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Fluorescence Investigation of the Binding of Pyrene to Hydrophobic Microdomains in Aqueous Solutions of Polysoaps

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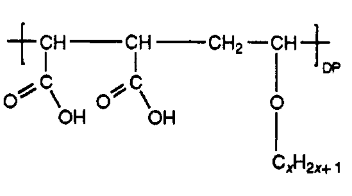
ABSTRACT: The binding of pyrene to a series of polysoaps of increasing hydrophobicity, the alternating copolymers of maleic acid and alkyl vinyl ether (alkyl = butyl, hexyl, decyl, dodecyl, and hexadecyl), has been investigated by spectrofluorometry and fluorescence decay time measurements, at constant pyrene concentration ($\approx 5 \times 10^{-7}$ M) as a function of the polysoap concentration and degree of neutralization. The plots of the ratio I_1/I_3 of the intensities of the first and the third vibronic peaks in the pyrene fluorescence emission spectrum against the polysoap concentration were found to have the sigmoidal shape expected for the binding of pyrene to the hydrophobic microdomains present in the polysoap solutions. The same was true for the results of fluorescence lifetime measurements. The quantitative analysis of the I_1/I_3 data required use of a polysoap concentration-dependent binding constant and allowed us to estimate the fraction of alkyl side chains not involved in the formation of microdomains. The concept of cmc in polysoap solutions was discussed in light of these results. Finally, the fluorescence lifetime data were used to infer information on the dynamics of polysoaps in solution.

1. Introduction

Polyamphiphiles, also called polysoaps,[1,2] are polymers or copolymers, ordered or disordered, where some or all repeat units are amphiphilic. In aqueous solutions polyamphiphiles form hydrophobic microdomains which have some of the properties of micelles of conventional amphiphiles, as for instance the capacity of solubilizing water-insoluble compounds.[1–4] It is generally accepted that polyamphiphiles have a zero critical micelle concentration (cmc) on the ground that since the local concentration of repeat unit hydrophobic groups is very large, it results in the formation of hydrophobic microdomains, however small the stoichiometric concentration of the polyamphiphile.[1–7] A largely unsettled problem is that of the value of the fraction of repeat unit hydrophobic groups involved in microdomains. There have been attempts[8–10] to determine this fraction, but this issue was sometimes neglected.[11–13]

This paper describes an attempt to evaluate these fractions in the case of a series of polyamphiphiles of various hydrophobicities. These polyamphiphiles are the much investigated alternated copolymers of maleic acid and alkyl vinyl ether,[8–10,14–19] of general chemical formula:



where DP is the degree of polymerization. These copolymers are referred to as PS*X* below. Their hydrophobicity can be controlled by adjusting the degree of neutralization, α, of the maleic acid moiety (at $\alpha = 1$, the two CO_2H groups are neutralized) and the carbon number, *X*, of the alkyl side chains. An extrinsic fluorescence probe, pyrene, was used to detect the presence of hydrophobic microdomains. Recall that pyrene is a much used fluorescent probe owing to its very low solubility in water, the long lifetime of its excited state, the large number of quenchers of its fluorescence, and the sensitivity of its fluorescence characteristics to the polarity of its immediate environment.[20,21] Pyrene has been extensively used for the study of microheterogeneous systems, such as micellar solutions[20–25] and, more recently, polyamphiphile solutions.[8–11,26,27]

In the present study, the binding of pyrene to the polysoap microdomains was investigated by spectrofluorometry and fluorescence lifetime measurements.

The measurements reported below involved three copolymers, PS10, PS12, and PS16, which have a well-characterized polysoap behavior, i.e. low viscous solutions even at moderate concentrations and a viscosity increasing with concentration in the whole concentration range (no maximum).[17,18] We also investigated two copolymers, PS4 and PS6, which behave like polysoaps at low α and like polyelectrolytes at higher α.[15,16] The conformational transition from the compact polysoap conformation to the extended polyelectrolyte conformation takes place in a fairly narrow range of α values, when the electrostatic repulsions between charged groups dominate the side chain attractive interactions.[9,11]

Our results permitted us to obtain estimates of the fraction of alkyl side chains involved in the formation of microdomains in aqueous PSX solutions, as a function of α and *X*, and to discuss the meaning of the cmc in solutions of polyamphiphiles.

2. Experimental Section

Materials. The sample of purified pyrene was the same as in previous investigations.[9,19]

The aqueous solutions of poly(maleic acid-*co*-alkyl vinyl ether) at the proper degree of neutralization by NaOH were prepared from the corresponding poly(maleic anhydride-*co*-alkyl vinyl ether) as described.[9,19] The origin and purification of these copolymers was reported.[9,19] The molecular weights of the PS*X* were not known but are believed to be above 50 000 in view of the values of the degrees of polymerization of the corresponding poly(maleic anhydride-*co*-alkyl vinyl ether). These degrees of polymerization were determined as part of this work by light scattering measurements and found to be 260, 700, and 4000 for PS4, PS12, and PS16, respectively. The concentration of PS*X* solutions is expressed in moles of repeat unit per liter.

Methods. The fluorescence emission spectra of pyrene solubilized in the investigated solutions were recorded using a Hitachi F4010 spectrofluorometer in the range 350–500 nm at an

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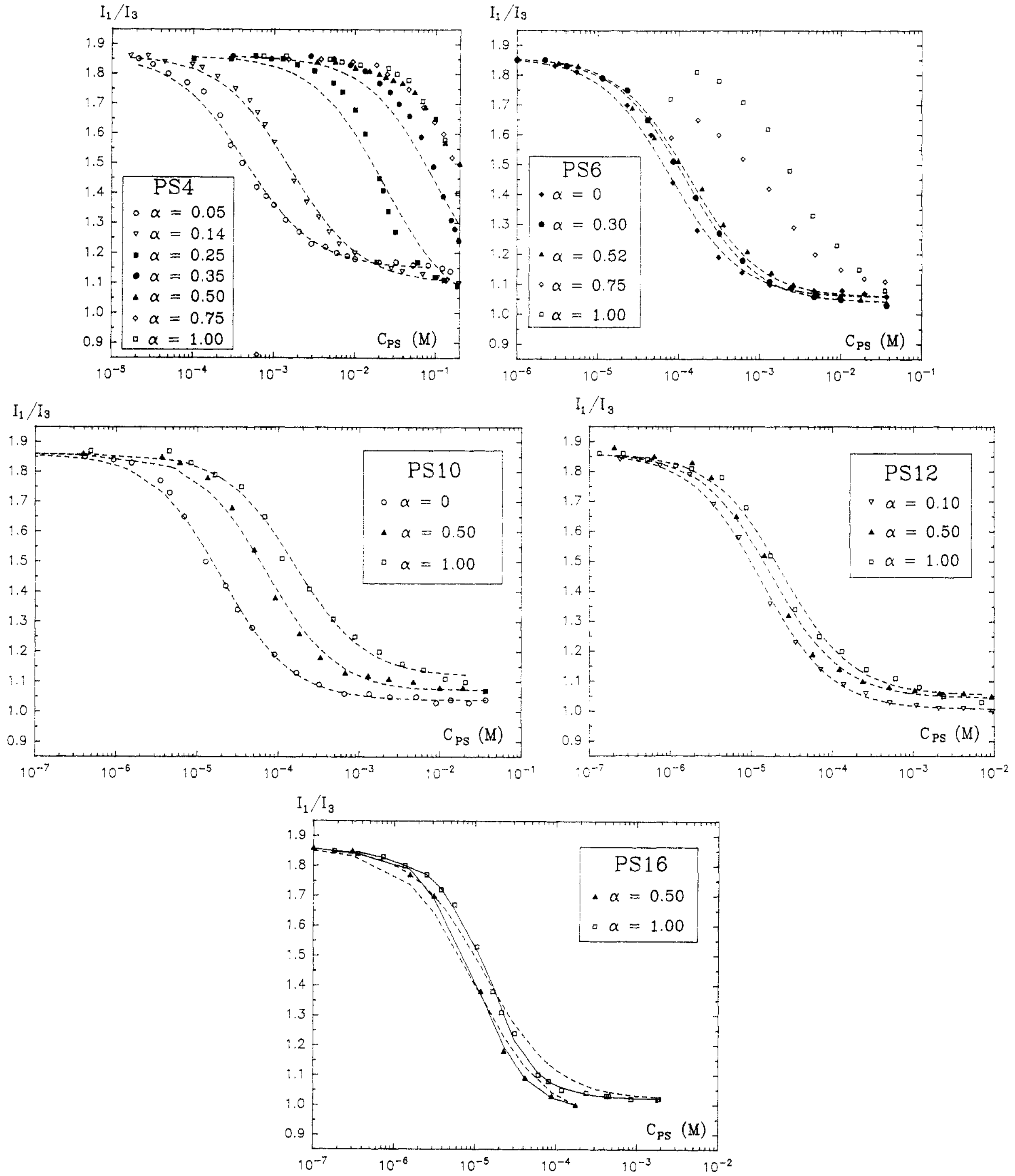

Figure 1. Variation of I_1/I_3 with C_{PS} at various α's for (A, top left) PS4, (B, top right) PS6, (C, middle left) PS10, (D, middle right) PS12, and (E, bottom) PS16. The broken line curves represent the best fits of the binding eq 3 to the data, assuming a single binding constant. In Figure 1E the continuous curves represent the best fits of eqs 3–5 (C_{PS}-dependent binding constant) to the data.

excitation wavelength of 335 nm. The ratios I_1/I_3 of the fluorescence intensities of the first and third vibronic peaks were then calculated. This ratio gives a measure of the polarity of the pyrene microenvironment.[20,21]

The lifetime of the pyrene excited state was determined using the single photon counting technique.[9,28] The excitation and emission wavelengths were 335 and 381 nm, respectively. The decay curves were fitted to a sum of exponentials (see below) using a weighted least-squares procedure.[9]

Pyrene was solubilized into the investigated solution as follows. The proper amount of a stock solution of pyrene in ethanol was introduced in a volumetric flask and distributed over the flask wall. The ethanol was evaporated under a stream of air, the PS*X* solution was introduced, and pyrene was solubilized by magnetic stirring for at least 12 h. The pyrene concentration was always about 5 × 10^{-7} M, a value slightly lower than its solubility in water.[29]

Aerated solutions were used throughout in spectrofluorometry. Lifetime measurements involved solutions deaerated by three freeze–pump–thaw cycles.

All experiments were performed at 25 °C.

3. Results

Parts A–E of Figure 1 show the plots of the ratio I_1/I_3 against the copolymer concentration, C_{PS}, for PS4, PS6, PS10, PS12, and PS16 at various values of the degree of neutralization α. In all instances I_1/I_3 shows a sigmoidal decrease upon increasing C_{PS}. As in fluorescence studies of aqueous solutions of similar copolymers[27] and of block copolymers[22] the decreases of I_1/I_3 stretch over a range of concentrations of more than two decades. Besides, the plots are shifted to larger concentrations upon increasing

α for a given polymer and upon decreasing side chain carbon number X for a given α, i.e., when the copolymer becomes less hydrophobic. The effect of α on the shift of the I_1/I_3 plots becomes more important as X is decreased.

As noted by Wilhelm *et al.*[22] "these sigmoidal curves have the shape anticipated for a binding isotherm plotted semilogarithmically". In the present case pyrene would bind to the microdomains. On the basis of results for the binding of pyrene to micelles of conventional surfactants,[30] the binding constant K is expected to increase with X or upon decreasing α, as the microdomains are then larger.[9] The interaction of pyrene with the microdomains can also be viewed as a partition of pyrene between microdomains and water. In dilute solutions, as in the present study, the partition coefficient K_p is related to the binding constant according to

$$K_p = 55.5K \quad \text{and} \quad K = C^{m}_{Py}/C^{w}_{Py}(C_{PS} + C^{m}_{Py}) \quad (1)$$

where C^{m}_{Py} and C^{w}_{Py} are the concentrations of pyrene in the microdomains and in water, expressed in moles per liter of solution. C^{m}_{Py} can be determined (see below). It has been found to be much smaller than C_{PS} and has been systematically neglected in the calculations.

At high C_{PS}, I_1/I_3 becomes constant and its values show a small decrease as X is increased. For instance, at α = 1.00, I_1/I_3 is equal to 1.11, 1.05, and 1.00 for PS10, PS12, and PS16, respectively. Similar changes were found at α = 0, indicating a more hydrophobic environment as X increases.

Changes in the pyrene excitation spectra at λ_{em} = 335 nm were found to correlate to the variations of I_1/I_3. As in the Wilhelm *et al.* study[22] the absorption maximum occurred at 333 nm when pyrene was solubilized in water (high I_1/I_3) and at 339 nm with a much larger intensity when pyrene was nearly completely solubilized in the microdomains (low I_1/I_3). In the intermediate range where pyrene partitions between water and microdomains, the two peaks were simultaneously present.

Two surprising observations were made in the cases of PS4 and PS6. First, at high C_{PS} values, I_1/I_3 decreased upon increasing C_{PS}, even at α values where, according to potentiometric results,[15,16] microdomains should no longer be present in the system (i.e. at $\alpha > 0.6$ for PS6 and 0.4 for PS4). These decreases occur at $C_{PS} > 0.04$ M (see Figure 1A,B), that is, a concentration much higher than in the potentiometric studies.[15,16] Second, for PS6 a variation of I_1/I_3 with time was observed at $\alpha > 0.5$. Thus I_1/I_3 increased upon dilution and no time effect was noted, until C_{PS} reached a certain value below which I_1/I_3 decreased as time elapsed. After a sufficient time, I_1/I_3 leveled out at a value close to that for $\alpha = 0.50$, at the same C_{PS}. A similar effect of time was observed in experiments where C_{PS} was increased with a slight hysteresis between dilution and concentration experiments. The time after which I_1/I_3 leveled out was shortened upon continuous stirring of the solution in both dilution and concentration experiments. No definite explanation can be given at the present time for the above two observations which may involve associations between different polymer chains.

The behavior of the decay of the pyrene fluorescence in copolymer solutions was found to be complex. All decays showed the presence of a fast process, with a decay time of about 50 ns, and of one or two slower processes, with decay times of about 200 and 390 ns. At low C_{PS}, when I_1/I_3 had a value close to that in water, the 200-ns process was predominant, whereas at high C_{PS}, when the value of I_1/I_3 was low and nearly constant, only the 390-ns process was observed. In the intermediate range, where I_1/I_3 decreased rapidly, the two slow processes were observed, in addition to the 50-ns one. Clearly, the 200- and 390-ns decays correspond to pyrene in aqueous and microdomain environments, respectively. The implications of this finding are discussed in the last paragraph of the discussion. Equation 2 was fitted to the decay curves, by taking

$$I(t) = I_1 \exp\left(-\frac{t}{\tau_1}\right) + I_2 \exp\left(-\frac{t}{\tau_2}\right) \quad (2)$$

τ_1 = 200 ns and τ_2 = 385 ns and starting the fitting procedure at time t = 100 ns, where the contribution of the 50-ns process is small with respect to those of the slower processes. We found this procedure to give more reliable results than a fit of the data to a sum of three exponential terms even when the decay times were fixed.

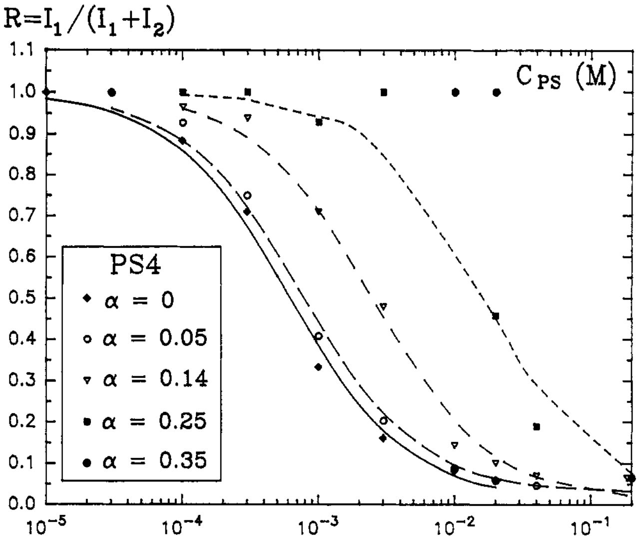

Figure 2. Variation of $R = I_1\tau_1/(I_1\tau_1 + I_2\tau_2)$ against C_{PS} for PS4.

The amplitudes I_1 and I_2 were obtained in this manner and the data plotted as $R = I_1\tau_1/(I_1\tau_1 + I_2\tau_2)$ against C_{PS} in order to compare them to the I_1/I_3 results. Figure 2 shows that the plots for PS4 at various α have the same sigmoidal shape as the I_1/I_3 plots. In fact, for a given α, the R vs C_{PS} and I_1/I_3 vs C_{PS} plots can be nearly superimposed after performing the required normation of ordinates. For this reason, the discussion below only concerns the I_1/I_3 plots.

4. Discussion

Partition of Pyrene between Polysoap Microdomains and Water. Before we discuss the results, recall that the measured values of I_1/I_3 depend on the wavelength of the excitation light,[22] being weighted toward the hydrophobic state (low value) at 339 nm and the hydrophilic state (high value) at 333 nm. At the excitation wavelength of 335 nm used in the present study the ratio is about equally weighted for the two states. Besides, the method used to determine the fractions of side alkyl chains involved in microdomains largely eliminates the effect of wavelength (see below).

If pyrene is partitioned between microdomains and water with a binding constant K and if the cmc of the system is zero, then I_1/I_3 writes[22,31]

$$I_1/I_3 = (I_1/I_3)_{PS} + [(I_1/I_3)_W - (I_1/I_3)_{PS}]\frac{1}{1 + KC_{PS}} \quad (3)$$

where $(I_1/I_3)_W$ and $(I_1/I_3)_{PS}$ are the values in water and in microdomains, respectively. The least-squares fit of eq 3

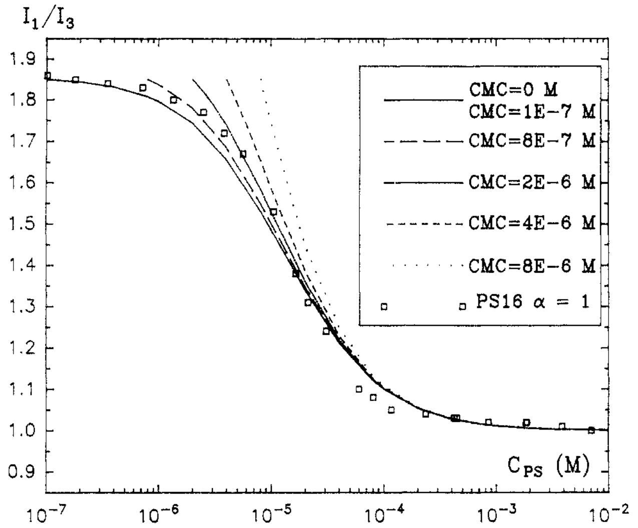

Figure 3. PS16. Fits of the binding eq 3, with C_{PS} replaced by C_{PS} − cmc, to the experimental data, with $K = 7.6 \times 10^4$ M^{-1} and various values of the cmc.

to the experimental results was first performed using $(I_1/I_3)_W$, $(I_1/I_3)_{PS}$, and K as adjustable parameters. The best fitting values of $(I_1/I_3)_W$ were all found to range between 1.84 and 1.87, i.e. independent of the system, as to be expected. Besides, we observed that the calculated curves led to values of $(I_1/I_3)_{PS}$ clearly lower than the experimental values at high C_{PS}, where I_1/I_3 levels out. Also, the calculated curves showed, in most instances, systematic deviations with respect to the experimental results, being above the experimental points at high C_{PS} and below them at low C_{PS}. This led us to fit eq 3 to the results using the experimental values of $(I_1/I_3)_W$ and $(I_1/I_3)_{PS}$, with K as the only adjustable parameter. The broken line curves going through the experimental points in Figure 1A–E were generated in this manner. These calculated curves nevertheless showed the same systematic deviations as described above. Two possible explanations can be given for these deviations: (1) the systems have a non-zero cmc, and (2) the partition of pyrene involves more than a single binding constant.

If the cmc is not zero, C_{PS} must be replaced by C_{PS} − cmc in eq 3. Figure 3 shows the I_1/I_3 vs C_{PS} plots generated using the modified equation, at C_{PS} > cmc, for various values of the cmc, and $K = 7.6 \times 10^4$ M^{-1}, a value equal to the reciprocal of the concentration at the midpoint of the I_1/I_3 vs C_{PS} plot going through the experimental results for PS16 at $\alpha = 1$ (this value gives K in the case of an ideal partition as assumed in deriving eq 3). The fits are not good, and the shape of the calculated curves differs from the experimental one. Also, the systematic differences noted at high C_{PS} between the curves calculated using the modified eq 3 and the experimental results are still present. Thus, a non-zero cmc does not permit us to account for the results.

We therefore tried to explain these results by using a C_{PS}-dependent partition coefficient. Three possible reasons can be given for such a variation. First, K may increase with C_{PS} because an increase of polysoap concentration decreases the self-ionization of the still unneutralized CO_2H groups on the copolymer. The microdomains become less charged and thus can solubilize more pyrene. Indeed, pyrene is less soluble in anionic than in nonionic micelles owing to its residual negative charge.[32] This explanation holds only for systems at relatively low α. However, the systematic differences noted above occurred at all α, even in the range 0.5–1 where the self-ionization then involves the second acidity of the maleic acid moiety. The pK_A of this group being rather high, no change of self-ionization is expected to occur in the concentration range covered. Besides, at $\alpha = 1$ no change of self-ionization can take place. Nevertheless the differences are still there. Thus the explanation in terms of self-ionization changes must be discarded.

A C_{PS}-dependent partition coefficient may also arise from the increase of ionic strength of the solution with C_{PS}. A higher ionic strength may result in an increase of the fraction of alkyl side chains involved in the formation of microdomains, similarly to the decrease of the cmc of conventional ionic surfactants upon increasing ionic strength and, in turn, in an increased pyrene solubility. This possibility has been checked by investigating the effect of additions of NaCl on the value of I_1/I_3 for selected PS*X* solutions. Additions of 0.35 mM NaCl to a 0.3 mM solution of PS4 at $\alpha = 0.05$ and of 10 mM NaCl to a 0.104 mM solution of PS10 at $\alpha = 1.00$ resulted in negligible changes of I_1/I_3. These results indicate that the effect of the ionic strength on K is very weak and cannot explain our observations in the concentration range where I_1/I_3 varies rapidly. The possible effect of the ionic strength is discussed further below.

A more likely explanation of our results is that K depends on the mole fraction of pyrene in the microdomains. Recall that the partition coefficient of a solubilizate between micelles of conventional surfactants and the bulk phase decreases as the solubilizate mole fraction in the micelles increases.[33–37] This variation reflects the change of solubilizate activity in the micelles, where its local concentration is high owing to the small micelle volume. A similar decrease of partition coefficient is expected for pyrene in microdomains as the average number of pyrene molecules per microdomain decreases monotonously upon increasing C_{PS}, that is microdomain concentration in experiments performed at constant total pyrene concentration, C_{Py}. We therefore attempted a fit of eq 3 to the results with K given by[33–37]

$$K = K_0(1 - bx_{Py}^{m})^2 \qquad (4)$$

where b is a constant and K_0 is the pyrene binding constant when the mole fraction of pyrene in the microdomains x_{Py}^{m}, given by eq 5, becomes vanishingly small. In eq 5

$$x_{Py}^{m} = \frac{C_{Py}^{m}}{C_{Py}^{m} + C_{PS}^{m}} \qquad (5)$$

C_{Py}^{m} and C_{PS}^{m} are the concentrations of pyrene and repeat units in the form of microdomains, in moles per liter of solution. For PS16 it can be safely assumed that $C_{PS}^{m} \approx C_{PS}$. Indeed, previous studies[9,38,39] suggested that all alkyl side chains are likely to be involved in microdomains. This assumption may not hold for PS*X* with shorter alkyl chains, particularly at high α. In these instances, however, the results contain information on the fraction of alkyl chains involved in microdomains, which can be extracted as shown in the next paragraph. At this stage we only consider PS16 at $\alpha = 1.00$. The fit of eq 3 with K and x_{Py}^{m} given by eqs 4 and 5 to the experimental results has been performed using for C_{Py}^{m} at a given C_{PS} the value calculated from

$$C_{Py}^{m} = C_{Py}\frac{(I_1/I_3)_W - (I_1/I_3)}{(I_1/I_3)_W - (I_1/I_3)_{PS}} \qquad (6)$$

where I_1/I_3 is the value of the ratio at C_{PS}. Figure 1E shows that the fit is good.

Equally good fits were obtained for the other I_1/I_3 plots with eq 3 and the simplified equation

$$K = K_0(1 - bC_{Py}^{m})^2 \tag{7}$$

as, then, the fraction of alkyl chains in microdomains was not known. C_{Py}^{m} was evaluated at each C_{PS} by applying eq 6 to the I_1/I_3 data.

Thus, the interpretation of the observed differences between the curves calculated from eq 3 with a single binding constant and the experimental results, in terms of a C_{PS}-dependent binding constant, appears to be valid on the basis of the goodness of the fits to the data.

The fact that the binding constant decreases upon increasing C_{PS} in experiments performed at constant C_{Py} has some bearing on the interpretation of similar results concerning the determination of the cmc of amphiphilic block copolymers in aqueous solution.[22,41] Various fluorescence properties showed sigmoidal variations upon increasing copolymer concentration in experiments performed at constant C_{Py}. Small departures from the variation calculated on the basis of a binding equilibrium with a single binding constant, in a concentration range where the pyrene molar concentration was comparable to the copolymer concentration, were interpreted as being due to the micellization of the block copolymer. The cmc's so determined were found to depend little on the molecular weight of the hydrophobic block and on the copolymer composition. Thus, an increase by a factor of 3.5 of the hydrophobic block weight fraction resulted in a decrease of the apparent cmc by a factor of only 2.[22] It is felt that these results should be reconsidered in light of the interpretation proposed above.

Fraction of Alkyl Side Chains under the Form of Microdomains. The fit of an equation such as (3) to the I_1/I_3 vs C_{PS} plots provides the binding constant of pyrene to the microdomains. A simple examination of the plots in Figures 1 shows that the midpoint of the decrease occurs at widely varying concentrations, from about 10^{-5} M for PS16 to about 10^{-2} M for PS4 at $\alpha = 0.25$. Such large changes reflect those of the binding constant K. For a homologous series of polyamphiphiles, as in the present study, these variations of K must be due to a change of volume of the microdomain pseudophase. Indeed, as they are obtained, the K (or K_p, eq 1) values refer to a binding per mole of host. When the host is modified, as is the case here by changing its volume at a constant number of moles, the variation of the binding constant should be nearly proportional to the variation of the volume where pyrene is solubilized.

In PSX systems, the volume of the host phase is proportional to $\beta(X,\alpha)v(X)$ where $\beta(X,\alpha)$ is the fraction of host molecules (side alkyl chains) in the form of microdomains and $v(X)$ the volume of the alkyl chain, given in cm^3/mol by[40]

$$v(X) = 16.2(X + 1) \tag{8}$$

Besides, one must take into account the fact that pyrene tends to solubilize close to the microdomain surface,[42,43] that is in a shell, cylindrical or spherical, depending on the microdomain shape, of thickness slightly larger than the average dimension of 1 nm of the pyrene molecule. A thickness of 1.25 nm was used below, which is equivalent to the length of 10 carbon–carbon bonds. As a result, for PSX, the effective volume for pyrene solubilization becomes

$$v_e(X) = v(X)F(X) = v(X)\frac{[(X + 1)^q - (X - 9)^q]}{(X + 1)^q} \tag{9}$$

where $q = 2$ or 3, depending on whether the microdomains are cylindrical or spherical. For $X < 10$, $F(X)$ is taken equal to 1.

Table 1. Values of the Pyrene Binding Constant *K* and of the Fraction of Alkyl Side Chains Involved in Microdomains on the Assumption of Spherical or Cylindrical Microdomains for the Various PSX[a]

polysoap	α	K (M^{-1})	β sphere	β cylinder
PS16	*0.00*	*1.3×10^5*	*1.00*	*1.00*
	0.50	1.0×10^5	1.00	1.00
	1.00	7.6×10^4	1.00	1.00
PS12	*0.00*	*7.9×10^4*	*0.75*	*0.70*
	0.10	7.4×10^4		
	0.50	5.6×10^4	0.69	0.64
	1.00	4.1×10^4	0.66	0.62
PS10	0.00	5.2×10^4	0.58	0.52
	0.50	1.4×10^4	0.20	0.18
	1.00	6.3×10^3	0.12	0.11
PS6	0.00	1.3×10^4	0.23	0.20
	0.30	8.2×10^3		
	0.52	5.4×10^3	0.12	0.11
PS4	*0.00*	*4.7×10^3*	*0.11*	*0.10*
	0.05	2.3×10^3		
	0.14	6.3×10^2		
	0.25	4.3×10^1		
	0.35	9.7×10^0		

[a] Italicized values correspond to extrapolated ones.

For PS16 at $\alpha = 1.00$ and, therefore at all α's, it can be safely assumed that $\beta(16,\alpha) = 1.00$.[9,38,39] The results for this copolymer can therefore be used as reference for calculating the values of $\beta(X,\alpha)$ for all other systems, if the values of $K(X,\alpha)$ have been first determined from:

$$\beta(X,\alpha) = \frac{K(X,\alpha)v(16)F(16)}{K(16,\alpha)v(X)F(X)} \tag{10}$$

The required values of $K(X,\alpha)$ have been obtained by setting them equal to the reciprocal of C_{PS} at the midpoint of the I_1/I_3 plot, that is at $I_1/I_3 = [(I_1/I_3)_W + (I_1/I_3)_{PS}]/2$, as for an ideal binding (see above). Clearly this procedure only yields approximate values of the binding constant. Nevertheless it is safe to assume that the differences between approximate and exact values cancel out in calculating $\beta(X,\alpha)$, which only involves ratios of binding constants. Table 1 lists the values of $K(X,\alpha)$, which are also represented in Figure 4.

For PS16, K is seen to increase slightly, by about 30%, as α decreases from 1.00 to 0.50. PS12 shows a slightly larger increase. As all alkyl chains of PS16 are in the form of microdomains at all α's, it is likely that the observed decrease of K reflects an increase of the binding constant due to the decreased microdomain electrical charge (see above). Note that the ionic strength of PSX solutions increases with α. If such an increase had the effect of increasing the fraction of alkyl side chains involved in microdomains (see discussion above), a decrease of $\beta(X,\alpha)$ would be expected upon decreasing α, contrary to our observations for PS10. Thus this result confirms the above conclusion that the ionic strength of the system plays only a minor role in determining the value of $\beta(X,\alpha)$.

Table 1 lists the values of $\beta(X,\alpha)$ calculated from eq 10, as indicated. The values obtained by assuming either spherical or cylindrical microdomains differ only a little. The rapid increase of $\beta(X,\alpha)$ with X, at a given α, is

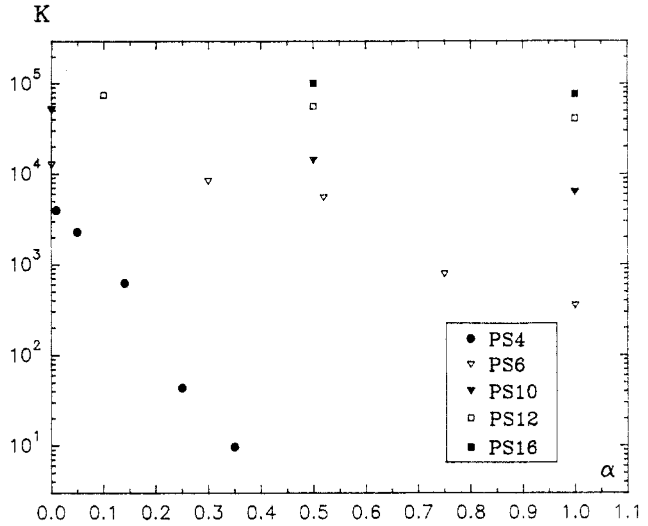

Figure 4. Variation of the binding constant K with α at various X's.

noteworthy. It supports a posteriori the assumption $\beta(16,\alpha) = 1.00$ made in calculating the values of $\beta(X,\alpha)$.

The low values of β found for PS4 and PS6 at low α, where microdomains are present, indicate that, as seen by cryo-transmission electron microscopy for PS10 at α = 1.00,[38] the microdomains are probably small and separated by long PSX segments with free alkyl side chains. For PS10 at α = 0 and PS12 at any α, the β values indicate that the successive microdomains in a given polymer molecule are rather close. Nevertheless, at this stage, the microstructure of the solution remains an open question. In particular, one cannot say whether the individual PSX polymer chains link end-to-end, as is the case in PS16 solutions, to form very long threadlike micelles.[38,39] Further studies by cryo-transmission electron microscopy may provide answers to this problem.

It is realized that the values of $\beta(X,\alpha)$ given in Table 1 represent only best estimates. Two other effects which have not been taken into account may affect them. The first one is that the shell in which pyrene is solubilized may not extend to the methylene group immediately connected to the head group, but to the next, or one after the next, CH_2. In eqs 8 and 9, X woul be replaced by $X - 1$ or $X - 2$, resulting in a small increase of β. For instance, for PS12 at α = 1.00 the values β = 0.70 or 0.73 would be found by replacing X by $X - 1$ or $X - 2$. The second effect arises because the solubility of pyrene in the microdomains is dependent upon the electrical charge and curvature of the microdomain. Pyrene solubilized in a small (highly curved) microdomain feels more the electrical charges of the polar groups than pyrene in a large, weakly curved microdomain. This would result in a smaller binding constant[30] and, thus, in a calculated value of β smaller than the real one. This effect cannot be quantified at the present time.

At this stage, recall that the β values for PS10 have been also determined from measurements of pyrene solubility.[9] These measurements involved the saturation of PS10 solutions at different α values by pyrene and the assumption that all decyl chains were in the form of microdomains at α = 0. The present work used solutions well below saturation and yielded at α = 0 and 1.00 β values of 0.5 and 0.11, as compared to 1.00 and 0.15 in the previous study. Thus the reported aggregation numbers of PS10 microdomains have to be revised to lower values (around 45 at α = 0 and 10 at α = 1.00).

cmc in Polysoap Solutions. As stated in the Introduction the cmc of polyamphiphiles has always been assumed to be equal to zero, that is micelle-like microdomains are present in polyamphiphile solutions, irrespective to their stoichiometric concentration. The shape of the I_1/I_3 vs C_{PS} plots (Figures 1) and the poor quality of the fit of eq 3, modified by the introduction of a finite cmc, to the data support the assumption that the cmc of polyamphiphiles in aqueous solution is zero. Here the cmc is identified as the concentration above which microdomains are present in the solution of a polyamphiphile having sufficiently long alkyl side chains, just as in the case of solutions of conventional amphiphiles.

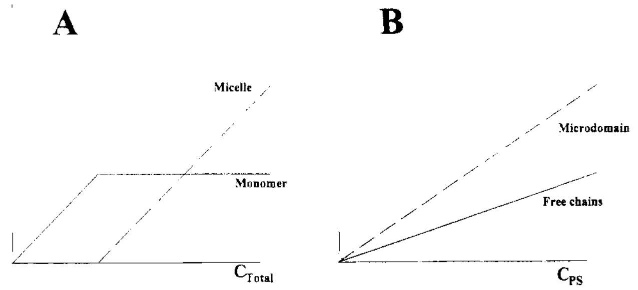

Figure 5. Variations of the micelle (microdomain) concentration and of the free amphiphile (side alkyl chain) concentration in solutions of conventional amphiphile (A) and polyamphiphile (B) with the total concentration C.

However in these solutions the cmc is also identified as the amphiphile concentration above which the concentration of free amphiphile starts differing from the total concentration, showing a small increase, remaining constant, or slightly decreasing depending on the nature of the amphiphile (nonionic, zwitterionic, and ionic, respectively), owing to the formation of micelles. Clearly, this definition does not apply to polyamphiphiles. There the concentration of alkyl side chains not involved in microdomains probably increases almost linearly with the polyamphiphile concentration, as indicated by the linear variation of the pyrene solubility in PS10 solutions,[9] for example, and the concept of cmc does not hold.

Figure 5 illustrates the variations of micelle (microdomain) concentration and of the free amphiphile (side alkyl chain) concentration in solutions of amphiphile and polyamphiphile.

Dynamic Aspects of the Polysoap Solutions. As noted in paragraph 3, in the range where I_1/I_3 decreases rapidly, the decay curves show two decay times: one corresponding to pyrene solubilized in water, the other to pyrene solubilized in microdomains.

This observation implies that the residence time of pyrene in microdomains is much longer that the lifetime of the pyrene excited state (400 ns), that is, longer than several microseconds. This lower bound value is consistent with the value of about 0.2 ms reported for the residence time of pyrene in micelles of conventional surfactants.[30]

The above observation also implies that the lifetime of PSX microdomains is long compared to the lifetime of the pyrene excited state. This sets a lower bound value of a few microseconds for the lifetime of these microdomains.

5. Conclusions

The binding of pyrene to a series of polysoaps, the poly(maleic acid-*co*-alkyl vinyl ether), has been investigated by spectrofluorometry and pyrene fluorescence lifetime measurements. The variation of the intensity ratio I_1/I_3 with the copolymer concentration has the shape expected for a binding equilibrium, with a binding constant increasing very much with the polymer hydrophobicity, that is a decreasing degree of neutralization of maleic acid and an increasing carbon number of the alkyl side chain. The deviations observed between the experimental results and curves calculated by assuming a single binding constant reveal that, as for conventional amphiphiles, the binding constant is concentration dependent, owing to the high activity of pyrene in the micelles. A method has been proposed and used to determine the fraction of alkyl chains involved in the microdomains. The results allowed us to discuss the concept of cmc in the case of polyamphiphile solutions. The lifetime data show that pyrene exists in two environments corresponding to water and microdomains in the range where I_1/I_3 decreases rapidly upon increasing C_{PS}. These data have been used to infer preliminary information on the dynamics of polysoaps in aqueous solutions.

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