Time-Resolved Fluorescence Studies of the Chain Dynamics of Naphthalene-Labeled Polystyrene-*block*-poly(methacrylic acid) Micelles in Aqueous Media

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ABSTRACT: A-B block copolymers of polystyrene-*block*-poly(methacrylic acid) and polystyrene-*block*-poly(*tert*-butyl methacrylate), both with short-chain oligovinyl-2-naphthalene moieties attached to the end of the polystyrene block, were prepared by anionic polymerization. After hydrolysis of the poly(*tert*-butyl methacrylate) blocks, micelles were prepared from the polystyrene-*block*-poly(methacrylic acid) copolymers and the photophysical properties were studied as a function of different ratios of the solvent system 1,4-dioxane/water. The fluorescence data were compared to that of micelles formed from the polystyrene-*block*-poly(*tert*-butyl methacrylate) copolymers in organic solvent mixtures of 1,4-dioxane/methanol. It was found that intramolecular excimer formation (which is controlled by the mobility of the pendant fluorescent groups and the polymer chain dynamics) is sterically hindered in the micellar cores as they become more compact. Both steady-state and time-resolved excimer fluorescence are significantly influenced by the gradual collapse of the micellar cores and the increase in segment density within the cores with an increasing content of water. Changes in lifetimes and preexponential factors for naphthalene fluorescence (monomer as well as excimer) were found to be sensitive indicators of micelle formation.

Introduction

For many years, studies of block-copolymer micelles have been carried out, resulting in a deeper understanding of micellar systems. Techniques such as light scattering, ultracentrifugation, viscometry, osmometry, size-exclusion chromatography, electron microscopy, and others have resulted in a wealth of data describing many micellar properties, including critical micelle concentration, the thermodynamics of micelle formation, the equilibrium between micelles and unimers, and micellar size.[1] More recently, steady-state and time-dependent fluorescence and fluorescence depolarization, along with fluorescence quenching experiments, have proven to be valuable tools in expanding the understanding of many micellar properties including micelle formation, micelle–unimer equilibrium, and micelle structure.[2]

Micelles are formed when block copolymers are dissolved in a selective solvent or solvent mixture and are typically composed of two distinct regions: (1) a compact micellar core consisting of the insoluble blocks and (2) an outer shell or corona which is formed from the solvated segments of the soluble blocks. The selective solvents are a thermodynamically good solvent for the block that forms the corona while simultaneously a thermodynamically bad solvent (or precipitant) for the other block which forms the core. The micellization of block copolymers is a reversible equilibrium process between micelles which are nearly monodisperse in mass and size and nonmicellized copolymers known as unimers. This equilibrium 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 (i.e., as the selectivity of the solvent increases, the association number also increases). NMR,[3] fluorimetry,[2d] and various other techniques[4] have been used in an effort to better understand the dynamics of micellization; however, there is little information on this topic at the present time.

Micellization in aqueous media is a topical subject of investigation due in part to its potential importance in medical and pharmaceutical applications. In this paper, we report fluorescence studies of micelle systems composed of block copolymers of polystyrene-*block*-poly(*tert*-butyl methacrylate) and polystyrene-*block*-poly(methacrylic acid), both labeled by a short chain of 2-vinylnaphthalene moieties at the end of the polystyrene block (four monomers on average). In aqueous media, excimer formation within this block is quite sensitive to the label mobility and this permits us to follow micelle formation and the properties of the micelle core as a function of the addition of the thermodynamically bad solvent. Water is an extremely strong precipitant for the polystyrene blocks, and hydrophobic interactions in the methacrylic acid micellar shell lead to unique behavior.[5] The result is that micelles in water are impossible to prepare by direct dissolution since equilibrium does not exist under these conditions. For such a case, micelles were prepared indirectly from a water/organic solvent mixture by dialysis.[2e] An alternative method, such as distilling off the organic solvent,[6] was not used since it was not convenient for this study.

Experimental Section

Materials. The diblock copolymer polystyrene-*block*-poly(*tert*-butyl methacrylate) (N4-SBM) was labeled by a short chain of four 2-vinylnaphthalene moieties at the end of the polystyrene block. Part of the N4-SBM was hydrolyzed to prepare polystyrene-*block*-poly(methacrylic acid) (N4-SMA).

The naphthalene-tagged diblock copolymers were prepared by anionic polymerization in tetrahydrofuran at −78 °C using

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cumylpotassium as an initiator. Initially, a small amount of 2-vinylnaphthalene was added to a solution containing the initiator, and short chains of polyvinylnaphthalene anions were synthesized. The average number of naphthalene groups, as determined by UV absorption spectroscopy, for these chains is 4, and their distribution of segment lengths is expected to obey a Poisson distribution.[7] Dried and purified styrene was then added to the solution, resulting in the preparation of living polystyrene blocks. A small amount of polystyryl anion solution was removed, and the reaction in this solution was terminated by the addition of degassed methanol. The resultant polystyrene was used in the determination of the molar mass and the distribution of molar masses of the polystyrene block and also for a comparison of the polystyrene fluorescence behavior with that of the copolymer. A single 1,1-diphenylethylene group was attached to the polystyryl anion to modify its reactivity, and *tert*-butyl methacrylate at −78 °C was added slowly to form the second block, which was then terminated by degassed methanol. The N4-SBM was filtered, precipitated in a water/methanol mixture, dried, and further characterized.

The molar mass and molar mass distribution of N4-SBM were determined by size-exclusion chromatography (SEC) in THF. The value of the molar mass (M_W) obtained from SEC was compared to M_W obtained from static light scattering. 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 contents of styrene and methacrylate were obtained from NMR. The values from these methods agree quite well.

Part of the N4-SBM was hydrolyzed for 5 h at 85 °C in 6 N aqueous HCl in 1,4-dioxane (the number of moles of HCl was 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 N4-SMA was filtered, precipitated in cold hexane, and then redissolved in 1,4-dioxane with subsequent freeze-drying of the solution. The degree of hydrolysis was etimated by NMR in a deuterated 1,4-dioxane/methanol mixture using tetramethylsiloxane as a reference standard. The molecular characteristics of the nonhydrolyzed sample are as follows: for the copolymer, from static light scattering, M_W 61 800; from SEC, M_W 60 300, M_n = 55 900, PD (polydispersity) = 1.08; for the polystyrene block, M_W 21 500, M_n 21 100, PD = 1.02; from NMR, the mole fraction of polystyrene, x_s = 0.45. The degree of hydrolysis of the hydrolyzed sample is 0.98.

Solvents. Spectral grade 1,4-dioxane and methanol were used as purchased (Aldrich). Deionized water was used to prepare the aqueous solvents.

Fluorescence Spectroscopy. Steady-state fluorescence spectra were recorded on a SPEX Fluorolog fluorimeter system described elsewhere.[8] Emission spectra for naphthalene-tagged polymers were collected with an excitation wavelength of 293 nm and span the range 310–510 nm. Lifetime measurements for naphthalene-tagged polymers were made at 340 nm for the monomer and at 410 nm for the excimer with an excitation wavelength of 293 nm.

Lifetime measurements were performed by the method of time-correlated single-photon counting. The system consists of a mode-locked frequency-doubled Nd:YAG laser (Quantronix Model 416) synchronously pumping a cavity-dumped dye laser (Coherent Model 701-3D) circulating Rhodamine 6G. The YAG produces a series of pulses at ca. 38 MHz and 532 nm with a pulse width <100 ps fwhm. The dye laser output is tunable in the range 570–620 nm and consists of pulses that are typically cavity dumped at ca. 1.9 MHz with a fwhm of ca. 8 ps. For the present experiment, the 586-nm output is frequency-doubled with an angle-tuned KDP crystal. Including all the effects of the electronics and laser pulse width, the instrumental response function is ca. 70 ps fwhm. The fluorescence is collected at right angles, focused into a Model DH-10 Instruments S.A. double monochromator, and detected by a Hamamatsu Model R-1564U-07 microchannel plate detector coupled to a Hewlett-Packard Model 8447F amplifier. The single-photon counting electronics are comprised of a Tennelec TC 454 Quad CFD, Ortec Model 566 TAC, and an Ortec Model 918A MCB interfaced to an IBM AT computer. The MCB has 8192 channels, and the time resolution is ca. 27 ps/channel for lifetime measurements. Fluorescence was detected through a dichroic sheet 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.

Naphthalene fluorescence is known to be quite sensitive to oxygen quenching. Bubbling with argon for long times resulted in slightly longer measured lifetimes (mainly for the long-lifetime components), although the reported value for 2-methylnaphthalene (ca. 59 ns)[9] was not obtained. A more efficient degassing technique such as freeze–pump–thaw cycles could not be employed since this results in the precipitation of micelles from solution. While the lifetimes we report are perturbed to some degree because oxygen was not completely removed from the solutions, the interpretations of this work are not affected.

Decay profiles are a convolution of the true sample decay and instrument response function. We perform deconvolution by iterative reconvolution through application of nonlinear least-squares curve fitting utilizing the Marquardt method.[10] The observed decay is given by the convolution integral

$$I_{obs}(t) = \int_0^t I(t-t')\, R(t')\, dt' \tag{1}$$

where $I_{obs}(t)$ is the observed decay, $R(t)$ is the instrument response function, and $I(t-t')$ is the true decay. The true decay is extracted by fitting to a multiexponential function given by

$$I(t) = \sum a_i \exp(-t/\tau_i) \tag{2}$$

while simultaneously minimizing χ^2 (see below). For the present work, three and four exponential fits were used. The goodness of fit is evaluated by two methods: (1) the minimization of χ^2

$$\chi^2 = \sum W_i [I_{obs}(t_i) - F(t_i)]^2 \tag{3}$$

where $F(t_i)$ is the calculated fitting function and the weighing factor W_i is defined as

$$W_i = 1/I_{obs}(t_i) \tag{4}$$

and (2) the weighted residuals given by

$$r(t_i) = [I_{obs}(t_i) - F(t_i)]/\sqrt{I_{obs}(t_i)} \tag{5}$$

which are plotted and evaluated. A plot of the residuals against time should show a data set randomly scattered about zero for an acceptable fit.

Static Light Scattering. The weight-average molar mass of molecularly dissolved copolymer and micelles in selective solvents was measured by a Sofica 42000 apparatus described elsewhere.[11] The refractive index increments necessary for the evaluation of light scattering data were measured under the condition of osmotic equilibrium between copolymer solution and mixed solvent as described by Tuzar et al.[12]

Quasi-Elastic Light Scattering. The apparent hydrodynamic radii of micelles (R_H) (measured at a low, albeit finite concentration) as well as unimers in good solvents were measured using 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 effective characteristic decay rate (Γ) was obtained from the first moment of the line width distribution using the cumulant method. The effective translational diffusion coefficient (D_T) was calculated from

$$D_T = \Gamma/q^2 \tag{6}$$

where $q = (4\pi n/\lambda_0)\sin(\theta/2)$ is the length of the scattering vector. R_H was then calculated using the Stokes–Einstein relation.

Since the values obtained from QELS correspond to the z-average diffusion coefficient ($(R_H^{-1})_z$), the following quantity is measured in systems containing both unimers and micelles

$$(R_H^{-1})_z = [w_U M_U (R_H)_U^{-1} + w_M M_M (R_H)_M^{-1}]/(w_U M_U + w_M M_M) \tag{7}$$

where the w_i and M_i are weight fractions and molar masses of individual components and the subscripts U and M represent unimer and micelle, respectively. Because M_M is much larger than M_U, the $(R_H^{-1})_z$ are very sensitive to relatively low concen-

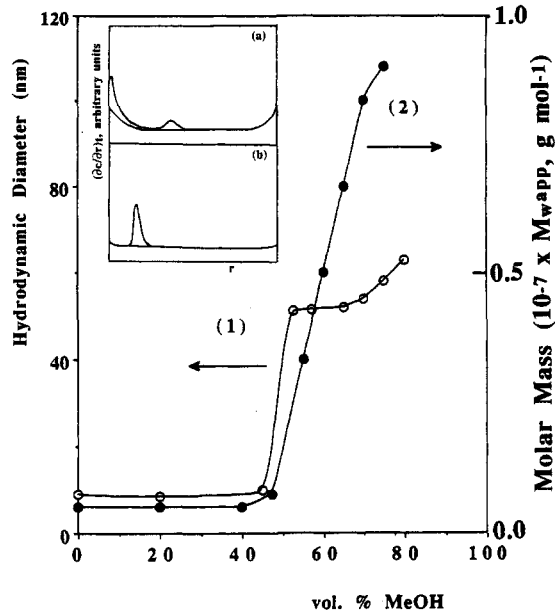


Figure 1. Apparent hydrodynamic diameter ($2R_H$) (curve 1) and apparent molar masses (M_w^{app}) (curve 2) for N4-SBM in 1,4-dioxane/methanol mixtures as functions of volume percent of methanol. Insert shows sedimentation velocity diagrams for N4-SBM in selective solvent mixtures: (a) 1,4-dioxane/methanol (60 vol %), $c = 3 \times 10^{-3}$ g cm^{-3}, 52 000 rpm, $t = 10$ min; (b) 1,4-dioxane/methanol (80 vol %), $c = 3 \times 10^{-3}$ g cm^{-3}, 22 000 rpm, $t = 15$ min.

trations of micelles in the solution. Once micelles are present, the measured values of $(R_H^{-1})_z$ approach the expected value for pure micelles even if unimers are present.

Ultracentrifugation. Sedimentation velocity measurements were performed using a Spinco Model E analytical ultracentrifuge with Schlieren optics. The apparent sedimentation coefficient (s) was evaluated using the standard technique of plotting $\ln r(t)$ vs $\omega^2 t$, where r is the distance of the peak maximum from the axis of rotation at time t and ω is the angular velocity of the rotor. The rotor ANJ with a double-sector 12-mm cell was operated at 25 °C and 20 000–50 000 rpm. The method of Gilbert[13] was applied to interpret the sedimentation diagrams of reversibly associating micellar systems.

NMR. NMR measurements were performed using a General Electric QE 300 (300-MHz) spectrometer. The samples were dissolved in solvent mixtures of deuterated 1,4-dioxane and methanol.

Results and Discussion

Nonhydrolyzed Sample N4-SBM in 1,4-Dioxane/Methanol Mixtures. The overall aim of this work is to study the behavior of water-soluble block copolymers in aqueous media with water as the basic component of the solvent mixtures and compare the behavior to that of similar samples which are soluble in organic solvents. Since the micellization of block copolymers in organic solvents has been studied in detail for several decades[1] and is much better understood than similar systems in water, results for the nonhydrolyzed sample in organic solvents will be presented first.

A detailed characterization of multimolecular micelles was performed by measuring the hydrodynamic diameter ($2R_H$) of particles in solution as a function of solvent composition by QELS. Since $(R_H^{-1})_z$ is measured by QELS, values pertinent to unimers are found only in good solvents. As soon as micelles are present in a solution (even as low a percentage as ca. 10–20 wt %), the measured $2R_H$ corresponds roughly to that of micelles. Curve 1 in Figure 1 shows the $2R_H$ values as a function of solvent composition. The curve is similar to that of M_w^{app} (Figure 1, curve 2).

Table I
Values of the Apparent Sedimentation Coefficients (s, 10^{13}s) for Nonhydrolyzed Sample N4-SBM in Mixtures of 1,4-Dioxane/Methanol

	52 vol % CH_3OH	56 vol % CH_3OH	60 vol % CH_3OH	70 vol % CH_3OH	80 vol % CH_3OH
slow peak	2.73	2.81	6.13		
fast peak		43.2	87.7	91.5	153.4

A prominent and steep rise with increasing content of methanol is observed at 45 vol % CH_3OH. In Figure 1, curve 2, the apparent weight average molar masses (M_w^{app}) measured by static light scattering are plotted as a function of solvent composition. Mixtures with less than 45 vol % methanol are good solvents for both blocks of N4-SBM. The sample dissolves molecularly, and the measured values of M_w^{app} are constant and equal to that of the unimer. Starting at 48 vol % CH_3OH, M_w^{app} rises rapidly with increasing content of CH_3OH, indicating the formation of multimolecular micelles. In mixtures with 54–60 vol % CH_3OH, a sedimentation velocity pattern typical for a reversible closed association[11,13] was obtained (Figure 1, inset, curve a). The relatively large area below the slowly sedimenting peak of unimer as compared to that of micelles indicates a high percentage of unimers in solution. In solvent mixtures with more than a 62 vol % methanol, the micellization equilibrium is shifted significantly in favor of micelles in the concentration range (2–3) $\times 10^{-3}$ g cm^{-3}, and only one fast sedimenting sharp peak of micelles is observed by the sedimentation velocity technique (Figure 1, inset, curve b). Typical values of the apparent sedimentation coefficients of micelles and unimers in selected solvents are given in Table I.

Study of the Segmental Mobility by Steady-State and Time-Resolved Fluorimetry. The head to tail oligomerization of several 2-vinylnaphthalene monomers at the end of the polystyrene block results in the possibility of a coplanar "sandwich" arrangement of neighbor-pendant naphthalene groups. These groups are the proper distance from each other for excimer formation, and if there is sufficient rotational freedom of pendant fluorophores and mobility of polymer chains, efficient excimer formation is observed.[14]

The steady-state emission spectrum of N4-SBM in 1,4-dioxane is composed of both the monomer emission close to 340 nm and a broad and intense excimer peak with a maximum at 410 nm (Figure 2, inset, curve a). In selective solvents, essentially all fluorophores are trapped in compact and dense micellar cores. The excimer intensity decreases for the case of a mild selective solvent for polystyrene (55 vol % CH_3OH; Figure 2, inset, curve b). In solvents containing 62 vol % or more CH_3OH, the micellar cores are even more compact (72 vol % CH_3OH; Figure 2, inset, curve c) and the excimer intensity further decreases. The excimer to monomer intensity (I_{410}/I_{340}) vs solvent composition is also presented in Figure 2, curve 1. The ratio of excimer to monomer intensity depends only slightly upon the solvent composition for good solvent mixtures. A pronounced drop in excimer fluorescence intensity is observed despite the fact that 400–1000 naphthalene groups (according to solvent selectivity) are located close to each other in a relatively small volume of the micellar core, ca. (1–2) $\times 10^4$ nm^3. The small degree of mobility in compact micellar cores prevents the fluorophores from reaching the required coplanar position with respect to each other within the fluorescence lifetime, although a small fraction of fluorophores form excimers from "contact pairs" for which no significant segmental rotation is required to produce the necessary cofacial

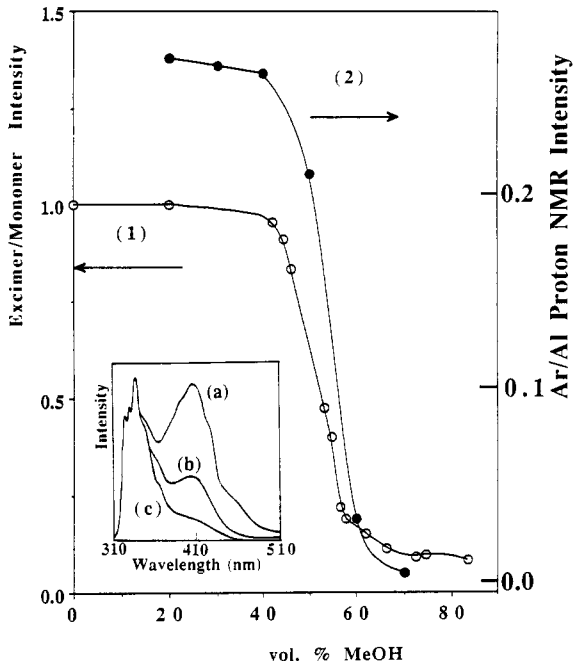


Figure 2. Excimer to monomer fluorescence intensity (I_{410}/I_{340}) (curve 1) and the ratio of aromatic to aliphatic proton NMR intensities (I_{Ar}/I_{Al}) (curve 2) for N4-SBM as functions of 1,4-dioxane/methanol solvent compositions. Insert shows steady-state emission spectra of N4-SBM in various solvent mixtures with excitation at 293 nm and emission at 310–510 nm: (a) pure 1,4-dioxane; (b) 1,4-dioxane/methanol (55 vol %); (c) 1,4-dioxane/methanol (72 vol %).

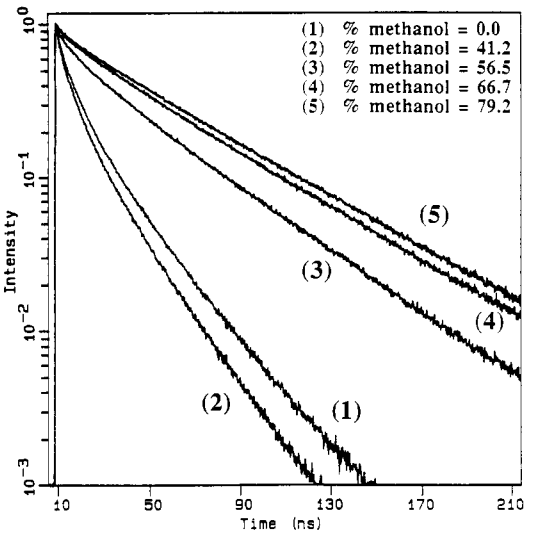


Figure 3. Time-resolved monomer fluorescence decays for N4-SBM in various 1,4-dioxane/methanol mixtures. Percentage of methanol by volume: curve 1, 0.0%, curve 2 41.2%, curve 3, 56.5%; curve 4, 66.7%; curve 5, 79.2%. Excitation at 293 nm, emission at 340 nm.

geometry (see later results).

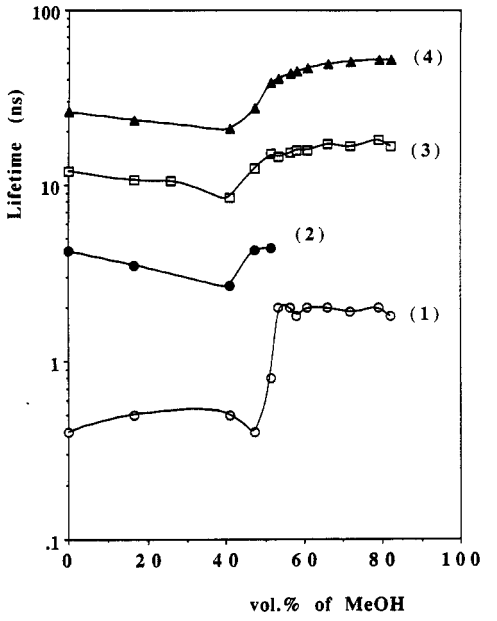
Independent information on the mobility of blocks in micellar cores and in shells was obtained by measuring the NMR intensities of aromatic protons in the core and aliphatic protons, which are mostly in shell, in various deuterated good and selective 1,4-dioxane-d_8/methanol-d_4 mixtures. When the mobility of protons on different segments of polymer chains is slowed, the respective peaks are broader and their integrated intensity decreases due to insufficient relaxation processes.[3] The concentration of the samples for NMR measurements was necessarily higher than that for fluorescence measurements (1.5×10^{-2} g cm^{-3}). The ratio of the integrated aromatic to aliphatic signal (I_{Ar}/I_{Al}) (Figure 2, curve 2) as a function of solvent composition is compared with the excimer to monomer signal ratio (I_E/I_M) (Figure 2, curve 1). The apparent changes in the mobility of the blocks in micellar cores are in good agreement with the time-dependent fluorescence measurements described below.

Steady-state excitation spectra of N4-SBM for monomer emission at 340 nm in good as well as in selective solvents are essentially identical to 2-substituted naphthalene moieties. In all cases, the peak for direct excitation of the naphthalene group is close to 293 nm. The absolute intensity decreases in micellar systems due to strong light scattering and high apparent OD (the bluish tint typical of micelles is observable by the naked eye). However, a new peak close to 260 nm appears (coinciding with polystyrene absorption), suggesting efficient sensitization of naphthalene by energy transfer from polystyrene (spectra not shown).

Analysis of the quenching of monomer fluorescence by excimer formation and of the buildup times for excimer fluorescence was found to be a good means for investigating polymer chain dynamics. Figure 3 shows several typical monomer decay curves for N4-SBM in good solvents (curves 1 and 2), in a mildly selective solvent (curve 3), and in strongly selective precipitants (curves 4 and 5) where micelles with compact cores are present. The distinct smooth curvature observed in good solvents is typical of monomer fluorescence quenched by excimer formation (but is not uniquely ascribable to this mechanism). Excimer formation is controlled by the rotational diffusion of pendant naphthalene groups along with the dynamics of the polymer backbone. Increasing the amount of CH_3OH in the region of good solvents shortens the lifetime, which seems to be a general effect when the overall polarity of the medium is increased for these naphthalenic systems. In selective solvents, there is far less quenching and the average lifetime increases significantly. This appears to be the result of the fluorophores being trapped in nonpolar micellar cores with extremely high microviscosity.

As described in the Experimental Section, three- and four-exponential functions were used for the deconvolution of experimental decay curves. The four lifetimes (τ_i) and corresponding preexponential factors (A_i) as functions of solvent composition are plotted in Figure 4, parts a and b, respectively. A significant increase in the longest lifetime, τ_4 (Figure 4a, curve 4), in the region 45–55 vol % CH_3OH and a sudden change in the corresponding coefficient A_4 (Figure 4b, curve 3) indicate that time-resolved fluorimetry can be used to monitor micelle formation. It is impossible to ascribe a physical meaning to these lifetime components except for the longest one, which corresponds to the behavior of nonquenched probes. Changes in the longest lifetime demonstrate the sensitivity of the naphthalene fluorophore to the microviscosity of the medium. In all systems, a small but nonnegligible contribution from very fast quenching was observed ($\tau_1 \approx$ 1–2 ns). Multiexponential decays with a small contribution of fast quenching are quite common in polymer systems containing pendant fluorophores covalently bound to the polymer chain.[15]

When the decay curves for excimer fluorescence at 410 nm in a good solvent are compared with those in selective solvents (Figure 5), significant differences are evident. In a good solvent (unimers only), the excimer decay curve shows a moderately fast buildup lifetime of ca. 6 ns (Figure 5, curve 1). When only micelles with compact cores are present, the decay is biphasic with a rapid initial decay (Figure 5, curve 3). The absence of a buildup implies that



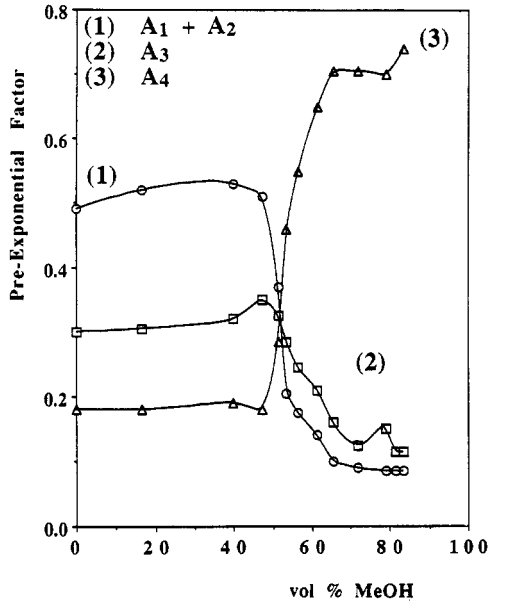


Figure 4. (a, top) Deconvolved monomer fluorescence decay lifetimes (τ_i) for N4-SBM as a function of 1,4-dioxane/methanol mixture composition with excitation at 293 nm and emission at 340 nm: curve 1, τ_1; curve 2, τ_2; curve 3, τ_3; curve 4, τ_4. (b, bottom) Corresponding preexperimental factors (A_i) as a function of 1,4-dioxane/methanol mixture composition: curve 1, $A_1 + A_2$; curve 2, A_3; curve 3, A_4.

excimers that form are present as "contact pairs" for which no significant segmental rotation is required to produce the necessary cofacial geometry. Curve 2 (Figure 5) is a combination of the two previously described cases for a mild selective solvent mixture where unimers and micelles with swollen cores coexist in equilibrium.

In mildly selective precipitants for polystyrene, micellar cores are swollen and a significant fraction of unimers coexist in equilibrium with micelles. Relatively fast rotational diffusion of pendant groups may be expected in partially collapsed unimer coils. The swelling of micellar cores by benzene in pure water (as described later) demonstrates that the buildup of excimer fluorescence may occur in swollen cores. Probes and excimers which are located close to the core/shell surface experience higher polarity, possibly lower microviscosity, and shorter decay

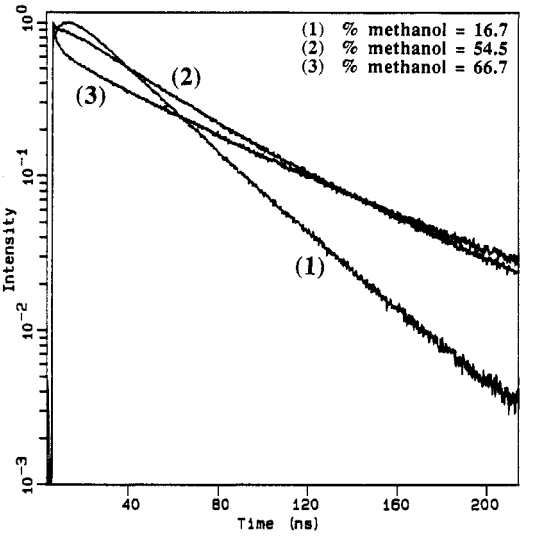


Figure 5. Time-resolved excimer fluorescence decays for N4-SBM in various 1,4-dioxane/methanol mixtures. Percentage of methanol: curve 1, 16.7%; curve 2, 54.5%; curve 3, 66.7%. Excitation at 293 nm, emission at 410 nm.

times than those trapped close to the center of micellar core.

The sudden changes in fluorescence properties with changing content of CH_3OH in the mild selective solvent region are in full agreement with the general understanding of micellar structure and micellar equilibrium.[1] Cores are formed by segments of insoluble blocks and are expected to be only slightly swollen by the solvent (1,4-dioxane in the present case) depending on the strength of the selective precipitant. In the case of polystyrene, the cores are nonpolar and glassy if no solvent is present (T_g for pure polystyrene is close to 100 °C).[16] In a glassy state, the mobility of polymer segments (and possibly side groups) is severely restricted. Micellization of block copolymers in selective solvents is an enthalpy-driven/entropy-controlled cooperative process,[17] and the properties of the insoluble blocks change sharply at the transition from the good solvent region to the selective region.

Hydrolyzed Sample N4-SMA in 1,4-Dioxane/Water Mixtures. Water is a very strong precipitant for polystyrene and a good solvent for polymethacrylic acid. Nevertheless, significant polyelectrolyte effects[18] and "specific interactions"[5] may occur in micellar shells which complicate micelle behavior. Buffer solutions were used to stabilize the behavior of the systems under study as most micellar properties were found to be more reproducible in buffers with moderately high ionic strength than in pure water. In this work we did not study changes in the polymethacrylic acid shell with pH, ionic strength, etc. This behavior is the subject of an ongoing investigation.[19]

All micellar solutions of water-soluble N4-SMA were prepared by direct dissolution in 1,4-dioxane/water (20 vol %), which avoids anomalous micellization (see later text). In this mixture, micelles with swollen cores are formed. Dialysis was performed in order to transfer micelles into mixtures progressively richer in water, and finally into pure water and aqueous buffers. During dialysis in solvents rich in 1,4-dioxane, both M_w^{app} and R_H of the micelles change. For water-rich solvents, only changes in R_H were detected, mainly due to a collapse of the micellar core as described elsewhere.[20] In mixtures with more than 40 vol % water, direct dissolution is impossible. Micelles which were prepared by dissolution in mixtures containing 30–40 vol % water gave less

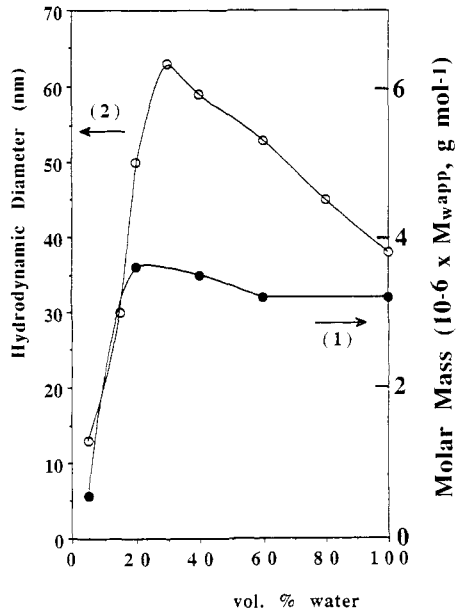


Figure 6. Apparent molar masses (M_w^{app}) (curve 1) and apparent hydrodynamic diameter ($2R_H$) (curve 2) for N4-SMA in 1,4-dioxane/water mixtures as functions of volume percentage of water.

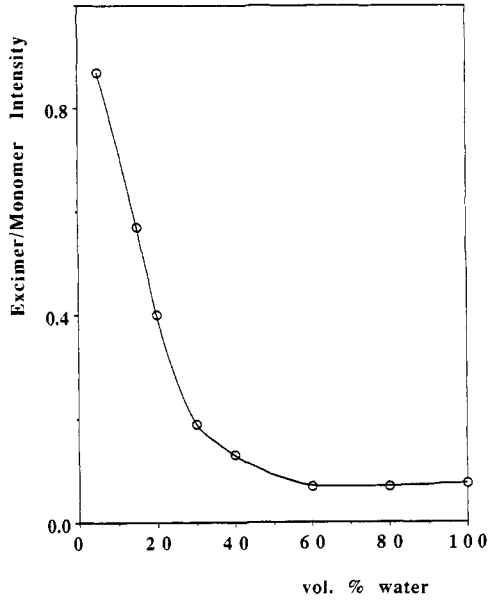
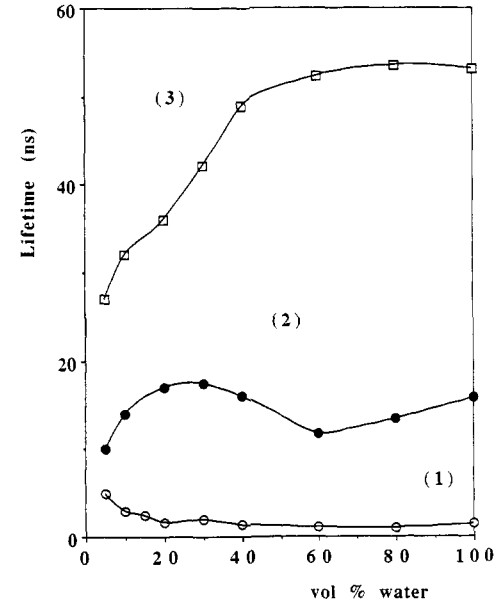


Figure 7. Excimer to monomer fluorescence intensity (I_{410}/I_{340}) for N4-SMA in 1,4-dioxane/water mixtures as a function of percentage of water. Excitation at 293 nm, excimer emission at 410 nm, monomer emission at 340 nm.



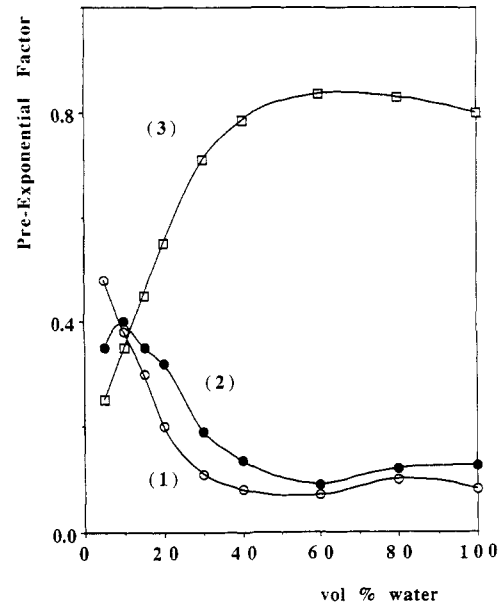


Figure 8. (a, top) Deconvolved monomer fluorescence decay lifetimes (τ_i) for N4-SMA as a function of 1,4-dioxane/water mixture composition with excitation at 293 nm and emission at 340 nm: curve 1, τ_1; curve 2, τ_2; curve 3, τ_3. (b, bottom) Corresponding preexponential factors (A_i) as a function of 1,4-dioxane/water mixture composition: curve 1, A_1; curve 2, A_2; curve 3, A_3.

reproducible results for R_H measurements than those obtained by dialysis from a 1,4-dioxane/water (20 vol %) mixture. In water-rich mixtures, the high density of insoluble blocks in the cores (with possible entanglements), together with the "specific interactions" of polymethacrylic acid in the shells, may hinder the equilibrium redistribution of individual polymer chains during micellar formation. It was shown earlier that the micellization equilibrium is "kinetically frozen".[20]

Characterization of micellar solutions was performed using light scattering. The M_w^{app} and $2R_H$ data are given in Figure 6 (curves 1 and 2, respectively). Micellization starts at 15 vol % water. In the narrow region close to 13 vol % water, anomalous micellization[1] along with the formation of a low-weight fraction of large particles was observed. It was found that once the large particles form, they survive in solution even after the solvent composition was changed by dialyzing into a water-rich solvent. This phenomena is avoided by starting in a water (20 vol %)/1,4-dioxane mixture.

The fluorescent properties of N4-SMA in mixtures with various water contents are generally similar to those of N4-SBM in organic mixtures described earlier. When Figure 7 is compared with Figure 2 (curve 1), which show the excimer to monomer emission of both samples, or Figure 8 with Figure 4, which show the time-resolved fluorescence characteristics, we note that there are obvious similarities. The primary difference is that the beginning of micellization occurs at a smaller volume percentage of the precipitant such that the excimer to monomer intensity, the lifetimes, and preexponential factors start to change significantly in the region of very low water content and vary over a broad region of solvent compositions. Presumably, this is due to the fact that water is a much

stronger precipitant of polystyrene than methanol. As the water content increases, the micellar cores gradually collapse in a relatively broad region of solvent compositions. The decreases in I_{410}/I_{340} (as well as in $2R_H$, Figure 6) with increasing content of water are quite gradual, which is surprising for such a strong precipitant as water. We believe that it is a result of the selective sorption of 1,4-dioxane into the cores and of the structural changes (hypercoiling) in polymethacrylic acid shells.[19] Refractive index increment values before and after dialysis[13] indicate that, in mixtures rich in 1,4-dioxane, considerable preferential sorption of the 1,4-dioxane into micellar cores takes place.

Small nonpolar molecules can be solubilized into the micellar cores of N4-SMA, and many types of fluorimetric studies could be employed for solubilization studies. The solubilized compound may be another fluorophore for energy-transfer studies, a quencher, or a "fluorescence-neutral" molecule that simply swells the core and indirectly changes the fluorescence properties of pendant fluorophores. Detailed solubilization studies using benzene as an absorbate will be the subject of a separate publication.[19]

Summary

Fluorescence measurements combined with light scattering and ultracentrifugation have been used to study micelle formation in polystyrene-*block*-poly(*tert*-butyl methacrylate) and polystyrene-*block*-poly(methacrylic acid) using methanol and water, respectively, as a precipitant from 1,4-dioxane solutions. These polymers have been end-tagged with an average of four 2-vinylnaphthalene groups. Steady-state and time-resolved fluorimetry in particular have been found to be very sensitive experimental techniques for studying micellization and micellar properties of labeled copolymers. The glassy state of polystyrene cores prevents rotational diffusion which is required for excimer formation from neighbor-pendant naphthalene groups, and only a very weak emission from a low fraction of "contact pairs" is observed. While these observations are consistent with a glassy polystyrene core, the retention of traces of 1,4-dioxane in the core cannot be ruled out.

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