changes in r_∞ which is unaffected by the uncertainties of r_0.

The anisotropy decays for N1SA-4 pure micelles are shown in Figure 4a, and those of N1SA-4 mixed micelles are shown in Figure 4b. The pure micelles have significantly smaller values of r_∞ than mixed micelles because of the effect of naphthalene–naphthalene energy migration; such energy migration was expected and was reported in a previous study of a similar type of micelle in aqueous media.[4] However, pure micelles in organic media (1,4-dioxane/methanol mixtures) do not exhibit energy migration.[6] This leads us to conclude that the core is more dense in aqueous media. Although the energy migration occurring in the pure micelle case precludes using the depolarization data to provide any useful information regarding probe mobility, the shape of the depolarization decays can still yield meaningful data. For N1SA-4 pure micelles, the initial fast decay component observed for all three pH's (see Figure 4a, curves 1–2) indicates that any molecular motion and/or energy transfer is extremely rapid among those chromophores able to depolarize (<60 ps). The remaining chromophores are locked into specific orientation with no further mobility possible. This is expected because the copolymer is tagged at the PS end, which is most likely to be in the core. Figure 4b shows the depolarization decays of N1SA-4 mixed micelles, for which we assume that we have eliminated most of the energy migration effects so that the probe mobility can be more accurately determined. The r_∞ values (0.120–0.130; see Table II) for the mixed micelle case are greater than those of the pure micelle case (0.0937–0.100; see Table II). The large r_∞ values for the mixed micelle case indicate that the probes reside in a restricted environment with little or no energy transfer. The decay of the anisotropy curves is much slower than those in Figure 4a. This implies that the probe is undergoing some hindered motion or residual

energy transfer on the time scale of ~5–10 ns, but the probe mobility is not sufficient for excimer formation on the basis of the steady-state data. In all cases of N1SA-4 micelles, the residual anisotropy is independent of pH, within experimental error.

The diblock copolymers labeled at the block boundary were prepared for studying the core/corona interfacial region. The anisotropy decays for SN1A-1 pure micelles are shown in Figure 4c which demonstrate much less depolarization than the corresponding pure end-tagged case (see Figure 4a). The lack of depolarization via energy transfer for SN1A-1 micelles as compared to N1SA-4 micelles is not so surprising when one considers a simple geometric argument. For N1SA-4 micelles, the aggregation numbers 262[15] and the radius of the core is about 150 Å. In the core, if two naphthalenes are within ca. 23 Å, they are depolarized via energy transfer. Therefore, on the basis of geometric spacing, the random placement of naphthalenes in the core (following a Poisson distribution) could lead to 30–40% depolarization. Applying the same type of geometric argument to SN1A-1 micelles shows that the probability for depolarization is much less. The anisotropy decays shown in Figure 4c are rather similar to those of the mixed end-tagged case (see Figure 4b) except for a more rapid decay to the limiting value. Since the r_∞ values are slightly higher than for the mixed micelle case discussed next, it seems reasonable to conclude that depolarization due to energy migration (if present) is less important than probe mobility in the mixed micelle. Although the interfacial region of SN1A-1 is believed to be fairly rigid, anisotropy data show that the probes do undergo some rotational mobility. The r_∞ values increase slightly with decreasing pH's (these differences are just outside experimental error). At pH 3.5 (see Figure 4c, curve 1), the corona is collapsed around the core, and probe mobility is more restricted (the highest r_∞ value). Increasing the pH to 7 or 11 (see Figure 4c, curves 2 and 3) expands the corona, and probe mobility is less restricted (the lower r_∞ value). Thus, on the basis of QELS and depolarization data, pH 7 and 11 seem to have very similar corona properties (see Figures 1 and 4c).

The anisotropy decays for mixed micelles of SN1A-1 are shown in Figure 4d. Analogous to the case of pure micelles, r_∞ values increase slightly with decreasing pH. The r_∞ value at pH 3.5 is very similar to the N1SA-4 mixed micelle or SN1A-1 pure micelle, but there is very little decay observed in $r(t)$. The unusual shape of the anisotropy decay at pH 11 (see Figure 4d, curve 3) provides a unique insight into the physical state of the interface. The striking feature of this decay is that it has a dip in the beginning. According to Michl and Thulstrup,[22] such decay can be interpreted as follows: at pH 11, the probe resides in a range of local environments. The environment with the shortest lifetime component is more mobile (faster rotation), and so the $r(t)$ drops to a lower value, while the longest lifetime component is less mobile (slower rotation), and thus the anisotropy rises to the highest value. At pH 7, there is some hint of a dip, but it is less dramatic and occurs at an even earlier time (see Figure 4d, curve 2). The depolarization data of SN1A-1 mixed micelles, especially at pH 11, further demonstrate that the true interfacial region is heterogeneous and not distinct. A quantitative interpretation of these data would require a specific rotational model for the motion of the pendant naphthalene chromophore.[23]

Summary

From the QELS data, we have determined that the spectra changes dramatically with pH. The striking change in the corona as a function of pH, however, has essentially no effect on the environment of the core, as for the case of N1SA-4 micelles. There is significant energy transfer in this case, which is not true for micelles in organic media.[6] This implies that the core is more compact in aqueous media because if the micelles are in organic media, the cores are likely to be swollen with organic solvent. In all cases, the mixed micelles seem to be more heterogeneous than the pure micelles. On the basis of time-resolved fluorescence decay data pH does influence SN1A-1 micelles. At pH 11, the decay is slightly faster (some naphthalenes are probably exposed to water). There does not seem to be as much energy transfer in pure SN1A-1 micelles as for N1SA-4, and the environment is more rigid (highest r_∞). For SN1A-1 mixed micelles, there is a clear effect of pH on the anisotropy decays and the residual anisotropy data. Changes in r_∞ as a function of pH further support the idea that the interfacial region is likely to be heterogeneous, since the corona dynamics appear to greatly influence the behavior of the interfacial region. This is consistent with time-resolved fluorescence decay data. Because of the residual anisotropy data and the excimer fraction, we would like to propose that the pure SN1A-1 micelles have a more rigid structure than the mixed micelles. Also, on the basis of our current studies,[24] fluorescence quenching is heterogeneous for mid-tagged micelles, which is consistent with an inhomogeneous interface. Since there is little or no energy transfer, the naphthalene groups are separated by at least ca. 23 Å from each other in the interfacial region. This is in approximate agreement with recent calculations of the area per emerging chain for these types of micelles.[25]

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