On the Dynamics of Pyrene Fluorescence Quenching in Aqueous Ionic Micellar Systems. Factors Affecting the Permeability of Micelles[1a]

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Abstract: Pyrene is solubilized in water by means of the surfactants sodium lauryl sulfate (NaLS) and cetyltrimethylammonium bromide (CTAB). These compounds form micelles with hydrocarbon like cores where the pyrene is situated. The pyrene is excited by a doubled ruby laser and the decay of the excited state observed by nanosecond pulse techniques. The rate of decay of the excited state in the presence of added quenchers which dissolve in the water phase, such as O_2, I^-, and triethylamine, describes the factors controlling the movement of the quencher across the micelle. The quenching efficiency decreases with time, an aging effect, which indicates that the micelle achieves greater order with time. In the NaLS micelles this effect may be accelerated by the addition of cations such as Mg^{2+} and with anions with the CTAB. In the NaLS case anions such as SO_4^{2-} and certain neutral molecules such as benzyl alcohol increase the quenching efficiency, and this is attributed to a disruption of the order of the micelles by the added solutes. By the use of mixed micelles with certain reactive groups the motion of the excited state of pyrene in the micelle may be observed. Esr studies and fluorescence polarization studies amplify the laser data. The CTAB micelles contain a large fraction of adsorbed Br^- ions which quench the pyrene excited state. The Br^- ions may be replaced by other ions which lead to a greater or smaller quenching of the excited state. In this case the excited state probe is used to determine the adsorption of ions on the micelle.

Since micelles play an important role in solubilization processes and represent models by which one may study chemical behavior in a microenvironment analogous to membrane systems, considerable effort has been made to study their static and dynamic properties by a variety of physical techniques. Polarized fluorescence measurements[2] have been used to determine the microviscosity of the interior of CTAB micelles in aqueous solution. These values are considerably larger than the bulk viscosities of liquid long-chain hydrocarbons. Recently, Dorrance and Hunter[3a] concluded from absorption and emission studies of pyrene, solubilized in long-chain-cationic micelles, that the micelle core has solid like properties at room temperature. Other fluorescence and photochemical studies have been carried out in micellar solution.[3b-e] Spin resonance techniques have been applied to study the position of solubilizates in the micelle[4] and the exchange rate of spin probes between the aqueous and micellar environment.[5] The movement of molecules across the water–micelle interface can be conveniently investigated by means of the nanosecond laser photolysis technique.[6] A fluorescent probe solubilized in the micellar interior is excited by the laser beam, and in the presence of substances which act as fluorescence quenchers the decay of the fluorescence emission is enhanced. The rate, at which the quencher enters the micelle and/or the fluorescent probe diffuses in the micellar core, determines the kinetics of this quenching process. Consequently a kinetic analysis of the time dependency of the fluorescence emission yields information about the permeability of the micellar shell with respect to the quencher and the movement of the solubilized fluorescent probe in the hydrocarbon interior of the micelle. Since permeability problems are of considerable interest in both the biochemistry of micelles and membranes and solubilization phenomena, the present study was directed to investigate the influence of chemical additives and aging time on the permeability of aqueous sodium lauryl sulfate (NaLS) micelles. These results are substantiated by esr and fluorescence polarization measurements. In addition, fluorescence quenching experiments were carried out with pyrene solubilized in aqueous cetyltrimethylammonium bromide (CTAB) micelles to study its diffusion rate in the micellar core and to check on the selective adsorption of counterions and neutral molecules in the Stern layer of these micelles.

Experimental Section

Materials. Sodium dodecyl sulfate (Matheson Coleman and Bell) and hexadecyltrimethylammonium bromide (Fluka, purum) were recrystallized from methanol. Pyrene (Kodak) was passed through silica gel in cyclohexane solution and then recovered. 2-Methylanthracene (Aldrich) was crystallized from ethanol. Di-*tert*-butyl nitroxide was purchased from Eastman-Kodak and used without further purification. Laboratory distilled water was redistilled from potassium permanganate.

Sample Preparation. Pyrene and 2-methylanthracene were solubilized in freshly prepared aqueous NaLS (0.1 *M*) and CTAB (0.01 *M*) solutions by stirring the mixtures for 4–5 hr at 70°. The concentration of solubilizate was finally checked by optical density measurements.

Apparatus. Laser photolysis experiments were carried out with a ruby laser Korad K1QP with outputs of 2 J and 250 mJ in the 694.2 and 347.1 nm lines, respectively. An Aminco-Bowman

(1) (a) Presented at the 165th National Meeting of the American Chemical Society, Dallas, Texas, April 1973. (b) The Radiation Laboratory is operated by the University of Notre Dame under contract with the U. S. Atomic Energy Commission. This is AEC Document No. COO-38-886.

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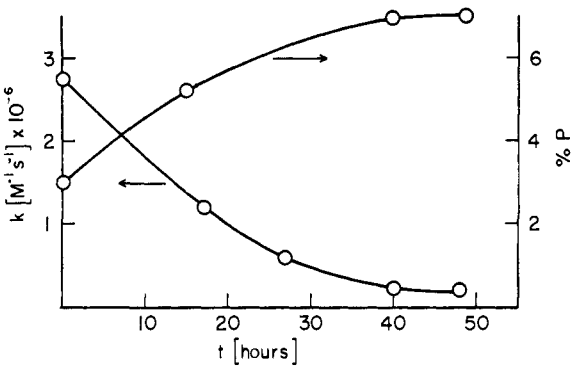

Figure 1. Right ordinate: pyrene fluorescence quenching rate constants k_2 as a function of the aging time of the micelles (10^{-4} M pyrene solubilized in 0.1 M NaLS; quencher, 0.4 M NaI). Left ordinate: influence of aging time on the degree of fluorescence polarization of 2-methylanthracene (10^{-6} M dimethylanthracene in 0.1 M NaLS; excitation wavelength 380 nm).

spectrophotofluorometer was used for fluorescence polarization measurements. Interference and cut off filters were placed in the excitation and emission channels to eliminate light from second order diffraction and scattering. Polacoat 105 polarizers with an effective range from 200 to 800 nm were used. The intensity of the fluorescence emission was measured at crossed ($I_{E\beta}$, $I_{\beta E}$) and parallel (I_{EE}, $I_{\beta\beta}$) positions of the polarizers. The degree of polarization is given by eq 1.[7] The subscripts denote the orientation of

$$P = \frac{I_{EE} - I_{E\beta}(I_{\beta E}/I_{\beta\beta})}{I_{EE} + I_{E\beta}(I_{\beta E}/I_{\beta\beta})} \quad (1)$$

the electrical vector of the light which passes the excitation (first letter) and emission channel (second letter), E meaning a vertical and β a horizontal orientation. In cases where scattering could not be avoided, corrections had to be made since scattered light is strongly polarized. This was carried out by subtracting fluorescence intensities obtained with a blank solution from the observed intensity value and inserting these corrected numbers in eq 1.

Esr spectra were recorded by a Varian V4502 spectrometer using a multipurpose cavity. The spectrometer was operated in the 100 kc mode.

Results and Discussion

Influence of Aging Time on the Permeability of NaLS Micelles. The observed rate constant for the first order decay of the fluorescence in aqueous solutions of micelles, containing solubilized pyrene as a fluorescent probe and a quencher Q in much higher concentration than the number of excited pyrene molecules, is given by

$$k = \ln 2/\tau_0 + k_2[Q] \quad (2)$$

τ_0 is the fluorescence half-lifetime of pyrene in the absence of perturbers and k is the second order rate constant for the quenching reaction. A series of investigations with different types of quenchers shows that k_2 depends on the time which passes between the preparation of the micellar solution and the performance of the quenching experiment. Results which were obtained with iodide as a quencher are shown in Figure 1. A final value of k_2, which is considerably lower than the initial value, is approached after about 40 hr equilibrium time of the micelles. Similar results were obtained using triethylamine as a fluorescence quencher. These are listed in Table I. If an aged micellar solution (solution equilibrated for 40 hr) is heated to the boiling point and then cooled, the properties of the cooled solution revert back to those of the unequilibrated fresh micellar solution; *i.e.*, the rate of quenching of the pyrene fluorescence is that obtained with a fresh micelle. As the effect is reversible, we conclude that no permanent chemical change in the micelle occurs over the equilibration period of 40 hr. The decrease in the rate constants of the quenching reaction is attributed to changes in micellar properties during an aging time of many hours. A completely equilibrated micelle may have a different alignment of the surfactant molecules than a fresh one, which would affect the separation of the charged head groups and the viscosity in the micellar interior. Alternatively the building up of a structured water layer on the surface of the micelle[8] may slow down the entry rate of the quencher into the micellar core. Epr and fluorescence depolarization measurements further elucidated this observation.

Table I. Effect of Aging Time and Mg^{2+} Additive on the Pyrene Fluorescence Quenching Rate Constant k_2, the Microviscosity of the NaLS Micelle Interior, and the Width of the High Field Esr Line of the DTBN Triplet

	k_2, M^{-1} sec^{-1}[a]	Microviscosities, cP[b]	Width of high field esr line, G[c]
Fresh micelle	2.5×10^8	15	0.54
48 hr equilibrated	1.15×10^8	36	0.86
Added 0.04 M Mg^{2+}	0.04×10^8	92	1.20

[a] Solution: 10^{-4} M pyrene in 10^{-1} M NaLS, 7×10^{-3} M triethylamine. [b] From the fluorescence polarization of 2-methylanthracene (10^{-6} M) solubilized in 0.1 M NaLS. Viscosities are calculated from eq 3 assuming $V_0 = 105$ Å.[2] [c] Solution: 5×10^{-4} M DTBN in 0.1 M NaLS.

Polarization Measurements. The degree of polarization of the fluorescence emitted from a molecule, as defined in eq 1, depends on the viscosity of the medium in which it is dispersed. If the dye is excited into the first absorption band, where absorption and emission oscillators are oriented parallel to each other, then the relation is given by eq 3,[2] where P_0 is the degree of

$$\frac{\frac{1}{P} - \frac{1}{3}}{\frac{1}{P_0} - \frac{1}{3}} = 1 + \frac{kT\tau}{\eta v_0} \quad (3)$$

polarization measured in an extremely viscous solvent, τ is the average lifetime of the molecule in the excited state, v_0 is its effective volume, and η is the viscosity.

Figure 1 shows the effect of aging time on the microviscosity of the interior of NaLS micelles with 2-methylanthracene solubilized as a fluorescent probe in the micelles. In hydrocarbon solvents the fluorescence decay time is 3.5 nsec and an effective volume of 105 Å[3] [8] was taken to calculate the viscosities listed in Table I. The trend of the degree of polarization values in Figure 1 reflects an increase in the rigidity of the micellar core with time until a limiting value is reached about 40 hr after the micelles have been prepared. There is an obvious correlation between the time behavior of the quenching rate constant and the microviscosity of the micelles. These results indicate a

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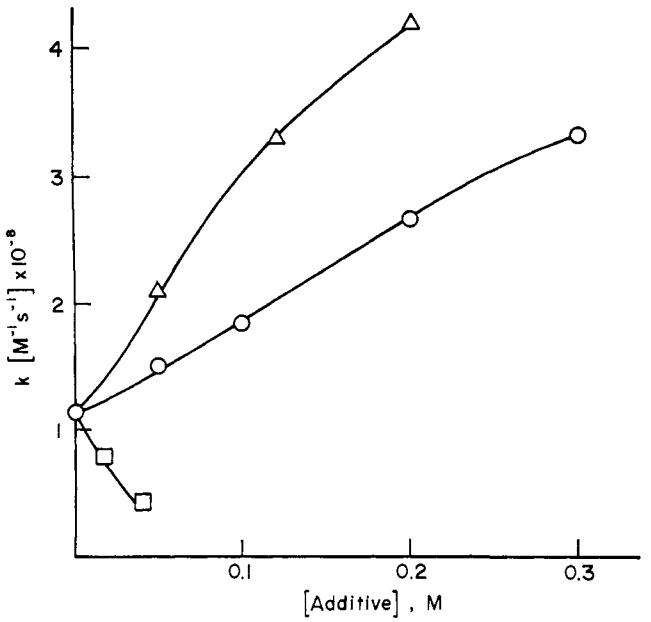

Figure 2. Influence of additives on the quenching rate constant k_2 of the pyrene fluorescence (10^{-4} M pyrene solubilized in 10^{-1} M NaLS; quencher, 7×10^{-3} M triethylamine) Additives: (□) $MgCl_2$, (○) Na_2SO_4, (△) benzyl alcohol

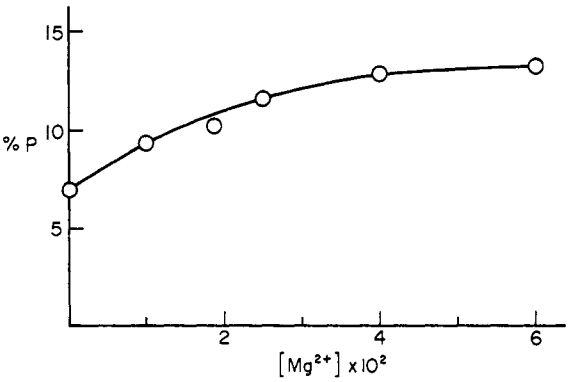

Figure 3. Change of the degree of fluorescence polarization of 2-methylanthracene upon the addition of Mg^{2+} ions: [2-methylanthracene] = 10^{-6} M; [NaLS] = 10^{-4} M.

rather high viscosity for the micelle interior, in agreement with the work of Weber[2] and associates, but do not go as far as to predict a solid like character as suggested by Dorrance and Hunter.[3a]

Esr Measurements. Esr measurements using di-*tert*-butyl nitroxide (DTBN) were used to further substantiate the above effects. At room temperature with a surfactant concentration of 0.1 M, 87.3% of the DTBN molecules are incorporated in the micellar phase.[5] The esr spectrum of this system consists of three lines produced by nitrogen hyperfine interaction. The line width of the hyperfine components is determined by the rate of spin relaxation, chemical exchange between the water and micellar phase, and rotational tunneling, the latter affecting predominantly the high field line. The change of the width of this line during the aging period of NaLS micelles is listed in Table I. A distinct broadening of the line is recognized indicating an increase in the spin rotational correlation time and therefore an increase in the viscosity of the spin probe environment during the equilibration time.

Influence of Additives on the Permeability of NaLS Micelles. As indicated previously, the rate constant k_2 for the reaction of a fluorescent probe solubilized in a micelle with a quencher, which moves between the micellar and aqueous phase, is influenced by the permeability of the interface between the two phases. Thus, permeability changes caused by interaction of the micellar molecules with added substances should show up in the observed k_2 values. Again pyrene solubilized in NaLS is a suitable system for such investigations, since singlet excited pyrene has an unusually long fluorescence decay time of several hundred nanoseconds and trimethylamine is a suitable quencher. The fluorescence data in this system show that the quenching event occurs in the micellar interior. Singlet excited pyrene reacts with triethylamine to form a heteroexcimer which, in polar medium, dissociates immediately into ion–radicals. In a nonpolar environment the exciplex fluoresces with a lifetime of 60 nsec and an emission maximum at 480 nm. In the laser photolysis of pyrene solubilized in aqueous NaLS this heteroexcimer emission becomes noticeable in the presence of triethylamine showing that the quenching event takes place in the hydrocarbon core of NaLS micelles.

The influence of various additives on the quenching rate constant k_2 is demonstrated in Figure 2. Both sulfate ions and benzyl alcohol increase the observed k value indicating that the permeability of the micelle water interface is greater in the presence of these substances. The explanation for this effect is found in the interaction of these molecules with the negatively charged head groups of the surfactant molecules. Phenylalkyl alcohol molecules are known to be adsorbed at micellar surfaces and it was suggested that they might push the heads of the surfactant ions further apart.[9] Such an "opening" of the NaLS micelles may enable the triethylamine quencher to enter the micelle more quickly. A similar mechanism may account for the increased quenching rate in the presence of sulfate ions. The sulfate concentration must be larger than that of the benzyl alcohol to yield an equal increment in k_2. This may be due to the fact that the sulfate concentration according to the negative surface potential of the micelles is smaller on the surface than in the bulk of the solution.

A marked decrease in the rate of the fluorescence quenching reaction is caused by relatively low concentrations of magnesium salts. Specific adsorption of Mg^{2+} concomitant with attraction of neighbored $-SO_4^-$ head groups of soap molecules is the most plausible explanation for this finding. A strong interaction of Mg^{2+} with dodecyl sulfate anions can be inferred from the critical micelle concentration of magnesium dodecyl sulfate which is ten times lower than the respective value for sodium dodecyl sulfate. The contraction of the head groups of surfactant molecules is expected to alter the viscosity of the micellar core, which can be examined by means of fluorescence polarization and esr measurements as described in the preceding chapter. Figure 3 shows how the degree of polarization of light, emitted from 2-methylanthracene solubilized in NaLS, changes upon the addition of Mg^{2+} ions to this solution. A Mg^{2+}

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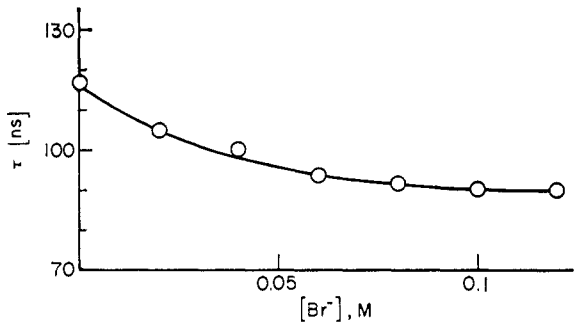

Figure 4. Effect of bromide concentration on the fluorescence decay time of CTAB solubilized pyrene: [pyrene] = 2.5×10^{-5} M; [CTAB] = 10^{-2} M, λ 400 nm.

concentration of 4×10^{-2} M causes an increase in the microviscosity of NaLS micelles from 37 to 92 cP (Table I). Contraction of the charged headings seems to enforce the interaction of the hydrocarbon chains which results in a more rigid micellar interior. Esr studies with aqueous NaLS micelles and solubilized DTBN spin probes confirm this conclusion. It can be seen from Table I that broadening of the high field line of the DTBN triplet is observed on addition of Mg^{2+} to such a solution. This is due to an increase in the spin rotational correlation time resulting from a higher viscosity in the micellar environment of the spin probe.

Fluorescence Quenching in Aqueous CTAB Micelles. It has been suggested[3b,c] that the fluorescence of aromatic probes in aqueous CTAB micelles is affected by the bromide counterions which may act as quenchers. The peculiarity of this system lies in the fact that the location of this quencher is restricted to the surface double layer of the CTAB micelles, while pyrene is incorporated in their interior. Such a situation favors an investigation of the movement of the fluorescent probe within the micellar core. In a first series of experiments, we examined the effect of bromide concentration on the fluorescence lifetime of solubilized pyrene by adding increasing amounts of sodium bromide to the micellar solution. The results, shown in Figure 4, can be understood in terms of the degree of dissociation of CTAB micelles. In the absence of additives and at room temperature these micelles are only to 15% dissociated[10] so that 85% of the bromide counterions are bound in the Stern layer of the micelle. An excited pyrene molecule, while diffusing in the hydrocarbon interior, eventually approaches the micellar shell where its interaction with adsorbed bromide leads to fluorescence quenching. Hence the fluorescence decay time should depend on the degree of coverage of the micelle with bromide ions. Addition of Br^- to the solution increases this coverage and consequently decreases the fluorescence decay time τ. The relative change in τ is small because only a little additional bromide can be adsorbed on the micelle by increasing the bulk Br^- concentration. The limiting value of τ reached at higher Br^- bulk concentrations is about 90 nsec. We can assume that under these circumstances the CTAB micelles are completely covered with bromide and every excited pyrene is quenched at the moment of its arrival at the micelle–water interface. Hence 90 nsec should be the average

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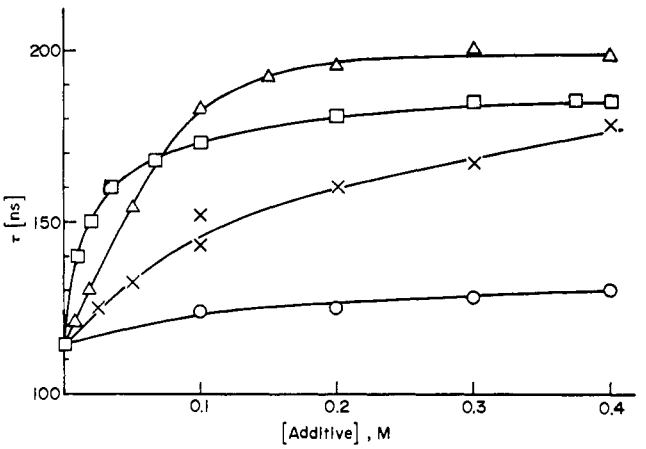

Figure 5. Effect of various additives on the fluorescence decay time of pyrene (2.5×10^{-5} M) solubilized in 10^{-2} M CTAB, λ 400 nm. Additives: (○) NaOH, (×) benzyl alcohol, (□) Na_2SO_4, (△) NaCl.

time the pyrene molecule needs to travel from the center to the shell of the micelle. Fick's diffusion theory yields for this time the expression

$$t = \bar{x}^2 3\pi\eta/kT$$

If in this equation we insert for the mean square path length $\bar{x}$ the CTAB micelle radius of 20 Å,[10] a microviscosity of 30 cP,[2] and an effective radius of the pyrene molecule of 3.2 Å, one obtains $t = 85$ nsec in agreement with the experimentally found value.

If the mechanism which we propose for the fluorescence quenching of pyrene solubilized in aqueous CTAB micelles is correct, replacement of the Br^- ions at the micellar surface by other ions which do not perturb the fluorescence should increase the observed fluorescence decay time. Since dodecyltrimethylammonium sulfate micelles are only 3% dissociated into ions,[10] sulfate ions should have a strong affinity for adsorption at the surface of dodecylammonium micelles. Hence it should be possible to replace bromide from CTAB micelles by added sulfate ions. It is shown in Figure 5 that addition of SO_4^{2-} to CTAB in fact yields an increase in the fluorescence decay time, which is expected if this type of a competitive adsorption takes place. Chloride ions show a similar effect; however, higher concentrations are necessary to get the same increment in τ reflecting a lower value for the binding constant of the chloride–micelle adsorption equilibrium than the respective value for sulfate. Hydroxide anions show very little binding affinity to dodecyltrimethylammonium micelles.

It is surprising that benzyl alcohol replaces bromide at the micellar surface as illustrated by the increased fluorescence decay time τ. However, it is also noted that the conductivity of aqueous micellar solutions of CTAB increases on the addition of phenylalkyl alcohols.[9] One explanation for the above data is that the degree of dissociation of CTAB micelles is higher in the presence of these alcohols. Selective adsorption of ions and neutral molecules at the surface of micelles plays an important role in the micellar catalysis of many reactions. The kinetics of the reaction of methyl chloride solubilized in aqueous CTAB with CN^- [11] for

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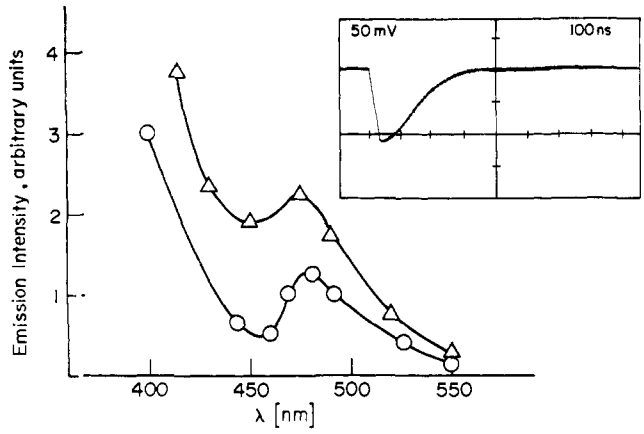

Figure 6. Emission spectra of the laurylamine excited pyrene heteroexcimer in *n*-heptane: [pyrene] = 10^{-4} *M*, [laurylamine] = 4×10^{-2} *M*, emission measured 80 nsec after the start of the laser flash. Mixed micelles of laurylamine and NaLS: [pyrene] = 10^{-4} *M*, [NaLS] = 5×10^{-2} *M*, [laurylamine] = 2.5×10^{-3} *M*, emission measured 100 nsec after the start of the laser flash. Insert: oscillogram of heteroexcimer emission formation and decay, λ 485 nm.

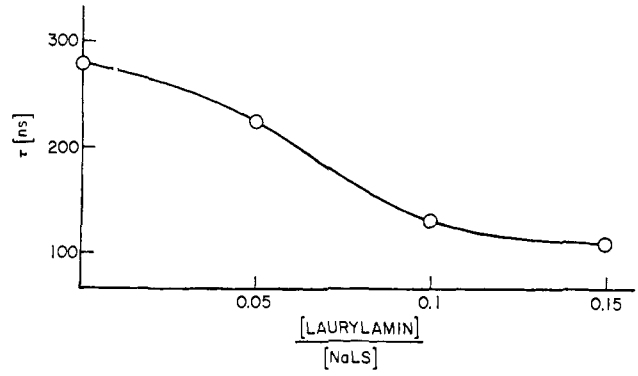

Figure 7. Decay time of the pyrene fluorescence in mixed micelles of NaLS and laurylamine: [NaLS] = 5×10^{-2} *M*.

instance could be readily explained by a competitive adsorption of CN^- and Br^- at the micelle–water boundary.

Kinetics of the Fluorescence Decay of Pyrene Solubilized in Mixed Micelles of NaLS and Laurylamine. Addition of laurylamine to aqueous micellar NaLS solutions leads to the formation of mixed micelles, in which the amino groups are expected to reside preferentially near the polar surface while the hydrocarbon ends extend in the micellar core. By analogy with triethylamine, laurylamine quenches the fluorescence of pyrene forming a heteroexcimer. Examination of this reaction in *n*-heptane gives a bimolecular quenching rate constant of 4×10^8 M^{-1} sec^{-1}. The laser photolysis of pyrene solubilized in mixed micelles of NaLS and laurylamine produces a fluorescence that is identical with the heteroexcimer emission in *n*-heptane. Figure 6 shows the heteroexcimer emission in both solutions with maximum intensity at 480 nm, and the fluorescence decay time at this wavelength is 60 nsec.

As heteroexcimer emission in the mixed micellar solutions is identical with that in heptane, it is concluded that at least some of the amino groups of the laurylamine molecules are located in the interior of the NaLS micelle. The distance between these amino groups and an excited pyrene molecule must be very small since the heteroexcimer emission is present immediately after the laser flash as shown by Figure 6. The remainder of the amino groups are located near the micellar surface. Their influence on the pyrene fluorescence decay time resembles that of the bromide ions in CTAB micelles as described in the previous section. Excited pyrene molecules have to move to the micellar surface to be quenched by the latter amino groups. Hence, there is a decrease of the pyrene fluorescence decay time (measured at 400 nm) on increasing the number of laurylamine molecules per NaLS micelle apart from the immediate quenching of adjacent amino groups. This decrease is shown in Figure 7. No heteroexcimer emission is observed from the quenching events which take place at the micelle–water interface. This is due to the high polarity of this region which causes an immediate, radiationless dissociation of the excited pyrene–laurylamine exciplex into radical ions.

Conclusion

The technique of nanosecond laser photolysis is particularly useful in studying the permeability of micelles, and we are extending the work to biological systems. A biological membrane or a vesicle made of lecithin and cholesterol may solubilize fluorescing probes such as pyrene, thus enabling us to study the factors contributing to the movement of molecules in these systems. The chemistry of micelles itself enables us to create molecular groups with a known geometry, in order to study the diffusion and energy transfer processes under set conditions. Preliminary work on the photoionization of pyrene in micelles provides significant information on the pathway of the liberated electron.

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