Release of Hydrophobic Compounds from Micellar Solutions of Hydrophobically Modified Polyelectrolytes

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The kinetics of release of the hydrophobic probes, pyrene and estradiol, from micellar aggregates of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(poly(ethylene oxide))-*g*-poly(acrylic acid) (Pluronic−PAA), have been studied. Release of the probes is bimodal with a fast and a slow component. Diffusion coefficients of 10^{-15}−10^{-14} cm^2/s characterize the slow mode of diffusion from the core of the micellar aggregates. The core of the aggregates consists mainly of poly(propylene oxide) (PPO) and possesses different properties from those of bulk PPO, with there being a higher activation energy for diffusion of pyrene and dramatic changes in the polarity of the microenvironment of the probe. The temperature-dependent fine structure of the pyrene spectra (I_3/I_1) in Pluronic−PAA above the critical micellization temperature (cmt) can be explained by a redistribution of pyrene from hydrophobic PPO domains into micelles.

Introduction

Amphiphilic block copolymers that spontaneously form micelles and other ordered structures ("self-assemble") are emerging as a distinct branch of colloid and polymer chemistry[1] that may eventually prove to have applications in areas such as drug delivery,[2−6] nanotechnology of metals, minerals, and semiconductors,[7−9] separation processes, and pollution control.[10−12] The most significant feature of the polymeric micelles is their capability of solubilizing compounds that are otherwise only marginally soluble in water.[1,13] Micelle-forming polymers usually comprise block or graft copolymers composing sequences of hydrophilic (most commonly a polyelectrolyte) and hydrophobic (polystyrene, poly(alkyl acrylate), etc.) segments.[1,14,15] With such polymers, it is possible to design a system that does not precipitate out of solution due to macroscopic aggregation, but is instead rather stable, while containing a large number of distinct microscopic domains. These stable microscopically separated micelle-like domains usually possess hydrophobic cores and highly hydrated coronas, analogously to micelles in some common surfactant solutions.[16] The dynamics of exchange between such polymeric micelles and the bulk solution is much slower than in their low molecular weight counterparts.[17]

Recently, we have introduced a novel class of micelle-forming graft-comb copolymers whereby poly(acrylic acid) (PAA) segments are grafted onto Pluronic poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO−PPO−PEO) block copolymers via C−C bonding.[2,3,18−32] These copolymers (Pluronic−PAA) combine the temperature sensitivity of Pluronics with the polyelectrolyte properties of PAA. Above certain temperatures, massive formation of micelle-like aggregates has been observed in aqueous solutions of Pluronic−PAA, using a

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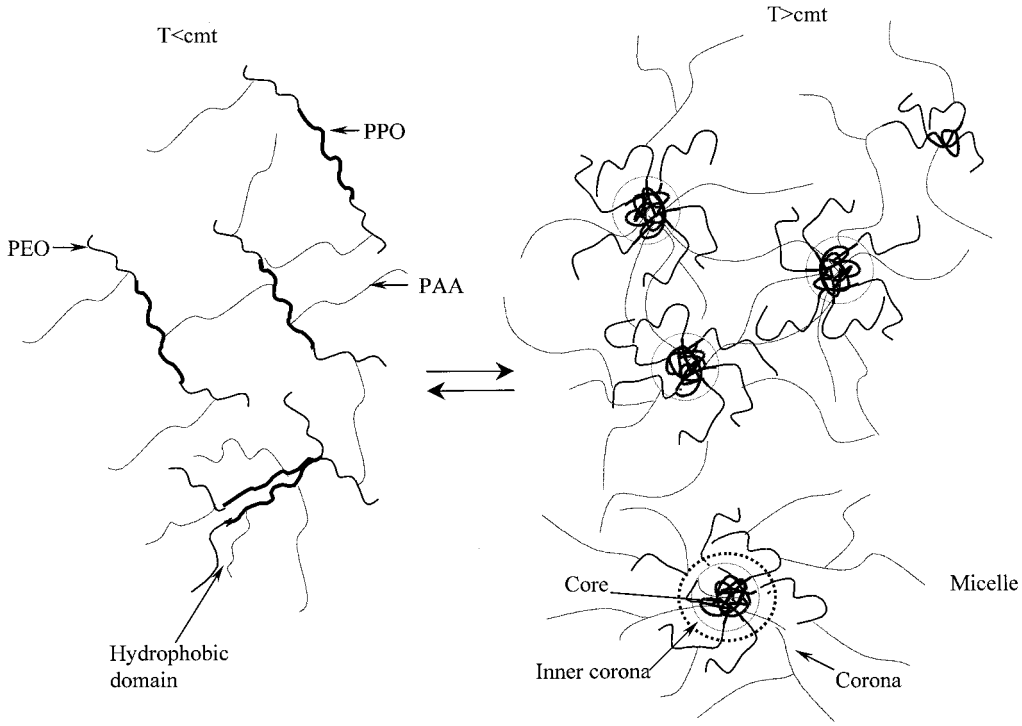

Figure 1. Scheme of self-assembly in aqueous solutions of Pluronic−PAA.

variety of methods such as light scattering,[2,3] NMR,[27] SEC,[25] SANS,[29] and DSC.[25] In these aggregates, the collapsed and substantially dehydrated chains of PPO form a spherical core, the adjacent PEO segments form a more hydrated layer around this core, and ionized PAA chains constitute an expanded corona.[29,30] SANS data indicate that the micelle-like aggregates are uniformly spaced.[32] The stability of the micellar aggregates at elevated temperatures is evidenced from the stability of gels where the aggregates serve as physical cross-links.[25] The complex structure of the aggregates in Pluronic−PAA solutions bears some resemblance to the three-layered "onion" micelles from poly(2-vinylpyridine)-*b*-poly(ethylene oxide).[33] The cores may belong to one (intramolecular aggregation) or several (intermolecular aggregation) Pluronic−PAA chains. Intramolecular aggregation is a pertinent feature of Pluronic−PAA below the well-defined critical micellization temperature (cmt). The small hydrophobic domains present in such solutions are capable of solubilization of pyrene below the cmt.[30] Above the cmt, intermolecular associations become dominant and lead to the formation of micelles with hydrophobic domains that are larger than those found at lower temperatures.[3,29] The temperature-driven self-assembly of Pluronic−PAA is schematically depicted in Figure 1.

Depending on their degree of hydrophobicity, small molecules such as estradiol can be solubilized into the micellar cores and in the regions bordering the hydrophilic corona of Pluronic−PAA.[30,32] This combined with its bioadhesive[2,21] and gel-forming properties[23] makes Pluronic−PAA an attractive candidate for use as a vehicle for the controlled release of pharmaceutically active proteins and drugs.[2,3] In previously reported studies of the kinetics of in vitro release of steroid hormones from Pluronic−PAA formulations, it was proposed that the rate of release is essentially bimodal.[19] The fast mode is associated with those hormone molecules that are entrapped in the expanded PAA corona of the Pluronic−PAA without solubilization into micelles. This mode of discharge of the hormone from the gel phase into an aqueous environment (which occurs via hydrodynamic erosion of the gelled structures) is strongly dependent on the mass transfer of the gel components, and thus macroviscosity of the Pluronic−PAA solution or gel. The second, much slower mode is associated with diffusion of molecules from the hydrophobic portions of the Pluronic−PAA aggregates. In support of this bimodal diffusion model, in vivo experiments (vaginal administration in sheep) demonstrated that a significantly longer time was required to obtain the maximum cumulative concentration of estradiol in blood when the concentration of Pluronic−PAA in the formulation was increased.[34,35] Since the hormone dose in these experiments was kept constant, the results show that increasing the fraction of the hormone in the Pluronic−PAA aggregates slows the overall rate of release. The present study was performed with the aim of elucidating the rate and mechanism of the release (slow mode) of some hydrophobic probes from the Pluronic−PAA solutions. Steady-state fluorescence methods were used to measure diffusion coefficients and to probe the microenvironment in the hydrophobic regions of Pluronic−PAA.

Experimental Section

Materials. Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(poly(ethylene oxide))-*g*-poly(acrylic acid) (CAS Reg. No. 186810-81-1) was synthesized by dispersion/emulsion polymerization of acrylic acid along with simultaneous grafting of poly(acrylic acid) onto the Pluronic backbone as described in detail elsewhere.[26] The ensuing copolymer is referred to as Pluronic−PAA. The backbone polymer, Pluronic F127 NF, was obtained from BASF Corp. (Parsippany, NJ) (molecular formula $EO_{100}PO_{65}EO_{100}$,

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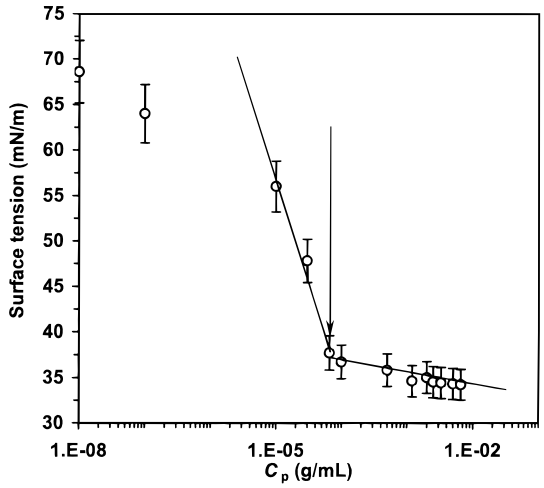

Figure 2. Surface tension as a function of Pluronic−PAA concentration (C_p) at pH 7.0 and 37 °C. Arrow indicates critical micelle concentration (cmc).

nominal molecular weight 12 600, molecular weight of PPO segment 3780 Da, 70 wt % EO, and cloud point in 1% aqueous solutions above 100 °C). The Pluronic−PAA was fractionated in dry tetrahydrofuran by semipreparative size-exclusion chromatography (SEC) as previously described.[24] The polymer consisted of 45% Pluronic and 55% poly(acrylic acid) as measured by FTIR and NMR.[26] The weight-average molecular mass and polydispersity indexes of the fraction used in this work were 3.5×10^6 Da and 3.2, respectively.

Pyrene (99%) was obtained from Aldrich Chemical Co. and was repeatedly recrystallized from absolute ethanol following sublimation. Poly(propylene oxide) (PPO) (average M_n ca. 3500, functionality ca. 1.7, hydroxyl number 25 mg of KOH/g), poly(acrylic acid) (PAA) (average M_w 2000), and poly(ethylene oxide) (PEO) (average M_n ca. 400) were obtained from Aldrich and used without further treatment. Steroid hormone β-estradiol (98%) was obtained from Sigma and used as received. All other chemicals, gases, and organic solvents of the highest purity available were obtained from commercial sources.

Procedures. To prepare solutions of Pluronic−PAA, polymer samples were dispersed in distilled water and gently stirred at 4 °C for 48 h. The pH was adjusted to 7.4 ± 0.1 with 5 M NaOH as needed. The solutions were filtered through Acrodisc nylon filters (Gelman Sciences) with pore diameters of 0.8 μm, deoxygenated by nitrogen flow, and stored at 4 °C. The polymer concentration was controlled to within ±0.005%. Formation of micellar aggregates was monitored by measurement of the surface tension of the copolymer solutions using the Wilhelmy plate method (Sigma 701 Automatic Tensiometer, KSV Instruments, Ltd.). The temperature was controlled to within 0.05 °C using a refrigerated bath/circulator. The platinum Wilhelmy plate was washed with acetone, rinsed in Milli-Q water, and flamed until red-hot before each measurement. Surface tension data for the Pluronic−PAA are presented in Figure 2. A change in slope was observed in the surface tension curve at a characteristic copolymer concentration, after which the surface tension values decreased more gradually. This change, indicating the onset of aggregation of Pluronic−PAA molecules, was observed at 6×10^{-5} g/mL, in accord with the previously reported critical micellization concentrations (cmc) in Pluronic−PAA solutions.[22] In a series of preliminary experiments, Pluronic−PAA solutions with concentrations ranging from 10^{-4} to 1 w/v % were stirred with magnetic bars for 96 h at 37 °C prior to measurement of the surface tension. Within experimental error (12%) no change in the cmc was observed, demonstrating that the micelles formed in the Pluronic−PAA solutions were stable.

The dynamic viscosity of PPO was measured at a shear rate of 16.8 1/s using a Brookfield LVDV+ rotational viscometer equipped with a SC4-34 spindle.

Micelle Loading and Release Measurements. Micelle loading and the kinetics of the release of hydrophobic probes from the micelles formed in the Pluronic−PAA solutions (pH 7.4) were examined by steady-state fluorescence studies. Fluorescence spectra were recorded using a Shimadzu Model RF-5301 PC spectrofluorophotometer (slit width of 5.0 nm for both excitation and emission) under controlled temperature conditions. Three scans were averaged, and the spectra were corrected for Rayleigh and Raman bands in blank polymer solutions. In the release experiments, a time course acquisition mode was applied. The optimal concentrations and conditions for loading of pyrene into Pluronic−PAA were established previously.[30] A 1 w/v % Pluronic−PAA sample loaded with 1 μM pyrene was allowed to equilibrate for 48 h at 37 °C and excitation (λ_{em} = 390 nm) and emission (λ_{ex} = 335 nm)[36] spectra were recorded. The ratio of the intensities of the third (384 nm) to the first (373 nm) vibronic peak (I_3/I_1)[37,38] in the emission spectra of the monomer pyrene were used to estimate the polarity of the pyrene microenvironment.[39,40] Fluorescence spectra of estradiol were measured in the UV region (λ_{ex} = 280 nm, λ_{em} = 307 nm).[41] Estradiol was loaded into micelles by injection of a 37.5 μg/mL stock solution in absolute ethanol (10 μL) into 2 g of 1 w/v % Pluronic−PAA solution (pH 7.4). The resulting gelled solution was equilibrated for 48 h at 37 °C. Optimum estradiol concentrations and loading conditions were established in a series of preliminary experiments.

To study the kinetics of release, the dilution method developed by Webber and co-workers[1,17,42] was applied. Dilution is followed by diffusion of the probe from the micelles due to the mass action law, until equilibrium distribution of the probe is restored. The fluorescence quencher present in the solution quenches only the accessible fraction of the probe located outside the micelle core. It is thus possible to estimate what fraction of the probe leaves the micelle over time.

In a typical release experiment, 100 μL of a micellar solution, which had been allowed to equilibrate at 37 °C, was quickly added to a 10-mm path length quartz fluorescence cell containing 1.9 mL of either water (pH 7.4) or thallium(I) nitrate solution (pH adjusted to 7.4), again at 37 °C. A small magnetic bar continuously stirred the contents of the cell. $TlNO_3$ was observed to quench the fluorescence of both pyrene and estradiol. The dilution factor was chosen so that the resulting Pluronic−PAA concentration (0.05 w/v %) was at least 5 times greater than the critical aggregation concentration (compare with Figure 2).

The sample was continuously excited at a fixed wavelength, and the intensity of the emission band was recorded every 0.02 min. Slit widths were set at 1.5 nm in all kinetics experiments. No appreciable instrumental drifts were observed for 1−2 h, as checked with the fluorescence standards. After about 1.5−2 h, significant oscillations of the intensity were observed, due to the loss of the probe to the cell walls.[17] Therefore, a typical acquisition time was set at 45 min. Fluorescence intensity data were analyzed using homemade software. Each kinetics experiment was run in triplicate, and the resulting intensity vs time plot was averaged producing an array of intensity data which was discreetly computed every 1 min (Figure 3). When introduced into aqueous solution, a fraction of the fluorescent probe loaded into micelles of the Pluronic−PAA is steeply released shortly after $t = 0$ causing an initial "burst" of fluorescence which keeps gradually increasing without the quencher (I_u) and decreasing with the quencher present (I_q). Standard deviations for data collected in one series of experiments were below 10%. Differential equations were solved numerically, by allowing the values for the diffusion coefficients to float until the best fit to the experimental data was obtained. Other computational details are given in the footnotes.

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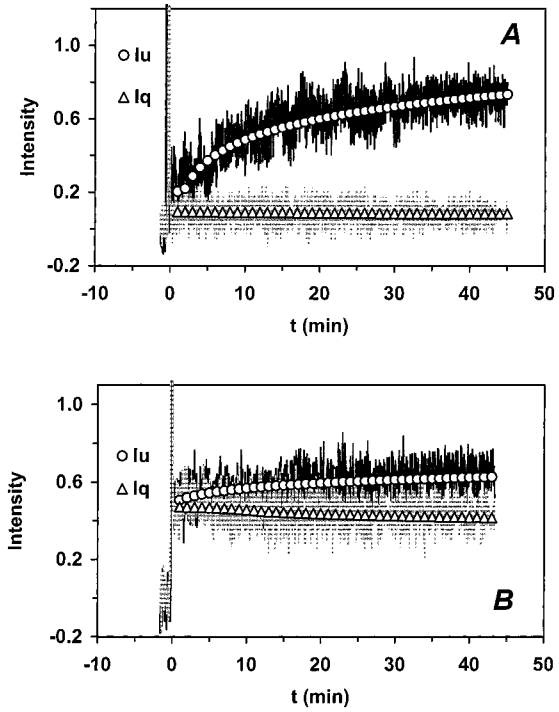

Figure 3. Kinetics of quenched (I_q) and unquenched (I_u) fluorescence intensities in aqueous solutions of Pluronic−PAA loaded with pyrene (A) or estradiol (B). Solid lines represent the spectrofluorophotometer output recorded every 0.02 min. Open points show average intensities computed discreetly every 1 min. A 1 w/v % solution of Pluronic−PAA containing 1 μM pyrene (A) or 0.7 μM estradiol (B) was quickly injected into water or 19 mM $TlNO_3$ aqueous solution, and the time commenced. T = 37 °C and pH 7.4 were kept constant throughout. For other experimental details, see text.

Results and Discussion

The present study was designed to determine the rate of release of pyrene and estradiol from Pluronic−PAA, since the translational diffusion coefficients of small molecules such as these are unknown.[43]

Dilution of the micellar Pluronic−PAA solution loaded with a probe is followed by a release process that can be examined by monitoring changes in fluorescence intensity with time (Figure 3). Pyrene and estradiol were chosen as probes in these studies since pyrene is a molecule with well-understood photochemistry and estradiol is a steroid hormone, which is fluorescent. In addition, both probes are very hydrophobic (*n*-octanol-to-water partition coefficients for pyrene and estradiol are 1.5×10^5 and 7.2×10^3, respectively[32]). Recently, we have shown insensitivity of the pyrene fluorescence in undiluted 1% Pluronic−PAA solution to the thallium ion concentrations.[32] These data, analyzed using Stern−Volmer plots, demonstrated that thallium ion does not penetrate into the PPO core of the micelles at temperatures above cmt.[32]

Following the two-phase model of Arca et al.,[42] the intensity of the fluorescence in the absence (I_u) or in the presence (I_q) of the quencher can be expressed as a sum of the contributions of the probe distributed in the micellar core (I_c) and in the aqueous (I_a) phase:[17]

$$I = I_c + I_a \tag{1}$$

The fluorescence contribution from the nth phase, I_n, is given by

$$I_n = \chi Q, \qquad \chi = kC\phi \tag{2}$$

where Q is the mole fraction of the probe in the nth phase, k is the instrumental constant, C is the total probe concentration, and ϕ is the quantum yield of the probe in the nth phase.

The total fluorescence intensity in the unquenched sample can be expressed as a sum of the fractions of the probe residing in the core and the solvent phases:

$$I_u = I_{core} + I_{aq} = kC(x_{core}\phi_{core} + x_{aq}\phi_{aq}) = kC\phi_{core}[x_{core} + (1 - x_{core})\beta]$$

where β is the ratio of the probe quantum yields in the aqueous phase and the micellar core phase.

Similarly, in the presence of the quencher that cannot penetrate into the core phase, the intensity of the quenched solution is[17]

$$I_q = kC\phi_{core}[x_{core} + (1 - x_{core})\beta\xi]$$

where ξ is the ratio of the intensities of the quenched to unquenched fluorescence in the aqueous phase alone.

Assuming that any probe molecules in the micellar core are completely inaccessible to the quencher, the time-dependent difference of the fluorescence intensities of unquenched and quenched samples can thus be expressed as

$$I_u - I_q = \chi\beta(1 - \xi) - \beta(I_q - \xi I_u) \tag{3}$$

where χ incorporates quantum yield of the probe in the micellar core.[44]

The fraction of the probe left in the micellar core ($Q_c(t)$) over time can be derived from eq 3 as

$$Q_c(t) = \frac{I_q - \beta\xi\chi}{\chi(1 - \beta\xi)} \tag{4}$$

By definition, the fraction of the probe released into the aqueous solution at any instant, $Q(t)$, is given by

$$Q(t) = 1 - Q_c(t) \tag{5}$$

It should be noted that, in all experiments, the rate of initial release was relatively large (10−15 s after the addition of the probe-loaded Pluronic−PAA solution). This phenomenon is due to the fast release of the probe from the hydrated regions of the micellar corona.[17] To estimate the fraction of the probe initially present in the aqueous phase (Q_0) prior to the increase of Q_t due to the slow release (i.e., after the initial burst release), we assumed that $Q_0 \cong Q'_t$, with Q'_t computed at $t = 1$ min. Such an assumption is consistent with the literature data suggesting that the initial burst release from micelles is complete in less than 30 s.[17,45,46] Based on the $I_u(t)$, $I_q(t)$ data (Figure 3), Q_0 was found to be 0.056 and 0.11 for pyrene and estradiol,

(43) Rotational diffusion coefficients can be estimated from our recent ESR measurements.[30]

(44) Equation 3 shows that the quantum yield ratio β and χ can be obtained from the slope and intercept, respectively, of the $(I_u - I_q)$ versus $(I_u - \xi I_q)$ plot. Using the computed I_u and I_q data shown in Figure 3 and independently measured ξ values we found β to be 0.920 and 1.19 for pyrene and estradiol, respectively, and χ to be 0.095 and 0.478 for pyrene and estradiol, respectively. The plots were linear (R^2 exceeded 0.98 in all experiments).

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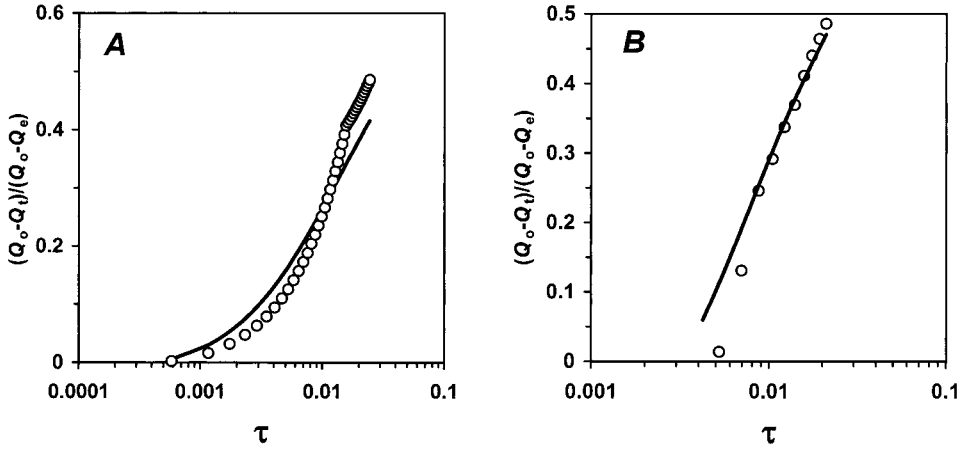

Figure 4. Kinetics of pyrene (A) and estradiol (B) fractional release at 37 °C. The computed and experimental release data are shown by solid lines and open points, respectively. Experimental data were obtained by averaging the results of five independent experiments (error bars are not shown for clarity). Time (τ) is presented in arbitrary units.

respectively. These Q_0 values are about 2-fold higher than the Q_0 values estimated from equilibrium partitioning experiments.[32] Using the method described here, higher values for Q_0 can be expected as a result of the release of probe molecules located in the PEO corona. Such a conclusion is reinforced by the fact that Q_0 for estradiol is greater than that for pyrene. Due to their different hydrophobicities, pyrene and estradiol will have different distribution patterns within the micelles, with a greater fraction of pyrene being localized within the micelles resulting in a lower value of Q_0 than for estradiol.

The rate of release of a probe with a constant diffusion coefficient D from a micelle with a spherical core of radius r_0 can be approximated by Fick's second law[17,47]

$$\frac{\partial \bar{C}}{\partial t} = D\left[\frac{\partial^2 \bar{C}}{\partial^2 r} + \frac{2}{r}\frac{\partial \bar{C}}{\partial r}\right] \qquad (6)$$

where $\bar{C}$ is the probe concentration inside the core. In a well-stirred solution of finite volume V and assuming that equilibrium has been attained at the interface, the following initial and boundary conditions apply:[47]

$$r > r_0,\ t = 0 \qquad \bar{C}(r) = 0$$

$$0 \le r \le r_0,\ t = 0 \qquad \bar{C}(r) = C_0 = \text{const}$$

$$r = r_0;\ t > 0 \qquad \bar{C}(t) = \frac{3\overline{VC}}{r_0 VC}\int_0^t J(t)\,\mathrm{d}t \qquad (7)$$

where $\bar{V}$ is the volume of the core, C = probe concentration in the solution, and J is the flux of the probe through the interface. Assuming the distributions of the probe in the core and in the solution to be uniform at all times, one can solve the system (6) and (7) analytically in terms of the fractional release of the probe, $(Q_0 - Q_t)/(Q_0 - Q_e)$.[48]

The computed and experimental release data are shown in Figure 4. Solid lines represent computed data, whereas open points are the data obtained by averaging the results of five independent experiments. The computed data are in reasonable agreement with the experimental data. At shorter times the model tends to overestimate $(Q_0 - Q_t)/(Q_0 - Q_e)$ (underestimating Q_t), while at longer times the model underestimates $(Q_0 - Q_t)/(Q_0 - Q_e)$ (overestimating Q_t). The shape of the computed curve depends on the time shift parameter and diffusion coefficient and, to a lesser extent, on distribution coefficient of the probe.[49] Unphysical fractional release data in the ranges below 0 and above 1 due to uncertainties in estimate of Q_0, Q_t, and Q_e at the limits of the time scale (i.e., at very short and very long times) were excluded from analysis.

The maximum standard deviations in experiments with pyrene and estradiol were 11% and 16%, respectively. The model fit was obtained for the averaged data only. In the calculations, values of 3.5 and 6.0 nm were used for the

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(48) Paterson (Paterson, S. *Proc. Phys. Soc. (London)* **1947**, *59*, 50) gives analytical solution of eq 6 with initial and boundary conditions (7):

$$\frac{Q_0 - Q_t}{Q_0 - Q_e} = 1 - \frac{2}{3\alpha}\sum_{n=1}^{\infty}\frac{\exp(-q_n{}^2 Dt/r_0{}^2)}{1 + q_n{}^2/9\alpha(\alpha+1)}$$

where the q_n's are the nonzero roots of

$$q_n \cot q_n = 1 + q_n{}^2/3\alpha$$

Here, Q_0, Q_t, and Q_e are the fractions of the probe in the solution at time zero, at time t, and at equilibrium, respectively, and $\alpha = 3V/4\pi R_c{}^3 K_{c/w}$, with $K_{c/w}$ being equilibrium partition coefficient of the probe between the core and the aqueous solution. We used experimentally obtained estimates $Q_0 = 0.056$, $Q_e = 0.62$, $\alpha = 0.60$ and $Q_0 = 0.11$, $Q_e = 0.47$, $\alpha = 0.90$ in the cases of pyrene and estradiol, respectively, to best fit the plots of $(Q_0 - Q_t)/(Q_0 - Q_e)$ versus time (in arbitrary units), to the experimental data.

(49) Distribution coefficient of the probe between 0.05% Pluronic−PAA solution (pH 7.4) and water (pH 7.4) was measured by the fluorescence technique.[17] A 100-μL volume of the probe-saturated polymer solution or water was dissolved in a known amount of THF. The fluorescence intensity of the resulting solution was measured and compared to the intensity versus [probe] calibration curve obtained in THF. No appreciable effect of 1−3% of water on calibration curves was observed. The distribution coefficient $K_d = (I_{micelle} - I_{water})/I_{water}$ was then measured. From the K_d value, the partition coefficient was estimated: $K'_{c/w} = K_d V_{water}/V_{micelle}$ core, where V_{water} and $V_{micelle}$ correspond to the volume of water in solution and micelle in the solution, respectively. The $K'_{c/w}$ values were found to be 1.66 ± 0.08 and 1.11 ± 0.06 for pyrene and estradiol, respectively. According to Crank (Crank, J. *The Mathematics of Diffusion*; Oxford University Press: New York, 1967; pp 88−91) at equilibrium $\alpha = 1/K'_{c/w}$, and $Q_e/Q_0 = K'_{c/w}/(1 + K'_{c/w})$. As the fraction of the probe that remains in the aqueous phase in the initially injected micellar solution (before release) is very low, approximation $Q_e \cong K'_{c/w}/(1 + K'_{c/w})$ is justified.[17] Hence, once $K'_{c/w}$'s were measured, we obtained the estimates for α and Q_e. Independent experiments can be used to verify the accuracy of the above estimates. Thus, it has recently been measured that micellar cores in the Pluronic−PAA solutions account for 52% of PPO,[29] and the weight fraction of PPO in the given Pluronic−PAA polymer is 0.11.[25] Then 1 mL of 0.05% Pluronic−PAA solution contains about 48 μL of PPO that represents micellar cores. Hence, using the $K'_{c/w}$ values we arrive at a partition coefficient of 5.8×10^4. This corresponds to about 69% of the partition coefficient found in independent experiments from I_3/I_1 [30] and 78% of the $K_{m/w}$ value obtained from equilibrium partitioning experiments.[32] We believe it is an excellent agreement given some uncertainty in the estimate of $V_{micelle}$.

r_0 in the case of pyrene and estradiol, respectively. These values were obtained for the radii of the PPO core and the PEO inner corona in the recent SANS study.[29] Different values of r_0 take into account differences in the distribution of pyrene and estradiol. The estimates for the equilibrium partition coefficient of the probe between the micellar core and aqueous solution were obtained experimentally.[49] Since the fractional release data in Figure 4 is plotted as a function of time for the experimental data and as a function of dimensionless parameter $\tau = (Dt/r_0^2)$ for the computed release curve, a time shift factor (τ^*) is required to achieve the best fit between the experimental and computed data and is given by[17] $\tau^* = r_0^2/D$. The D values obtained from the fitting shown in Figure 4 were 5.7 ($\pm$1.5) $\times$ 10^{-15} and 18 ($\pm$6.1) $\times$ 10^{-15} cm^2/s for pyrene and estradiol, respectively. The uncertainty in the estimates of D was $\pm$26% and $\pm$34% for pyrene and estradiol release, respectively, based on multiple release experiments with independent fits of the data. The higher D value obtained for estradiol, despite pyrene's smaller molecular size, reflects the model's assumption that pyrene diffuses from a sphere with a smaller radius. The model cannot account for possible nonuniform distribution of the probe between the core and the "inner corona".[17] In the case of etradiol, the D value should be ascribed to an effective diffusion coefficient that is a function of diffusion coefficients in the core and in the "inner corona".[17,47]

Overall, the obtained diffusion coefficients were 10^2–10^4-fold higher than the values reported by Teng et al.[17] for diffusion of pyrene from micelles with cores composed of glassy polymers such as polystyrene or poly(*tert*-butyl acrylate). On the other hand, Michaels et al.[50] obtained values ranging from 9 $\times$ 10^{-10} to 6 $\times$ 10^{-7} cm^2/s for the diffusion coefficient of steroid hormones in rubbery polymers (such as low-density polyethylene, poly(dimethylsiloxane), poly(ethylene-*co*-vinyl acetate), and polyurethane). The diffusion coefficient of pyrene in poly(isobutyl methacrylate) above the glass transition temperature (T_g) of the polymer was found to be of the order of 3 $\times$ 10^{-12} cm^2/s.[51] Thus the D values obtained herein lie between values measured for bulk glassy and for rubbery polymers.

The core of the Pluronic–PAA micelles consists mainly of substantially dehydrated (hydration levels <20%) PPO blocks.[29,52] This PPO is amorphous with the segmental motions within its polymer chains (so-called α-relaxation process[53]) having a T_g of about −70 °C at atmospheric pressure (for a molecular weight of 4000).[54] Therefore, in our experiments the PPO in the micellar core is always in its rubbery state. The diffusion coefficients obtained in the present study would be considered to be unusually low for a homogeneous polymeric system in its rubbery state. Such low values can be found if the structure of the micellar core is of a heterogeneous nature. A substantial body of evidence exists to indicate that massive grafting of poly(acrylic acid) segments in Pluronic–PAA during synthesis occurs on both the PEO and PPO segments of Pluronic.[25,29] Such grafting restricts the mobility of the PPO blocks and acts as a permanent cross-link, leaving

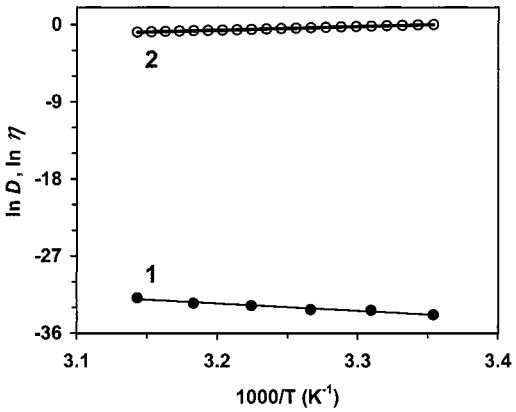

Figure 5. Temperature dependencies of diffusion coefficient (D) (1) of pyrene in Pluronic–PAA solution and viscosity (η) (2) of poly(propylene oxide) expressed in Arrhenius coordinates.

only about one-third of the Pluronic molecules in Pluronic–PAA capable of participating in the micelle-like aggregates.[29] These cross-linked, constrained domains of the micellar core may then be responsible for the low D values that are observed. In support of this hypothesis, Andrady and Sefcik[55] observed that using 7-fold shorter PPO segments as cross-links in PPO networks reduced the diffusivity of carbon monoxide by a factor of 200. In the case of larger molecules such as pyrene and estradiol, the effect of cross-linking on D will be much greater. It is also possible that the heterogeneity of the micellar aggregates can also increase the contribution of the fast diffusional modes, but the method applied in the present study does not allow for an estimation of such an effect.[17]

To highlight the differences between the core of micellar aggregates in Pluronic–PAA and those in bulk PPO, we studied the temperature dependence of the viscosity, the diffusion coefficient of pyrene, and the ratio of the third to the first vibronic peaks of pyrene loaded in either Pluronic–PAA and PPO (Figures 5 and 6). In these studies, the molecular weight of the PPO chosen (4000) roughly corresponded to that of the PPO segment of Pluronic F127.

As micellar aggregates in 1% aqueous solution of Pluronic–PAA do not form below 20 and melt above 45 °C,[22,23] we studied diffusion in the range of 25–45 °C. The Arrhenius plots (Figure 5) yielded apparent activation energies (E_a) of 35 and 72 kJ/mol for the PPO viscosity (η) and the diffusion coefficient of pyrene in Pluronic–PAA solution (D), respectively.

The activation energy for η is in excellent agreement with the value of 33 kJ/mol reported by Nivaggioli et al.[56] from kinematic viscosity data for PPO of a lower molecular weight. An activation energy of 34 kJ/mol,[56] measured using the monomer-to-excimer intensity ratio of dipyme, was obtained for the microviscosity of PPO. These values indicate that there is good agreement between the activation energy for the viscosity and for D. The E_a of 72 kJ mol^{-1} for the diffusion coefficient of pyrene in Pluronic–PAA solution is in sharp contrast with these observations. Such a high activation energy suggests the occurrence of temperature-induced changes in the micellar cores. Any such changes that do occur can be monitored by the changes in spectra of pyrene (I_3/I_1).

The I_3/I_1 values for 1 w/v % Pluronic–PAA solution are presented in Figure 6 along with the I_3/I_1 ratio for 0.5% aqueous solutions of PAA, bulk PEO, and bulk PPO

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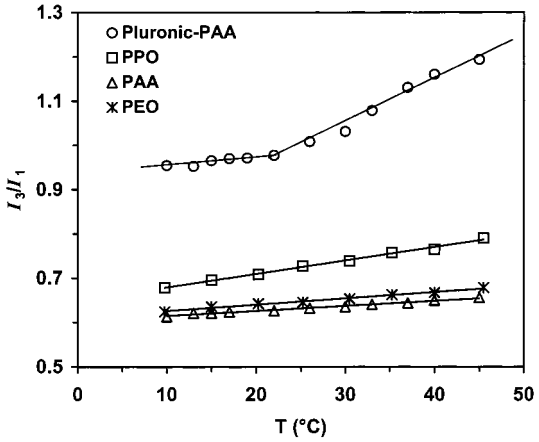

Figure 6. Temperature dependencies of the ratio of the third to the first vibronic peak intensities (I_3/I_1) of pyrene loaded into 1 w/v % solution of Pluronic−PAA, 0.5% PAA, PPO, or PEO.

homopolymers. While a linear increase of I_3/I_1 with temperature was observed for the homopolymers, the I_3/I_1 ratio for Pluronic−PAA exhibited a sharp increase in slope at cmt.[30,57]

Analysis of the data in Figure 6 reveals several unusual features in the Pluronic−PAA solutions, when compared to Pluronic solutions. First, it appears that even at temperatures below cmt the pyrene microenvironment is less polar in Pluronic−PAA than in PPO, as the I_3/I_1 ratio in the former is ca. 25% larger. This observation is in accord with the recently reported existence of hydrophobic microdomains in Pluronic−PAA.[29,30] Second, at temperatures above the cmt, the slope of the I_3/I_1 vs T plot is 3.5-fold higher than in PPO or in Pluronic solutions.[57] It is quite evident that since the temperature dependence of I_3/I_1 for each component of the Pluronic−PAA aggregate (i.e., individual PPO, PEO, and water) is much smaller than for the aggregate itself, there must be an aggregate rearrangement process (Appendix). The increase in slope above the cmt is indicative of structural changes in the microenvironment of pyrene above this temperature. Light scattering[3] and SANS[29] experiments demonstrated that there is an increased number of large scattering species in Pluronic−PAA solutions with increasing temperature. The structural changes may be caused by dissociation of small hydrophobic domains (that did not participate in micellar aggregates below cmt) followed by incorporation into micelles above the cmt. Above 45−50 °C, all of the aggregates in Pluronic−PAA begin to "melt".[23] The temperature-dependent dynamics of such a restructuring will alter the degree of partitioning and rates of diffusion of hydrophobic probes in the micelles. These temperature-dependent changes are, in general, a distinctive feature of the Pluronic−PAA copolymers.[23,30]

Conclusions

The rate of release of two hydrophobic probes, pyrene and estradiol, from micellar aggregates formed in aqueous solutions of an associative polymer, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-(poly(ethylene oxide))-*g*-poly(acrylic acid) (Pluronic−PAA), has been shown to have fast and slow modes. The fast mode is a result of a "burst" release (<1 min) of 5−11% of the total amount of the probe initially loaded into the micellar Pluronic−PAA solution. Under physiological conditions (37 °C, pH 7.4) the slow release mode is characterized by diffusion coefficients of the order of 10^{-15}−10^{-14} cm^2/s and can be ascribed to diffusion of the probes out from the core of the micellar aggregates. This finding correlates well with in vitro and in vivo experiments that have suggested bimodal release kinetics via the relatively fast erosion of the Pluronic−PAA gels followed by diffusion from the micellar aggregates.[34,35] The core of the aggregates is evidently heterogeneous and possesses properties different from those of the bulk poly(propylene oxide) (the major constituent of the micellar core in Pluronic−PAA). The apparent activation energy for the diffusion of pyrene is high and is suggestive of structural changes in the micellar core with increasing temperature. The temperature dependence of the ratio of the intensities of the third to the first vibronic peak of the pyrene emission spectra (I_3/I_1) indicates that, in comparison to bulk poly(propylene oxide), dramatic changes in the polarity of the pyrene microenvironment in Pluronic−PAA occur. At temperatures below cmt, the pyrene microenvironment is less polar than in PPO, suggesting the existence of discrete hydrophobic domains. The temperature dependency of I_3/I_1 in Pluronic−PAA above the cmt may be explained by a redistribution of pyrene between such domains and the micelles.

Appendix

Analysis of I_3/I_1 vs T plots for Pluronic solutions (see refs 40, 56, 57 herein) is based on an approximation generally valid for binary solvent systems A and B:[58,59]

$$I_3/I_1 \cong (xI_{3A} + yI_{3B})/(xI_{1A} + yI_{1B})$$

where I_{1A}, I_{1B}, I_{3A}, and I_{3B} are the emission intensities of the corresponding vibronic bands of pyrene in solvents A and B, respectively. Here, x and y are corresponding mole fractions of the components A and B, respectively. Since for pyrene the I_3 is considered to be independent of the microenvironment,[60] the above equation reduces to

$$I_3/I_1 \cong x(I_3/I_1)_A + y(I_3/I_1)_B$$

Similarly, one can assume for Pluronic micelles:

$$(I_3/I_1)_{\text{micelle}} \cong x(I_3/I_1)_{H_2O} + y(I_3/I_1)_{PEO} + z(I_3/I_1)_{PPO}$$

where $x + y + z = 1$.

If temperature variation of x, y, and z is negligible, then

$$\mathrm{d}(I_3/I_1)_{\text{micelle}}/\mathrm{d}T = x\,\mathrm{d}(I_3/I_1)_{H_2O}/\mathrm{d}T + y\mathrm{d}(I_3/I_1)_{PEO}/\mathrm{d}T + z\mathrm{d}(I_3/I_1)_{PPO}/\mathrm{d}T$$

and x, y, and z can be calculated from temperature dependencies of the I_3/I_1 plots in the corresponding solvents.

It can be readily seen that similar assumptions lead to unphysical values of x, y, and z in Pluronic−PAA solutions (Figure 6). Hence, Pluronic−PAA exhibits temperature-dependent fractional content.

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