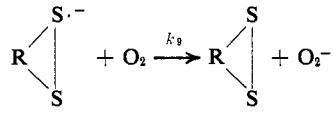
The faster rate of disappearance of dithiothreitol free radical in the presence of molecular oxygen can be represented by eq 9 and 10, where $k_9 = (1.37 \pm 0.15) \times 10^9\ M^{-1}\ \text{sec}^{-1}$ and $k_{10} < 10^2\ M^{-1}\ \text{sec}^{-1}$.[16,17]



(9)

$$O_2^- + O_2^- + 2H^+ \xrightarrow{k_{10}} O_2 + H_2O_2 \qquad (10)$$

Acknowledgment. We wish to thank Dr. A. O. Allen for his constructive criticism and for having read the manuscript. Thanks are also due to Mr. D. Comstock for his excellent technical assistance.

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A Novel Fluorescent Probe. Use of Time-Correlated Fluorescence to Explore the Properties of Micelle-Forming Detergent[1]

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Abstract: A technique is described which utilizes the fluorescence lifetime of naphthalene as a probe for solutions containing micelle-forming detergents. The processes of micellar catalysis and inhibition have been directly observed using the probe. A direct measure of the partitioning of naphthalene between the micelle and aqueous phases has been made and the results are compared with a simple model treating the micellar system as a simple two-phase distribution. The naphthalene probe provides important information concerning the solvent properties and the effective local oxygen concentration of the micelle phase for several detergents. A comparison is made with other types of fluorescence probes commonly used in systems of biological interest.

One of the most fascinating properties of biological systems is their ability to achieve chemical efficiency and specificity by means of organization of reactants.[2] Recently, attempts to map out the distinct microenvironments of biological macromolecules, such as proteins and polynucleotides, have been the subject of intensive research. In particular, investigations of organized multimolecular aggregates such as membranes have received special attention.[3] Fluorescent probes[4] have been elegantly and effectively employed to study the structure of such organized systems. The general idea behind a fluorescent probe is that a molecule, whose fluorescent emission can serve as a sensor of microenvironments, will display a specific affinity for a given site (*e.g.*, the active site of an enzyme relative to bulk solution) *and* will also display *distinct* fluorescent properties which uniquely characterize each environment. In practice a common technique is to employ a probe which is highly fluorescent in apolar environments and is virtually nonfluorescent in water.[4]

Thus, the fluorescent probe technique usually depends on the assumption and hope that any observed fluorescence arises predominately from probe molecules that are bound or associated with the apolar regions of the macromolecules. A decided disadvantage of the fluorecence probe technique is the empirical finding that relatively large and highly polar (or ionic) molecules are required in order to observe a sensitivity of fluorescent properties to medium polarity. Clearly, a probe which has considerable molecular size and is inherently polar is very likely to cause significant perturbations of the very apolar regions which are being explored. Furthermore, one can imagine complications in an analysis due to solvent reorientation at the local environment of the probe upon electronic excitation (indeed, large spectral shifts of emission relative to absorption are a common feature of the commonly employed fluorescence probes). It is quite possible, therefore, that time-dependent fluorescent properties can severely complicate and even vitiate interpretations of results on both a quantitative and qualitative level.[5]

We report here the use of a fluorescent probe that is based on precise measurements of fluorescence decay constants which allows a distinction between two or more different environments available to the probe. The specific method described here is the time-correlated fluorescence of naphthalene dissolved in aque-

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(3) (a) D. Chapman, Ed., "Biological Membranes," Academic Press, New York, N. Y., 1968; F. Snell, J. Wolken, G. Iverson, and J. Lam, Ed., "Physical Principles of Biological Membranes," Gordon and Breach, New York, N. Y., 1970; (b) E. Sackmann and H. Trauble, *J. Amer. Chem. Soc.*, **94**, 4482, 4492, 4499 (1972), and references therein.
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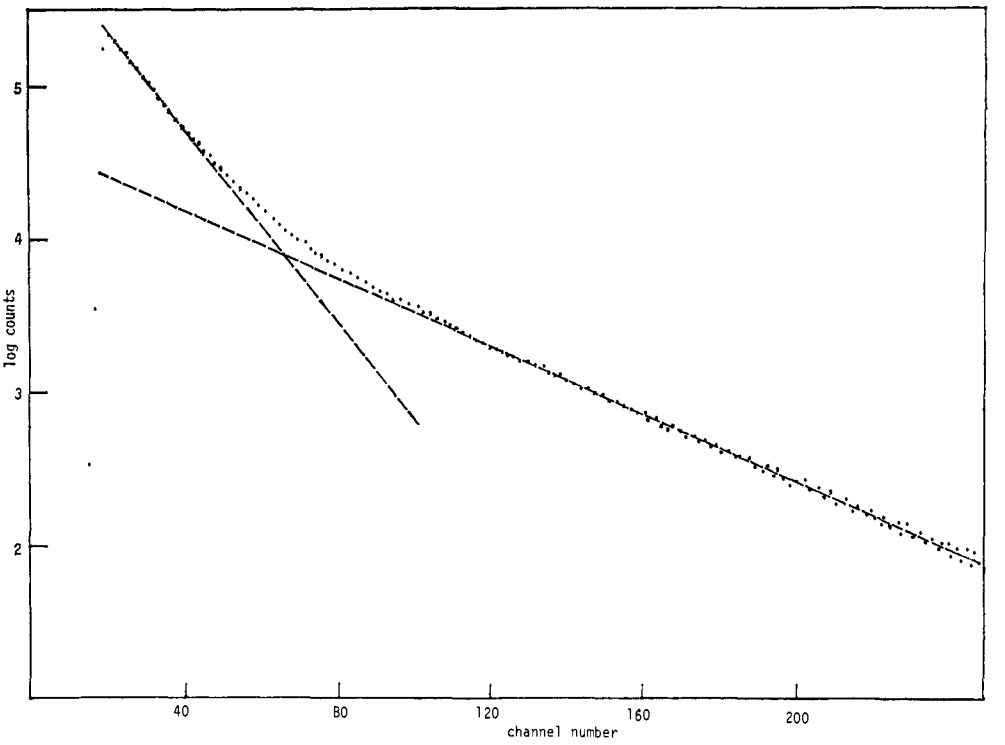

Figure 1. Fluorescence decay curve of naphthalene (2×10^{-4} *M*) in HDTBr (2×10^{-2} *M*) measured using the single photon counting technique. Extrapolation of the long component (τ_b = 34 nsec) to zero excitation time (channel 21) yields a value of 0.11 for the coefficient of the component. The short component (a = 0.89) corresponds to a lifetime of 10 nsec. The multichannel analyzer was calibrated at 9.25×10^{-10} sec per channel for this experiment.

ous solutions of micelle-forming detergents.[6] This technique has the important property of employing small, nonpolar molecules without requiring a different spectral distribution of probe emission or a significant difference in fluorescence intensity in the different environments being explored. As a result of these properties, relatively subtle differences in each environment are revealed by differences in fluorescence decay constants.[5] Furthermore, this technique is, in principle, adaptable to a wide dynamic range and should provide a means of obtaining kinetic information which is not easily available from the use of other probes.

Experimental Section

The detergents used were purified by recrystallization and exhaustive extraction with ether. Naphthalene was recrystallized from ethanol and sublimed. Water was distilled from potassium permanganate. The inorganic salts were reagent grade and used as received.

The fluorescence decay curves were obtained using the single photon counting technique.[7] An air spark flash lamp[8] gave a pulse with a half-width ~2 nsec. The fluorescence was monitored at 324 nm. The multichannel pulse height analyzer (256 or 512 channels) was calibrated at about 0.5 nsec per channel and the number of counts in the peak channel was generally 50,000 to 100,000.

The decay curves were analyzed by three methods: (a) visual extrapolation of the long lifetime component (to time = 0) followed by subtraction of these data to analyze the remaining shorter component; (b) computer analysis based on a modification (by Professor C. Cantor) of the method of moments analysis of Isenberg and Dyson;[9] and (c) comparison with synthetically generated decay curves. All three methods were in good agreement (±1 nsec unless otherwise indicated).

Fluorescence spectra were recorded on an Hitachi Perkin-Elmer MPF2A spectrofluorimeter. Fluorescence quantum yields were related to the known value of 0.19 for naphthalene[10] in deoxygenated cyclohexane using solutions of matched optical density. No evidence for any naphthalene excimer emission was noted in any of the experiments.

The absorption and emission spectra of naphthalene in water, cyclohexane, and detergent solutions are quite similar. Subtle differences were noted but do not require any significant corrections in the experimental data.[11]

Results and Discussion

1. Fluorescence Decay of Naphthalene in Solutions of Cationic Micelle-Forming Detergents. The fluorescence decay curve of naphthalene (2×10^{-4} *M*) in aqueous solutions containing micelle-forming detergents consists of two readily resolvable exponential components (Figure 1). The intensity of fluorescence as a function of time, F(t), for such a system follows eq 1, where a is the fraction of naphthalene emission

$$F(t) = a \exp(-t/\tau_a) + b \exp(-t/\tau_b) \qquad (1)$$

from a micellar environment, b is the fraction of naphthalene emission from an aqueous environment, τ_a and τ_b are the lifetimes of naphthalene in micellar and aqueous environments, respectively, and t is time in seconds.

In Table I values for the fluorescent lifetimes and coefficients are given for naphthalene in solutions containing several representative concentrations of hexadecyltrimethylammonium chloride (HDTCl). The contribution (*a vs. b*) of each component is dependent

(6) For a comprehensive review of physical properties of micelles and chemistry in micelles see: E. J. Fendler and J. H. Fendler, *Advan. Phys. Org. Chem.*, 8, 271 (1970).
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(8) T. Tao, Ph.D. Dissertation, Columbia University, 1969.
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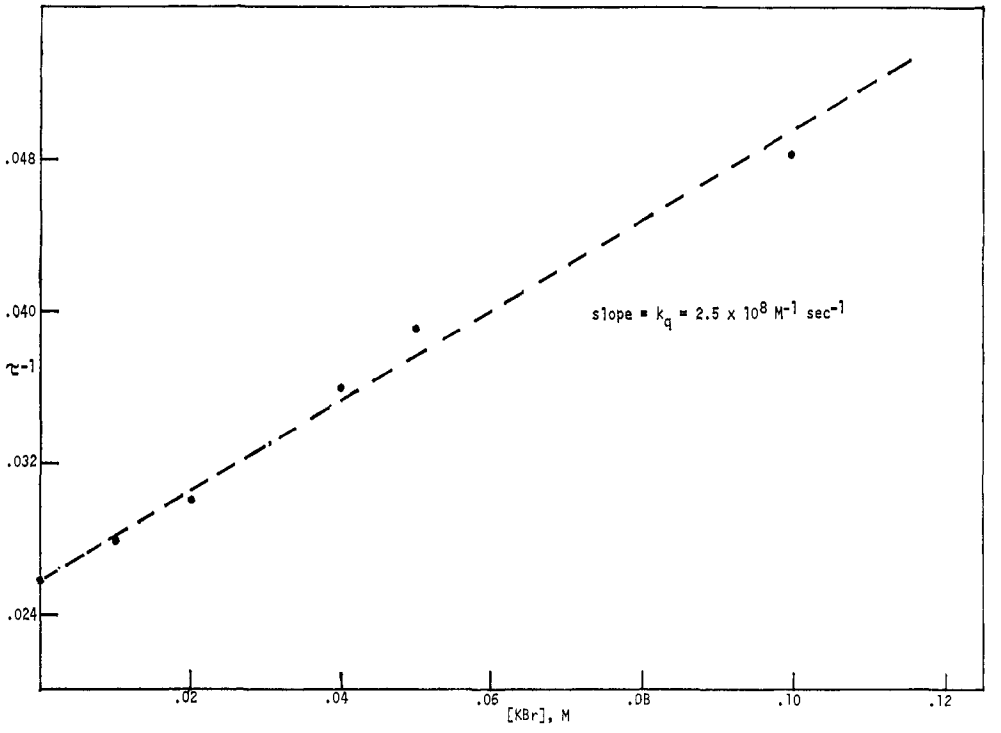

Figure 2. Quenching of naphthalene fluorescence by KBr in water. Units of τ^{-1} are sec^{-1} ($\times 10^9$).

Table I. Contributions to the Fluorescence Decay of Naphthalene in Aqueous Solutions Containing Hexadecyltrimethylammonium Chloride (HDTCl)

[HDTCl], *M*	a_m	τ_a, nsec	b_w	τ_b, nsec
0			1.00	39
0.01	0.50	23	0.50	39
0.05	0.70	23	0.30	39
0.09	0.77	23	0.23	39
0.13	0.84	23	0.16	39

on the detergent concentration in a manner which is suggestive of a simple distribution of naphthalene between the micelle phase and the aqueous phase. The normalized coefficient *a* increases at the expense of *b* as the detergent concentration is increased. The component $b \exp(-t/\tau_b)$ is readily assigned to the fluorescence of naphthalene in water because τ_b is *identical* with the fluorescence lifetime of naphthalene as measured independently in aqueous solutions. The magnitude of τ_a, however, is dependent on the local solvent properties of the micellar interior as experienced by the solubilized naphthalene molecules (*vide infra*).

Somewhat different results are obtained using hexadecyltrimethylammonium bromide (HDTBr) (Table II). Comparison of the fluorescence decay coefficients using HDTBr with those using HDTCl shows a greater fraction of naphthalene associated with HDTBr for a given detergent concentration. As the detergent (HDTBr) concentration is increased, the contribution of micellar component is increased accordingly so that at 0.02 *M* HDTBr 93% of the naphthalene is associated with the micelle. The actual percentage is determined from the observed coefficients using eq 2, which corrects for differences in fluorescence quantum yield

$$\frac{C_{micellar}}{C_{total}} = \left(1 + \frac{b_w \tau_b \epsilon_{mic} \Phi^F_{mic}}{a_m \tau_a \epsilon_{water} \Phi^F_{water}}\right)^{-1} \quad (2)$$

Table II. Contributions to the Fluorescence Decay of Naphthalene in Aqueous Solutions Containing Hexadecyltrimethylammonium Bromide (HDTBr)

[HDTBr], *M*	a_m	τ_a, nsec	b_w	τ_b, nsec	% inside Exptl	% inside Calcd
0			1.00	39		
0.001			1.00	39	0	8
0.002	0.25	16	0.75	38	34	48
0.003	0.52	13	0.48	37	63	64
0.004	0.59	14	0.41	36	70	72
0.005	0.70	13	0.30	36	80	78
0.01	0.86	11	0.14	35	91	88
0.02	0.88	10	0.12	34	93	94
0.03	0.91	11	0.09	33	94	96
0.06	0.94	10	0.06	34	96	98

and extinction coefficient between the two environments.

More striking are the fluorescence lifetime values in HDTBr, which are not only lower than those found for HDTCl, but are sensitive to detergent concentration. Bromide ion was found to quench the fluorescence of naphthalene with a rate constant, k_q, of $\sim 2 \times 10^8$ M^{-1} sec^{-1} (Table III) (obtained from the slope of a plot of τ^{-1} *vs.* [Br^-] according to the Stern–Volmer expression, $\tau^{-1} = \tau_0^{-1} + k_q[Br^-]$, Figure 2). This result implies that the bromide ions associated

Table III. Quenching of Naphthalene Fluorescence in Water by Bromide Ion

[KBr], *M*	τ, nsec
0	39
0.01	36
0.02	33
0.04	28
0.05	26
0.10	21

with the micelle as counterions are effective in quenching the naphthalene which is "dissolved" in the micelle. Indeed comparison of the relatively small decrease in the fluorescence lifetime ($\tau \sim 39$ to 34 nsec at 0.02 M HDTBr) of the naphthalene in the aqueous phase *vs.* that in the micelle phase ($\tau \sim 23$ nsec without bromide, $\tau \sim 10$ nsec with bromide) indicates a micellar "catalysis" of the quenching reaction resulting from the high local concentration of bromide ion.[12]

It is interesting to compare the distribution in the HDTBr system with that predicted from a simple model of a two-phase distribution. The relative solubility of naphthalene in water *vs.* hydrocarbon is *ca.* 1/2500 (determined spectrophotometrically). The volume of the micelle phase can be approximated from the micellar radius and the known aggregation number[13] which is 80 for the HDTBr micelle. At 0.01 M HDTBr (assuming that the total detergent concentration minus the critical micelle concentration represents the detergent molecules comprising the micelle volume) a value of 0.34% for the micellar volume is obtained. Thus, one can predict a value of 7.7 for the ratio of naphthalene in the micelle phase *vs.* the aqueous phase (or 89%) at this concentration. Values calculated for other detergent concentrations are given in Table II and the agreement with the observed values is remarkably good at concentrations sufficiently in excess of the known actual critical micelle concentration of HDTBr (9.2×10^{-4} M) to ensure that most of the detergent is associated into micelles.

2. Fluorescence Decay of Naphthalene in Solutions Containing Cationic Detergents with Added Bromide. The addition of bromide to an aqueous solution containing naphthalene and a micelle-forming detergent results in a decrease in the value of τ_b consistent with the independently measured rate constant for quenching of naphthalene fluorescence in water (Table IV).

Table IV. Effect of Salt Addition on Fluorescence Decay of Naphthalene in Micelle-Containing Aqueous Solutions

[HDTBr], M	[HDTCl], M	[KBr], M	a_m	τ_a, nsec	b_w	τ_b, nsec
0	0.01	0	0.50	23	0.50	39
0	0.01	0.005	0.58	18	0.42	35
0	0.01	0.01	0.62	17	0.38	34
0	0.01	0.02	0.69	13	0.31	32
0	0.01	0.04	0.69	12	0.31	28
0.02	0	0	0.88	10	0.12	34
0.02	0	0.02	0.87	7	0.13	31
0.02	0	0.04	0.86	8	0.14	31

The quenching effect is enhanced for the micellar component τ_a as the local concentation of bromide counterions increases in the vicinity of the micelle. Comparison of τ_a values for comparable [Br^-] concentrations in Tables II and IV shows that the quenching is somewhat reduced in HDTCl solutions *vs.* HDTBr solutions, reflecting competition between chloride (which does not quench) and bromide for counterion "sites."

The nature of the quenching mechanism by bromide has not been explored but could be another example of nucleophilic addition to the photoexcited aromatic or a charge-transfer quenching. Bromide ion (but not chloride) has been found to competitively quench the photoinduced nucleophilic substitution reactions of nitroaromatics without leading to any net product formation;[14] *i. e.*, the bromide probably adds to the aromatic ring but then is eliminated in a subsequent step.

3. Fluorescence Decay of Naphthalene in Solutions of Anionic Micelle-Forming Detergents. In either sodium dodecyl sulfate (SDS) or sodium tetradecyl sulfate (STS) solutions only one fluorescence lifetime is apparent (Table V). That this represents emission from

Table V. Effect of Bromide Ion on the Fluorescence of Naphthalene in Aqueous Solutions Containing Anionic Detergents

[SDS], M	[STS], M	[NaBr], M	$\tau_{micelle}$, nsec
0.03			60 (±4)
0.03		0.04	64
0.03		0.08	64 (±3)
	0.02		69 (±2)
	0.02	0.07	71
			39
		0.04	28
		0.08	22

naphthalene inside micelles rather than in the bulk water is suggested by the lifetimes found when bromide ion is added. In solutions containing anionic detergents the naphthalene associated with the micelle should be protected from quenching by bromide ion. Indeed, addition of sodium bromide to such solutions leads to *no decrease in the lifetime.* (Sodium bromide was used due to its greater solubility in SDS solutions compared with potassium bromide.) The slight *increase* observed probably reflects a general salt effect on the solvent properties of the anionic micelle. These observations are consistent with previously described models for micellar catalysis[6] whereby the bromide ion, in the role of a counterion to the cationic micelle, is highly concentrated around the fraction of the naphthalene "dissolved" in the micelle. Conversely, the naphthalene dissolved in the anionic micelle is protected from quenching by bromide by virtue of the unfavorable interaction between anions and the anionic detergent.

The remarkably large fluorescence lifetime of naphthalene in the anionic micelles reflects the nature of the micellar interior with respect to two variables: polarity (or, more specifically, the presence of water) and oxygen concentration, both of which exert substantial effects on the naphthalene lifetime. In Table VI are listed the values of the naphthalene fluorescence lifetime in a variety of solvents and mixed solvents (air saturated and deoxygenated). The values range from 17 nsec in aerated cyclohexane to 110 nsec in

(12) From the change in lifetime a value of the product k_q[Br^-] can be obtained which allows comparison of the reactions in the micellar and aqueous phases. The values for 2×10^{-2} M HDTBr for k_q[Br^-] are 47×10^6 in the micelle phase *vs.* 2×10^6 in the water phase. Thus the effective micellar catalysis under these particular conditions is a factor of 23 which could result from an effective increase in the *local* concentration of bromide ([Br^-]), an increase in the rate constant (k_q) for quenching, or both.

(13) H. V. Tartar, *J. Colloid. Sci.*, **14**, 115 (1959).

(14) E. Havinga, R. O. deJongh, and M. E. Kronenberg, *Helv. Chim. Acta*, **50**, 2550, (1967); J. H. McCain and R. L. Letsinger, *J. Amer. Chem. Soc.*, **91**, 6425 (1969).

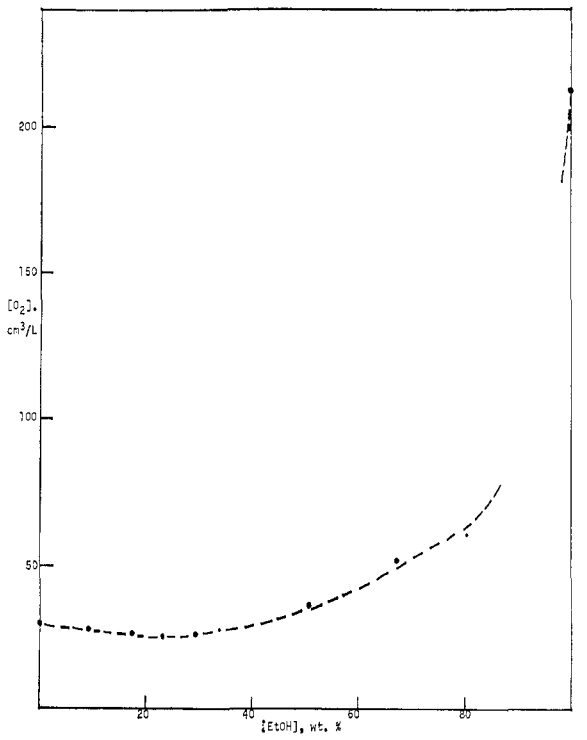

Figure 3. Oxygen solubility in ethanol–water mixtures. Ethanol concentration in g per 100 g of EtOH–H_2O solution. See "Gmelin Handbuch der Anorganischen Chemie," System No. 3, p 456.

Table VI. Effect of Solvent and Oxygen on the Fluorescence Lifetime of Naphthalene

Solvent	Conditions	τ, nsec
Cyclohexane	Degassed	108
Acetonitrile	Degassed	110
Ethanol (95%)	Degassed	52[5a]
Water	Degassed	45
Cyclohexane	Air saturated	17
Acetonitrile	Air saturated	18.5
Water	Air saturated	39
CH_3CN:H_2O (1:3)	Air saturated	48
CH_3CN:H_2O (1:1)	Air saturated	39
CH_3CN:H_2O (3:1)	Air saturated	30

degassed acetonitrile or cyclohexane. The larger drop in cyclohexane or acetonitrile relative to water upon aeration is due to the higher solubility of oxygen in the former (for air-saturated solutions, $[O_2] = 2.6 \times 10^{-4}$ M in water, 4.9×10^{-3} M in cyclohexane). Comparison of the values (degassed) shows a drop in τ as the hydroxylic character of the solvent is increased (CH_3CN to EtOH to H_2O; τ = 110 to 52 to 45 nsec). The combination of this effect with the variable solubility of oxygen in these solvents gives rise to an inverted order of τ in aerated solutions.

Since the detergent solution is a sort of "mixed solvent" system, it is of interest to note that oxygen solubility in mixed solvent systems involving water is not a linear function of solvent composition but remains very low over a wide range of water concentration (Figure 3). Of particular interest in this regard is the study of naphthalene fluorescence in mixed acetonitrile–water solvents (Table VI) which demonstrates this lack of parallel. For the solution containing 25% CH_3CN and 75% H_2O, a higher τ value was observed than in either solvent neat. Thus, the τ values observed in

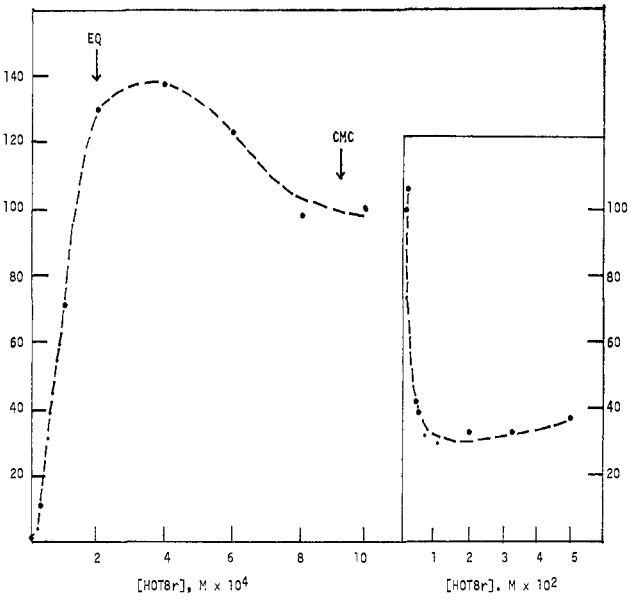

Figure 4. Effects of HDTBr on the fluorescence intensity (495 nm) of 8-toluidinyl-1-naphthalenesulfonate (2×10^{-4} M) in water. The arrows indicate the concentrations of HDTBr corresponding to an equivalent of HDTBr (EQ) and the critical micelle concentration (CMC).

the cationic *vs.* anionic detergent systems could reflect more polar character, a greater solubility of oxygen, or both in the former.

Comparison with Other Fluorescent Probes

Wide use of various aminonaphthalenesulfonates as probes for hydrophobic microenvironments in proteins[4,15] and membranes[5a,b] is based on the fact that these compounds exhibit strong fluorescence in apolar solvents (at least in solvents less polar than water) and extremely weak fluorescence in water. For example, 8-anilino-1-naphthalenesulfonate has a fluorescence quantum yield in water of 0.004 (λ^f_{max} 515 nm) and a yield in *n*-butyl alcohol of 0.5 (λ^f_{max} 462 nm). When associated with apomyoglobin the fluorescence yield has been found to be near 1 (λ^f_{max} 454 nm).[5e] The nature of these probes, however, raises some important questions. Why should a charged and highly polar compound such as this seek a hydrophobic environment?[5c,16] Are the binding sites perturbed by the character of the functional groups and the large size of the probes?[17] In particular, is this the case after electronic excitation, resulting in possible time-dependent emission properties? Is the nature of their binding dependent on specific interactions?

We have examined the fluorescence behavior of 8-toluidinyl-1-naphthalenesulfonate in the presence of varying concentrations of HDTBr and found that indeed the fluorescence yield of 1,8-TNS is enhanced by at least a factor of 100 at the most favorable detergent concentration. However, we note two disturbing features: (1) the solutions become noticeably opalescent, indicative of a heterogeneous suspension, and

(15) G. H. Dodd and G. K. Radda, *Biochem. J.*, **114**, 407 (1969).
(16) D. F. H. Wallach, E. Ferver, D. Selin, E. Weidekamm, and H. Fischer, *Biochim. Biophys. Acta*, 203, 67 (1970).
(17) W. Kesslauer, J. Cain, and J. K. Blasie, *Biochim. Biophys. Acta*, **241**, 547 (1971).

(2) the most abrupt change occurs not at the critical micelle concentration (Figure 4) of the detergent ($\sim 10^{-3}$ *M*), as would be expected if the probe associated with the micelle, but at the equivalent concentration of the probe indicating a 1:1 association complex. Davis has also reported recent results using dansylglycine as a probe for a variety of micelle-forming detergents and found similar difficulties and peculiarities.[18]

It seems clear that probes of this type are not suitable for providing information on the *micellar* environment. Indeed it may be necessary to critically reevaluate the results obtained from other systems; *e.g.*, the effect of oxygen on fluorescence yields will probably play an important role in addition to the environmental polarity.[19]

The smaller size and lack of polar or ionic functional groups in naphthalene should greatly reduce the degree of perturbation of a hydrophobic microenvironment. It is certainly easier to understand why naphthalene partitions favorably into a hydrophobic environment. As we have shown, naphthalene does not exchange environments during the singlet lifetime. Furthermore, the lack of any significant difference in the absorption and emission spectra of napthalene in a variety of media indicates a minimal interaction of the electronically excited species with the environment.

The fact that bromide ion (but not chloride) quenches naphthalene fluorescence is a bonus feature with regard to the micellar system for it allows a detailed study of the factors influencing interactions between a substrate dissolved in a micelle and a counterion of the micelle. This is a typical situation for micellar catalysis of chemical reactions.[20] Furthermore, this technique is suitable for studies involving competition between various counterions and the effects of denaturants on micellar catalysis. Figure 5 summarizes the inhibition and catalysis of naphthalene quenching by bromide.

Implicit in the analysis of the observed decay curves is that each excited naphthalene molecule remain in the environment in which it was excited (until the singlet state is electronically deactivated). If this were not the case each component would exhibit deviation from exponential decay, the extent of which would be related to the relative rates of singlet deactivation *vs.* the rates of "immobility" between the phases. We specifically looked for evidence of this effect, in an attempt to measure a typical rate for this potentially competing process, but found none. Thus the system appears to be relatively static over the time scale involved in these experiments ($\sim 10^{-8}$ to 10^{-7} sec). Nmr line broadening techniques have been used to set an upper limit ($<10^{-4}$ sec) for the lifetime of an average benzene molecule in a sodium dodecyl sulfate micelle.[21] It seems that an average lifetime for a micellar-bound substrate is somewhere between these limits. In this regard, the exchange rate of a paramagnetic surfactant between the aqueous and micellar phases has been measured at 10^{+5} sec^{-1} at room temperature using epr techniques.[22]

(18) G. A. Davis, *J. Amer. Chem. Soc.*, **94**, 5089 (1972).
(19) For an example of the use of oxygen quenching of fluorescence as a probe of oxygen concentration in microenvironments see: W. M. Vaughan and G. Weber, *Biochemistry*, **9**, 464 (1970).
(20) R. R. Hautala and R. L. Letsinger, *J. Org. Chem.*, **36**, 3762 (1971).
(21) T. Nakagawa and K. Tori, *Kolloid Z.*, **194**, 143 (1964).

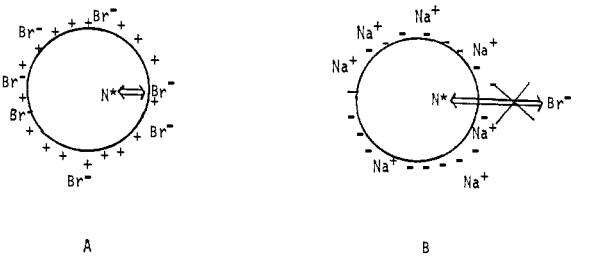

Figure 5. Crude representation of photoexcited naphthalene (N*) in a cationic micelle (A) and an anionic micelle (B). The fluorescence quenching of bromide is facilitated in A by virtue of the role played by bromide as a counterion of the micelle. In B interaction between N* and bromide is precluded by the repulsion of bromide from the negatively charged micelle.

Comparison with Other Recent Work Dealing with Fluorescent Probes in Micelles

Since the submission of this paper, three relevant papers concerned with the fluorescence of micelle-solubilized aromatic hydrocarbons have appeared in the literature.[23-25] The work of Patterson and Vieil[23] is in general agreement with our data. These workers found that bromide ion decreased the fluorescence lifetime of HDTCl solubilized anthracene. Their conclusions are quite analogous to ours.

A recent publication by Dorrance and Hunter,[24] however, comes to the conclusion that the effective concentration of oxygen in micelles from HDTBr is very low. Their conclusions are based mainly on absorption and static emission studies of pyrene. They comment that pyrene mobility in the micelle is indicated by the observation of excimer emission, but the quenching of pyrene fluorescence by oxygen seems to be inhibited (either by a very low local concentration of oxygen or by some feature of the micellar exterior which immobilizes the oxygen molecules). The work of Pownall and Smith,[25] employing a kinematic method, also suggests a high viscosity (about 100 to 200 cP) of the micellar interior.[26]

Since these conclusions differ from ours, we have made some preliminary measurements of pyrene fluorescence lifetimes in aerated HDTCl, HDTBr, and STS (Table VII). Our results show that the fluorescence

Table VII. Fluorescence Lifetimes of Pyrene under Various Conditions

Solvent	τ_F, nsec
Cyclohexane[a]	20
Cyclohexane[b]	202
Cyclohexane[c]	370
Water[a]	131
Water/0.015 *M* HDTBr[a]	120
Water/0.015 *M* HDTCl[a]	156
Water/0.015 *M* STS[a]	175

[a] Air-saturated solutions. [b] Nitrogen-purged solutions. [c] Vacuum-degassed solutions.

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lifetime of pyrene in HDTCl micelles is considerably shorter than for nitrogen-purged or vacuum-degassed cyclohexane solutions. Furthermore, the fluorescence lifetime of pyrene in HDTBr micelles is shorter than in HDTCl micelles and the longest fluorescence lifetimes are found for pyrene solubilized in STS micelles. Thus, our results with pyrene are qualitatively similar to those for naphthalene, except that the solubility of pyrene in water was so small in our experiments that no ultraviolet absorption from aqueous pyrene could be detected.

We also point out that excimer emission can complicate fluorescence analysis in the case of pyrene. In the concentration range used for our experiments (10^{-6} to 10^{-7} M) no excimer emission was observed. Further work is in progress to elucidate the role of oxygen quenching in micellar environments.

Conclusions

In summary, although our work is somewhat preliminary in nature, we believe that our results demonstrate that use of time-correlated studies will be capable of affording considerable understanding of phenomena occurring in micelle solutions including both dynamic and structural information by *direct* observation of processes in the micelle phase and bulk solution. The use of micelle-forming detergents to organize and direct chemical reactions is generally based on measurement of some average property of the solution. In most, if not all, previous studies, it has been necessary to assume that a dissolved substrate is totally associated with the micelle or to deduce from kinetic analysis the magnitude of the observed properties which derive from the micellar and aqueous phases. Some interesting effects, which have been previously unrecognized, are suggested by this work. There appears to be a difference in the effective oxygen concentrations in the hydrocarbon core of different micelles. This could result from either a lower (kinetic or equilibrium) concentration of oxygen in the micellar interior or from a tremendous decrease in the quenching constants for aromatic singlet states by oxygen in a micellar environment. The "catalysis" and "inhibition" of quenching of singlet states by oxygen have been put forth and are supported by the strong quenching of solubilized naphthalene fluorescence by bromide ion in cationic micelles and the resistance to bromide ion quenching of naphthalene solubilized in anionic micelles. It is also possible that water is playing a special role in quenching naphthalene fluorescence; *i.e.*, water penetration is greater into the cationic micelle than into the anionic micelle.[27]

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Nuclear Spin Relaxation in Di-*tert*-butyl Nitroxide

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Abstract: Measurements of the transverse and longitudinal relaxation times of the protons in the di-*tert*-butyl nitroxide radical have been made at a series of radical concentrations and at three different frequencies. The relaxation times are accounted for by intermolecular and intramolecular electron–proton interactions. The intermolecular contribution to the relaxation times is explained in terms of the electron–proton dipole–dipole interaction. The intramolecular dipolar interaction is used to account for the longitudinal relaxation times, while the transverse relaxation times are determined by both the dipolar interaction and the Fermi contact interaction.

The interaction of the unpaired electrons on transition-metal ions or organic free radical with nuclear spins provides an efficient mechanism for nuclear spin relaxation. Investigations of nuclear relaxation times can provide information on electron–nuclei separations, scalar coupling between the spins, and correlation times for molecular motion. There have been a number of studies of the intermolecular interaction between the electron spins on transition-metal ions and solvent nuclei.[2] Intermolecular electron–nuclear interactions between organic free radicals and solvent nuclei have also been investigated.[3] A study of the relaxation times of the protons in di-*tert*-butyl nitroxide has been reported.[4] The nuclear relaxation times are normally dominated by the electron–nuclear dipole–dipole and Fermi contact interactions. The dipolar interaction can be modulated by rotational or translational motion or by various types of exchange interactions. The Fermi contact interaction is modulated by interactions which affect the lifetimes of the electron spin states.

Nuclear magnetic resonance spectra of organic radicals can be obtained from molecules in which the lifetimes of the electron spin states are short compared with the reciprocal of the hyperfine splitting constant.[5] In this case, the hyperfine field generated by the electron at the nucleus is rapidly averaged and one observes a

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