Fluorescence Studies of Amphiphilic Poly(methacrylic acid)-*block*-Polystyrene-*block*-Poly(methacrylic acid) Micelles

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Received March 1, 1991; Revised Manuscript Received July 8, 1991

ABSTRACT: A-B-A block copolymers (A = poly(methacrylic acid), B = polystyrene) have been prepared by anionic polymerization. These amphiphilic copolymers can form stable micelles in water/1,4-dioxane mixtures as well as in water or an aqueous buffer. These micelles are presumed to have a polystyrene core and poly(methacrylic acid) shell. The ability of the micelles to solubilize and release hydrophobic species was studied by fluorescence methods, primarily using pyrene as a fluorescence probe. The following processes were studied: (1) the effect of pyrene loading on monomer/excimer fluorescence ratio and quenching by Cu^{2+}; (2) the rate of exchange between micelles containing pyrene and other aromatic species by the time dependence of either their monomer/excimer ratio or sensitized fluorescence after mixing micelles; (3) the time dependence of the fluorescence quenching of pyrene following the addition of small molecules (*N*,*N*-dimethylaniline, CCl_4). The following conclusions were obtained: (1) a significant fraction (ca. 20–30%) of the pyrene molecules were on or near the polystyrene–water interface (this depends on loading); (2) diffusion of the probe out of the micelle is the rate-determining step in the release and exchange of large hydrophobes. This process is very slow in its later phases and probably represents slow diffusion from the core of the polystyrene region of the micelle.

Introduction

In a companion paper[1] it was demonstrated that block copolymers of styrene and methacrylic acid can form stable multimolecular micelles in aqueous media with well-defined aggregation numbers and shapes (as measured by static and dynamic light scattering). These micelles can solubilize various materials and release them into the aqueous environment. It has been demonstrated by Winnik et al. that fluorescence methods can give insight into the morphology of complex polymeric particles as well as provide a direct measure of exchange phenomena.[2] In this paper we report fluorescence studies of micelles prepared from a block copolymer of styrene and methacrylic acid. The primary fluorescence probe we use is pyrene, although some other fluorescence species are also examined to exploit the phenomenon of Förster energy transfer. Fluorescence quenching studies were carried out to establish the rate of incorporation of small molecules into the portion of the micelle that contains the pyrene probe. As might be expected, the rate at which a quencher acts on the embedded pyrene depends drastically on its molecular size and its solubility in the polystyrene core.

While our studies of A-B and A-B-A block copolymer (where A is poly(methacrylic acid) and B is polystyrene) micelles in aqueous environments are just beginning, certain conclusions have emerged: (1) these micelles are very stable and hydrophobic molecules are well solubilized in them and very slowly released into the aqueous phase; (2) the amount of a hydroprobe that can be loaded into the micelle depends on the details of the preparation as does the accessibility of the probe to quenchers and the rate of exchange of the probe between micelles.

Experimental Section

Copolymer Sample. The details of the sample preparation and both molecular and micellar characterization are reported elsewhere.[1,3] We repeat just the most important facts: The triblock copolymer used in this study is a poly(methacrylic acid)-*block*-polystyrene-*block*-poly(methacrylic acid), with a weight-average molar mass M_w of 16.5×10^3 g mol^{-1} and polydispersity index $M_w/M_n = 1.2$. The sample forms micelles with polystyrene cores and poly(methacrylic acid) shells spontaneously in a H_2O/40 vol % 1,4-dioxane mixture ($M_w^{micelle} = 5.2 \times 10^6$ g mol^{-1}, hydrodynamic radius R_H = 25 nm). When the sample is transferred into aqueous environments (free of 1,4-dioxane) by means of a dialysis procedure, M_w does not change within experimental error; i.e., the association number (the number of the copolymer molecules forming a micelle) remains at approximately 315. Hydrodynamic radii in water, phosphate buffer (pH = 7), and water with 0.1 M LiCl were 30, 19, and 18 nm, respectively.

Two methods were used to load the pyrene probe into these micelles. In method A the pyrene was dissolved in the initial water/1,4-dioxane mixture and was incorporated during the dialysis. In method B the aqueous micellar solution was prepared by dialysis, and while remaining in the dialysis tubing, it was exposed to an exterior solution of pyrene-saturated water and equilibrated for 3–5 days. Not surprisingly, the amount of pyrene that can be incorporated with these two methods is different, with method A producing a maximum loading of ca. 1.88×10^{-5} M in a solution of ca. 0.12 mg of micelle/mL of water, which is ca. 3 times more than the maximum loading with method B.[4] On the other hand, the pyrene incorporated by method B appears to be more tightly bound and less accessible to quenchers that are confined to the aqueous environment, as will be discussed in the Results section.

All fluorescence probes were obtained from commercial sources and purified as follows: (1) pyrene (Kodak, recrystallized 2 times from ethanol), (2) dimethylaniline (Aldrich, used as received, (3) 9,10-diphenylanthracene (Aldrich, recrystallized 2 times from benzene), (4) *N*-propyl-4-bromo-1,8-naphthalimide[5] (purified by liquid chromatography).

Optical Methods. Two steady-state fluorimeters were used. For higher resolution excitation or emission spectra we used a Spex Fluorolog 2 system. For the dynamic fluorescence measurements a Tracor Northern 6100 series diode array system was used. For some experiments a home-built stopped-flow apparatus was employed. Both instruments and the stopped-flow system have been described in an earlier publication.[6] However, for most exchange or quenching experiments the rate of change was extremely slow (days), which necessitated the use of a shutter system controlled by the triggers available from the Tracor control unit. This system permitted a brief exposure of the solution to excitation light while the fluorescence spectrum was obtained by the diode array system (ca. 0.5 s). The shutter then closed for

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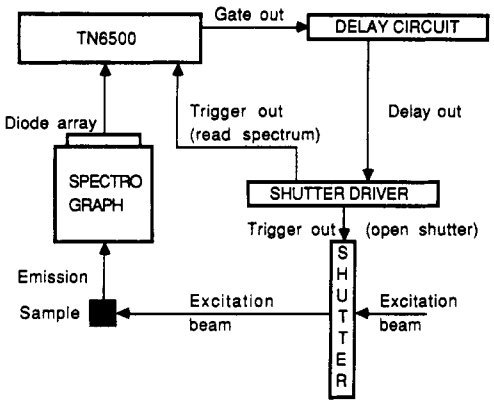
Scheme I



a period of time from a few seconds to 10 min until the cycle was repeated (see Scheme I). Thus an array of fluorescence spectra was obtained. The intensity of fluorescence over a range of wavelengths assigned to a particular species (pyrene, exciplex, etc.) could be summed and the intensity or ratio of intensities plotted to provide kinetic data.

All absorption spectra were obtained using a Hewlett-Packard 8413 diode array UV–vis spectrophotometer.

Results

(a) Partition Coefficient of Hydroprobes in PS–MA Micelles. One of the most fundamental properties of these systems is the partitioning of a hydrophobic probe molecule between the bulk water and the micelle. This can be characterized by means of a partition coefficient ($K_{M/W}$), as discussed in the following. While most of our fluorescence experiments center on pyrene, we also use other hydrophobic probes and hence determined $K_{M/W}$ for these species as well. The method was very simple. A cell was constructed in which the two halves were separated by a dialysis membrane like that used in the preparation of the micelles. A known volume of a solution of micelles loaded with probe was placed on one side of the cell and pure water on the other. Alternatively a saturated aqueous solution of the probe was exposed to a micellar solution. For pyrene, absorption spectroscopy was used to determine the pyrene concentration on both sides. For materials with lower inherent water solubility (e.g., diphenylanthracene or anthracene), a calibration curve based on fluorescence was constructed. After equilibration and measurement of the probe concentration on both sides of the cell, fresh water could be added and the system reequilibrated. This permitted a correction for the amount of probe that was absorbed onto the dialysis material, but this correction was found to be negligible. Once the concentration of the probe was known for each side of the cell, the dimensionless partition coefficient was calculated in terms of moles (m) of probe per gram of micelle as follows:

$$K_{M/W} = (m_{mic}/W_{mic})/(m_{aq}/W_{aq}) = (V_{mic}/V_{aq})(W_{aq}/W_{mic})\{([P]_{mic} - [P]_{aq})/[P]_{aq}\} \quad (1)$$

where V denotes the volume of the micelle or solvent side of the cell, [P] is the molar concentration of the hydrophobe, and W is the mass of water or micelle. The excess concentration of probe in the micelle side of the cell, given by $[P]_{mic} - [P]_{aq}$, is ascribed to solubilization by the micelle. There is no evidence to suggest that the micelles can penetrate the dialysis material. If so, the value of $[P]_{aq}$ would be overestimated and the value of $K_{M/W}$ would be underestimated. For pyrene and diphenylanthracene $K_{M/W}$ was determined to be 2.2 (±0.2) × 10^5 and 1.1 (±0.2) × 10^5, respectively. However, in the typical solution of micelles, in which there are ca. 0.12 mg of micelles per mL of water, approximately 4% of the pyrene is in the bulk water phase because of the very large excess of water.

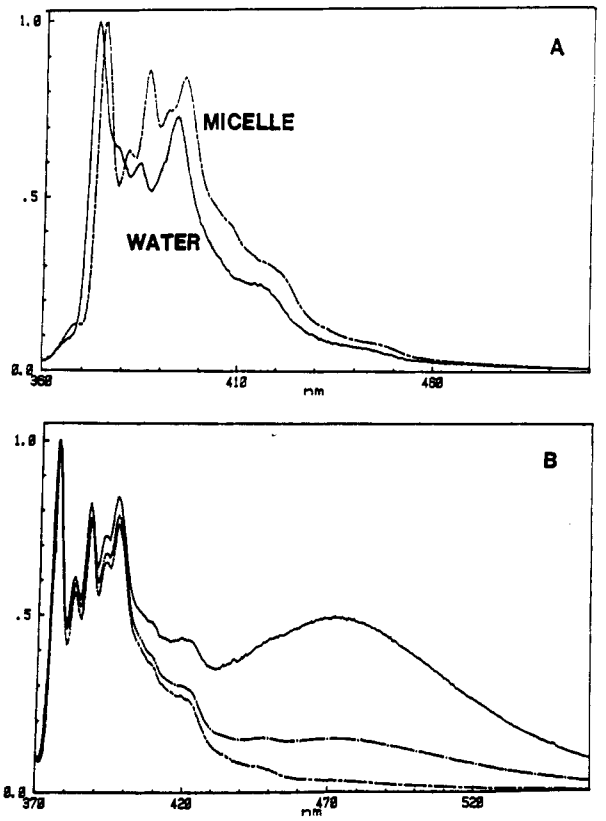


Figure 1. (A) Comparison of fluoresence of pyrene in H_2O (solid line) and solubilized in H_2O by PS–MA micelles (broken line); scaled to equal to intensity for ease of comparison (note change of wavelength axis). (B) Fluorescence of pyrene in PS–MA micelles (preparation method A) as a function of pyrene loading (concentration of pyrene = 0.031, 0.019, and 0.0080 g of pyrene/g of micelle with 0.162 mg of micelle/mL of H_2O).

(b) Fluorescence Spectroscopy of Pyrene in Micelles as a Function of Loading and Preparation. Pyrene is a widely used fluorescence probe because its vibrational structure is sensitive to polarity[7] and it produces a distinct excimer fluorescence under conditions of sufficiently high concentration and mobility.[8] In the present case, the fluorescence is characteristic for pyrene in a nonpolar environment for all loadings and preparation conditions (see Figure 1a) and the "I_1/I_3 ratio" is similar to that for pyrene in a polystyrene (PS) film. Thus on this basis it seems that essentially all the pyrene is residing in the PS micellar core. It is difficult to estimate exactly how much pyrene might be associated with the poly(methacrylic acid) (PMA) shell. On the basis of the I_1/I_3 ratio alone, we estimate an upper limit of ca. 10%. We note that if the pH of the solution is raised from 5.6 to 11 by the addition of NaOH 5–18% of the pyrene is lost, depending on the loading (more is lost for higher loading). For equivalent loadings slightly more was lost for preparation method A than for preparation method B. Thomas et al.[9] have shown that pyrene solubilized in poly(methacrylic acid) is excluded from the polymer at higher pH. Thus this experiment provides some insight as to the fraction of pyrene that resides in or near the PMA shell (also see Cu^{2+} quenching results discussed later).

Our I_1/I_3 results are similar to those reported by Dowling and Thomas[10] for pyrene solubilized by an acrylamide–styrene copolymer that has block character by virtue of

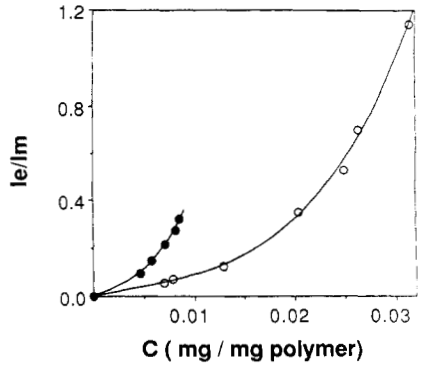


Figure 2. Pyrene excimer to monomer ratio (I_e/I_m) as a function of pyrene concentration in PS–MA solution for preparation A (open circles) and B (filled circles).

the micelle polymerization method used in its preparation. They ascribe this to the compartmentalization of the pyrene in the polystyrene region of this polymer, similar to our supposition. We note that Dowling and Thomas can achieve ca. 5×10^{-6} mmol of pyrene per mg of their polymer (1.0×10^{-3} mg/mg) while we attain ca. 1.6×10^{-4} mmol of pyrene per mg of polymer (3.2×10^{-2} mg/mg). However, this difference is primarily because of the much larger polystyrene content of our copolymer. The mole ratio of pyrene to styrene at maximum loading is ca. 1.7×10^{-2} and 2.5×10^{-2} for the Dowling and Thomas polymer and our micelles, respectively. Dowling and Thomas do not report any excimer fluorescence and their highest loading, which implies that the pyrene moieties are well separated from each other. This is in contrast to our micelles, as described next.

As the loading of the pyrene is increased, the magnitude of the excimer fluorescence increases (see Figure 1B). This effect is demonstrated by the plot of the relative excimer/monomer intensities vs. the overall pyrene concentration in the solution (as described above, the majority of pyrene resides in the micelles) in Figure 2 for preparation methods A and B. Since method A achieves a much higher loading than method B, the maximum value of the ratio of excimer to monomer fluorescence (I_e/I_m) is much larger in the former case. However, for equivalent loadings I_e/I_m is higher for method B. This can be rationalized if the pyrene moieties are localized in the outer shell of the PS core in the method B preparation. As can be seen in Figure 2, the [Py] dependence of I_e/I_m is not linear and in fact can be fitted quite well to a quadratic dependence. This is expected since a minimal loading of the micelle is required before one would expect a significant probability of two pyrene molecules being within an interaction distance of 3–5 Å, as needed for excimer formation.[8] If there is significant diffusion of pyrene within the PS core, then the average pyrene separation can be larger than this value. Preliminary fluorescence lifetime studies demonstrated minimal buildup time for the pyrene excimer, implying that pyrene pairs are directly excited and that little pyrene diffusion occurs during the ca. 200-ns excited-state lifetime.

(c) Rate of Pyrene Exchange from the Time Dependence of I_e/I_m. Copolymer micelles containing pyrene are mixed with micelles without pyrene and as the pyrene molecules are exchanged the I_e/I_m ratio decreases. The time scale of this ratio change reflects the rate at which this exchange occurs. Data of this type are presented in Figure 3 for preparations A and B (note that the initial I_e/I_m values are quite different, reflecting the different pyrene loadings for these two preparation methods). The absolute value of I_e/I_m is arbitrary since it depends on the

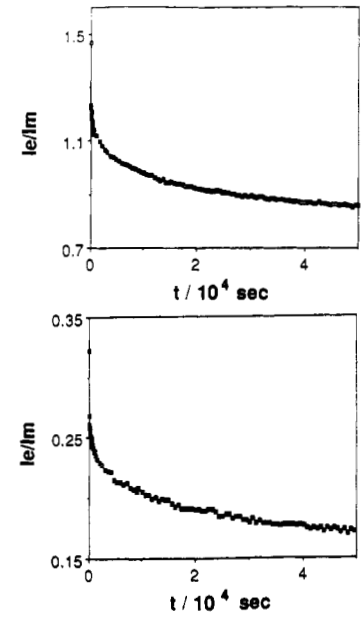


Figure 3. I_e/I_m ratio as a function of time after mixing pyrene-loaded micelle with pyrene-free micelles. Upper panel, A preparation; lower panel, B preparation.

specific wavelength range assigned to these two features (see Experimental Section). There is a relatively rapid initial change in I_e/I_m during the first 10–20 min, followed by a very slow equilibration that is not complete in a day. There is no qualitative difference in the exchange dynamics between these two preparations, implying that the pyrene moieties are held equally strongly for both types of micelles. The kinetics of this exchange process were essentially unchanged if the solutions were slowly stirred,[11] demonstrating that the rate of micelle–micelle collision is not rate determining. Thus it seems likely that the rate-determining step is pyrene diffusion within the PS core and that this is not significantly different for these two preparations nor is the diffusion a strong function of pyrene loading.

(d) Rate of Pyrene–Aromatic Exchange from the Time Dependence of Sensitized Fluorescence. Micelles can be prepared containing a variety of hydrophobic species, including molecules that can be sensitized by pyrene via the Förster dipole–dipole mechanism.[12] In this case the rate of donor to acceptor energy transfer is given by

$$k_{DA}(R) = (1/\tau_D)(R_0^{DA}/R)^6 \qquad (2)$$

where τ_D is the normal fluorescence lifetime of the donor, R is the separation of the donor and acceptor, and R_0^{DA} is a parameter that depends on the photophysical properties of the donor–acceptor pair, including the overlap of the donor emission with acceptor absorption. The R_0^{DA} value for pyrene (Py)–diphenylanthracene (DPA) is 2.89 nm,[13] and for *N*-propyl-4-bromo-1,8-naphthalimide (BNPI), R_0^{DA} is estimated to be 5.8 nm.[14]

This kind of experiment has been carried out exactly like the previous set, i.e., micelles containing pyrene and an acceptor are mixed and we observe the increase of acceptor fluorescence. While there is some direct excitation of acceptor at 342 nm (i.e., at λ_{max} for the pyrene), this occurs from the moment the micelles are mixed, and the steady increase of the acceptor fluorescence (plotted as I_A/I_{Py} in Figure 4) arises from sensitized fluorescence. The full spectra for BNPI–Py at different times following mixing are presented in Figure 5. We note that the spectral separation of the Py fluorescence from that of BNPI is

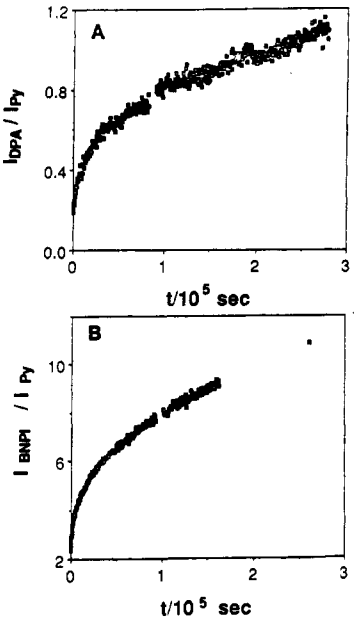


Figure 4. Ratio of I_A/I_{Py} vs time for acceptor indicated: (A) acceptor = diphenylanthracene; (B) acceptor = BNPI.

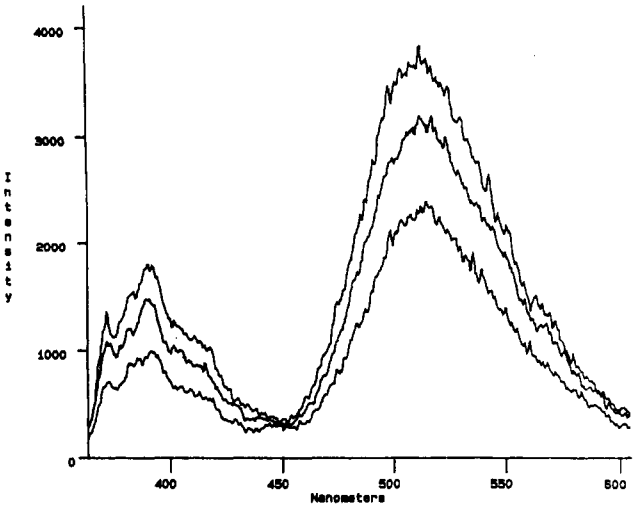


Figure 5. Steady-state fluorescence spectra at 47, 5020, and 56 289 s after mixing BNPI–micelle with pyrene–micelle.

much more favorable than the Py–DPA case but this does not significantly degrade the quality of the kinetic data.

Qualitatively the time dependence of the I_A/I_{Py} curve is like that of the I_e/I_m curve, with a rapid change for the first hour, followed by a slower approach to equilibrium that requires more than 1 week. Upon closer comparison of the I_A/I_{Py} and I_e/I_m curves, it is seen that there are distinct differences in the shape, with the former having a more pronounced curvature at long times. We have not attempted to analyze these kinetic curves based on a specific diffusion and exchange model as yet, but we speculate that these differences arise in part from the fact that sensitized fluorescence can occur over relatively long range (ca. R_0^{DA}) while excimer formation is a short-range phenomenon (ca. 3 Å).

(e) Fluorescence Quenching Studies of Pyrene in Micelles. The objective of these studies is to learn where the pyrene molecules are located and how rapidly small molecules can penetrate the micelle. One must always be concerned that the quencher can modify the micelle itself.

In the first set of experiments $CuSO_4$ was used as a quencher since the Cu^{2+} ion cannot penetrate a PS core and hence is expected to quench only those pyrene

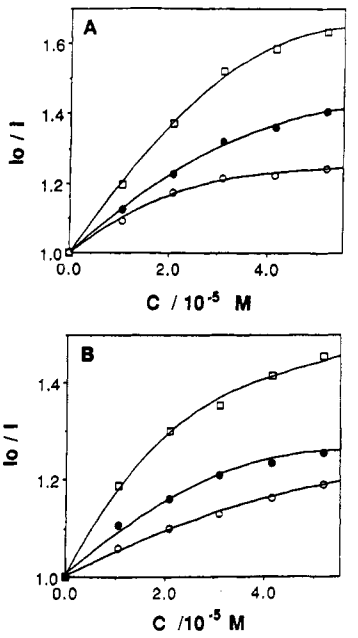


Figure 6. Plot of I_0/I vs $[Cu^{2+}]$ for pyrene–micelles: (A) as a function of pyrene loading by method A (□, ●, ○ correspond to 0.032, 0.0064, and 0.0027 g of pyrene/g of micelle) and (B) prepared by method A (□) and B (●) with equal pyrene loading (0.0073 g/g) at pH 5.6. The latter polymer at pH 8.4 (○) is also shown.

molecules located in the methacrylic acid shell or at the PS core–H_2O interface. It is anticipated that a certain fraction of pyrenes will be inaccessible to the Cu^{2+}, as is reflected in the saturation behavior of the I_0/I curves in Figure 6 (I_0/I represents the ratio of unquenched to quenched fluorescence). As can be seen from Figure 6A, the value of I_0/I at the saturation limit decreases steadily as the loading of pyrene decreases for the micelles prepared by method A. This implies that at higher loadings a larger fraction of the pyrene molecules are in contact with the aqueous phase. Two micelle samples were prepared by method A and B respectively in such a way that the pyrene content was essentially identical (this requires considerable trial and error). The I_0/I curve in Figure 6B demonstrates that the fractional quenching at saturation was higher for method A than for method B (32% and 23% of available pyrene, respectively). While this is not a huge difference, it is surprising to us that pyrene molecules that are solubilized after the micelles are formed (method B) are more isolated from the aqueous environment that those incorporated during the micelle formation (method A). We also note that these Cu^{2+} quenching results agree with the loss of pyrene upon raising the pH of these solutions (see Results section b). Thus for the highest loading of pyrene using method A preparation (1.9×10^{-5} M) we conclude that ca. 18% of the pyrenes are intimately associated with the MA shell and ca. 32% of the pyrenes are in at least partial contact with the aqueous phase.

N,*N*-Dimethylaniline (DMA) will interact with the excited state of many aromatic chromophores either to produce an exciplex fluorescence in nonpolar media or simply to quench the excited singlet.[15] DMA is sparingly soluble in water (9.57×10^{-3} M at saturation at ca. 25 °C) but is expected to be preferentially absorbed in the PS core of the micelle. When 100 μL of a saturated aqueous solution was added to 2 mL of pyrene-loaded micelle solution (0.12 mg/mL), the pyrene fluorescence was quenched and the DMA–pyrene exciplex fluorescence increased. This is illustrated by the steady-state spectra (Figure 7) and by the kinetic plot of the ratio of the ex-

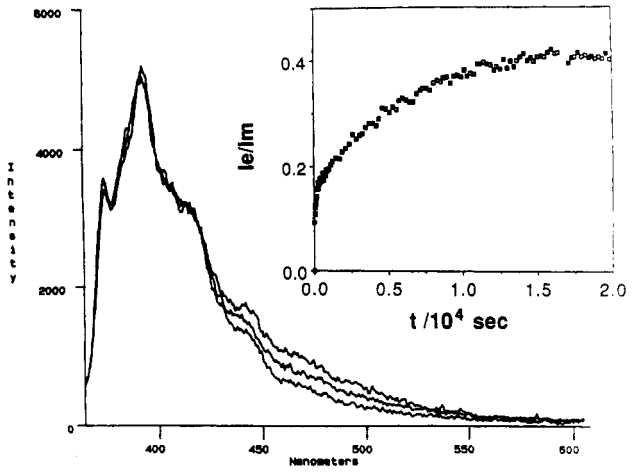


Figure 7. Steady-state spectrum of pyrene solubilized in PS–MA micelle 17.6, 1924, and 14 410 s after addition of DMA. Insert: Plot of exciplex to monomer as a function of time.

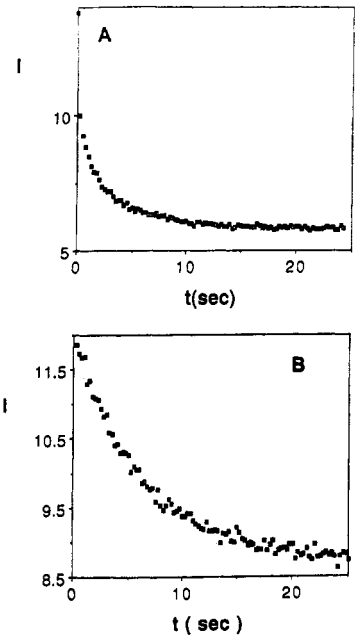


Figure 8. (A) I/I_0 vs time after addition of CCl_4-saturated H_2O to pyrene–micelle solution. (B) Decrease of pyrene–micelle fluorescence during exposure to 342-nm light.

ciplex to pyrene fluorescence (I_{ex}/I_{Py}) (insert in Figure 7). This plot is very similar to those presented earlier (e.g., Figures 3 and 4) but the time required to equilibrate is considerably shorter. This is expected in part because the DMA is initially in the bulk solution and does not have to diffuse out of one micelle before entering another. The DMA molecule may also soften the PS core,[16] which in turn will enhance the diffusion constant. It is significant that we observe exciplex fluorescence, since this can arise only from the pyrene–DMA pairs that reside in the PS core. This does not imply that all pyrene–DMA encounters are in the core since some pyrene quenching could be occurring in an environment exposed to the aqueous phase, as was the case for Cu^{2+} quenching.

Finally, CCl_4 was employed as a quencher using the stopped-flow apparatus. Equal volumes of a saturated H_2O–CCl_4 solution and a pyrene-loaded micelle solution were mixed. The final CCl_4 concentration after mixing was 2.5×10^{-3} M, which was much higher than any other quencher studied. In this case the quenching was very rapid, equilibrating within 10–15 s (Figure 8A). It is not surprising that a small molecule like CCl_4 can penetrate the PS core so rapidly, as well as soften it, similar to the effect postulated for DMA. However, part of this quenching is the result of the diminution of pyrene fluorescence that occurs under continuous exposure to 342-nm light even in the absence of quencher (Figure 8B). Note that this was not a problem in the experiments described in the foregoing studies because the exposure to excitation was never longer than ca. 0.5 s per data point. This "photobleaching" of fluorescence is not accompanied by a loss of absorbance in the solution; it occurs equally in both aerated and nitrogen-purged solutions, and it is reversed when the solution sits in the dark. We suggest that photoexcitation stimulates the release of pyrene into the aqueous phase, where the fluorescence is known to be much lower.[17] In the dark the pyrene is reabsorbed. Presumably this is the result of local heating near the pyrene that results from the deactivation of excited pyrene molecules by radiationless transitions. This will be the subject of future experimental work.

Discussion

Using fluorescence techniques primarily with pyrene as a fluorescence probe, we have examined the solubilization and release properties of polymer micelles formed from triblock poly(methacrylic acid)-*block*-polystyrene-*block*-poly(methacrylic acid) in aqueous solution. The expected structure of these micelles consists of a relatively compact polystyrene core with a more swollen poly(methacrylic acid) shell. The micelles are very effective in solubilizing hydrophobic aromatics,[18] with a partition coefficient on the order of 10^5 with respect to water.

It is known that poly(methacrylic acid) can solubilize hydrophobic species.[9] We find from the effect of pH and the accessibility to Cu^{2+} ions that at the highest pyrene loading as much as 18% of the pyrene molecules may reside in the polyacid shell layer at pH 5.6 and ca. 30% are exposed to the aqueous phase. For equivalent pyrene loadings the values depend slightly on the method used to introduce the pyrene into the micelle.

The rate of pyrene intermicellar exchange was monitored by the change in excimer fluorescence when pyrene-loaded micelles were mixed with pyrene-free micelles. Similar experiments were conducted using Förster energy transfer. Qualitatively the results are similar: a relatively rapid change in either monomer/excimer or donor/acceptor fluorescence ratio occurs at early times, with a much slower approach to equilibrium at longer times (on the order of a week). This process is rate-limited by diffusion of the probe into and out of the micelles, since stirring the solution did not change the time dependence. We have not attempted to model these time-dependent curves as yet, but we presume that they can be understood in the context of standard diffusion–adsorption theory. This will be the subject of later research.

Smaller molecules such as *N*,*N*-dimethylaniline (DMA) or CCl_4 are sparingly soluble in water but can be solubilized by the micelle. These species penetrate the micelle quickly and can quench the pyrene fluorescence and/or produce exciplex fluorescence on a time scale 10 to 10^5 faster than the time scale of the large-molecule exchange. This can be understood qualitatively as the result of two simultaneous effects: (1) the quencher is in the bulk aqueous phase and hence no diffusion out of a micelle is required; (2) these quenchers may "plasticize" the PS core and hence further enhance the rate of quenching via more rapid diffusion.

Finally we observed an unexpected phenomenon of reversible photobleaching of the pyrene probe. We suggest

that this could be a manifestation of reversible "photorelease" of the pyrene into the aqueous environment. This could point toward some additional applications of these materials and will be the subject of continuing research.

Acknowledgment. We acknowledge the financial support of the Department of the Army (Grant No. DAAA LO3-90-G-0147). S.E.W. thanks the Robert A. Welch Foundation for partial support of this work (Grant F-356).

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Registry No. (PS)(MA) (block copolymer), 124916-37-6; BNPI, 100865-05-2; DMA, 121-69-7; CCl_4, 56-23-5; $CuSO_4$, 7758-98-7; 9,10-diphenylanthracene, 1499-10-1; pyrene, 129-00-0.