(conformation D) are similar, which suggests similarity in their conformations. In contrast, the amide III region of VM-CH_3OH is different. In particular, the disappearance of the high-frequency component of the amide III vibration may reflect the loss of hydrogen bonds in the more polar solvent.

Deuteration of VM in CCl_4 solution (Figure 4) corroborates the assignment of the 1248- and 1313-cm^{-1} peaks of VM-CCl_4 to amide III vibrations; these vibrations appear at 1252 and 1307 cm^{-1} in crystalline VM (conformation D). The upward shift of the 1307-cm^{-1} peak to 1313 cm^{-1} may account for its apparent increase in intensity in solution, since it is superposed on a second peak between 1325 and 1330 cm^{-1}. In CH_3OH (Figure 3d) the intensity of the 1250-cm^{-1} peak is reduced to roughly half that of the 1275-cm^{-1} peak, and no amide III peak appears above 1300 cm^{-1}.

Conclusion

This study has focussed on the effect of different solvents on the amide and ester C=O stretch frequencies of valinomycin. Using the Raman spectrum of the solid-state conformation D as a reference, tentative conclusions were drawn about the extent of hydrogen bonding of the amide and ester C=O groups. We find evidence suggesting that hydrogen-bonded ester C=O groups may be present in VM dissolved in nonpolar solvents; this in turn may indicate that conformer D is among the conformers present.

Increasing the polarity of the solvent shifts the conformational equilibrium of VM to forms containing fewer hydrogen bonds; these results are consistent with findings using other techniques.[8-11] A plot of ν_{CO} vs. dielectric constant of the solvent reveals an *increase* in ν_{CO} for amide C=O groups and a *decrease* in ν_{CO} for ester C=O groups. This may reflect weakening or rupture of *amide* C=O hydrogen bonds induced by polar or hydrogen bonding solvents and a concomitant increase in solvent interaction with *ester* C=O groups.

Further Raman studies will be made of VM incorporated into bilayers, as has recently been done for the membrane protein opsin.[27]

Acknowledgments. We acknowledge helpful discussions with Drs. R. C. Lord, W. L. Duax, B. C. Pressman, G. Phillies, B. Tokar, and R. Sanches. This work was supported by an NEI Fellowship to one of the authors (K.J.R.), the Research Corporation, and an NIH Biomedical Sciences Support Grant (NIH-5-S05-RR07047-08).

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Environmental Effects on Vibronic Band Intensities in Pyrene Monomer Fluorescence and Their Application in Studies of Micellar Systems

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Abstract: The fluorescence intensities for various vibronic fine structures in the pyrene monomer fluorescence show strong solvent dependence. In the presence of polar solvents, there is a significant enhancement in the intensity of the 0–0 vibronic band at the expense of other bands. This strong perturbation in the vibronic band intensities is more dependent on the solvent dipole moment than on the bulk solvent dielectric constant. This suggests the operation of some specific solute–solvent dipole–dipole interaction mechanism. The strong perturbation of the vibronic band intensities has been used as a probe to accurately determine critical micelle concentrations and also to investigate the extent of water penetration in micellar systems.

Fluorescence probe analysis is becoming an important area in biophysical studies of multimolecular aggregates such as micelles[2] and membranes.[3] Studies with pyrene as a fluorescence probe have received special consideration.[4,5] Pyrene has several interesting photophysical properties which make it suitable for use as an effective probe, notably the long life-

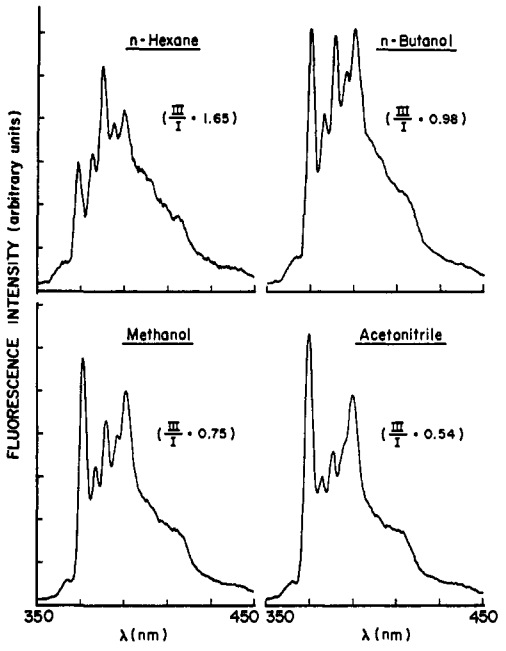

Figure 1. Solvent dependence of vibronic band intensities in pyrene monomer fluorescence: [pyrene] = 2 μM; λ_{excit} = 310 nm.

time of pyrene monomers (τ_M^{fl} = 450 ns and Φ_M = 0.60 in cyclohexane) and efficient formation of excimers. There have been extensive studies on the photophysics of pyrene: its electronic spectrum and state assignments,[6] kinetic details of excimer formation,[7] spectral pressure effects,[8] formation and kinetics of excited states,[9] photoionization,[10] delayed luminescence,[11] and quasilinear spectra,[12] yet there have been few studies directed toward environmental effects on the fluorescence spectrum of pyrene.

The solvent dependence of vibronic band intensities in pyrene monomer fluorescence was first investigated by Nakajima.[13-16] The intensities of the various vibronic bands were found to show a strong dependence on the solvent environment. In the presence of polar solvents there is an enhancement in the intensity of the 0-0 band at the expense of others. As with the Ham bands of benzene, the 0-0 band in the UV absorption and fluorescence of pyrene undergo perturbations in intensities due to vibronic coupling.[13,14] The earlier studies were extended to cover isomeric solvents[15] as well as chlorinated aromatic solvents. Though the intensity enhancements of forbidden vibronic bands were found to be due to solute-solvent interactions, no conclusions could be reached as to the exact mechanisms involved in the above solute-solvent interactions. Both solvent dipole moment and dielectric constants were found to be important in these effects. We have reinvestigated this solvent perturbation of vibronic bands in further detail, quantitatively, in a wide variety of solvents. The solute-solvent interaction involved in the perturbations appear to be complex. Our conclusions are essentially those of Nakajima, viz. the major contributions to vibronic band intensities is from specific solute-solvent dipole-dipole coupling, although other effects due to π-orbital interactions between solute and solvent and bulk dielectric constant of solvent cannot be neglected. The strong perturbations in the vibronic band intensities have been used, later, as a fluorescence probe in the study of micellar aggregates. The variations in the vibronic band intensity ratio have been used to accurately determine critical micelle concentration and also to investigate the extent of water penetration into micelles.

Experimental Section

Steady state fluorescence spectra were recorded on an Aminco-Bowman spectrophotofluorimeter using narrow (0.2-mm) slits.

Table I. Principal Vibronic Bands in Pyrene Monomer Fluorescence[a]

Peak	λ, nm	ν, cm^{-1}	Distance from 0-0 line	Assignment (IR/Raman active)	Vibrational mode and symmetry type
I	372.51	26 845	0	0-0	
	378.23	26 439	406	0-406 (R)	a_g (ω)
II	378.95	26 389	456	0-456 (IR)	b_{1g} (τ)
	379.58	26 345	500	0-500 (IR)	b_{1g} (τ)
III	383.03	26 108	737	0-737 (IR)	b_{1g} (κ)
	384.00	26 042	803	0-803	a_g (κ)
	387.99	25 774	1071	0-1071 (R)	a_g (δ)
IV	388.55	25 737	1108	0-1108 (R)	b_{1g}
	389.08	25 702	1143	0-1143 (R)	a_g (δ)
	390.42	25 613	1232	0-1232 (R)	a_g (δ)
	391.80	25 523	1322	0-1322 (R)	($a_g + b_{1g}$) (κ)
	392.49	25 478	1367	0-1367 (R)	b_{1g}
	392.85	25 455	1390	0-1390 (R)	a_g (ω)
V	393.09	25 439	1406	0-1406 (R)	a_g (ω)
	395.34	25 295	1551	0-1551 (R)	a_g
	396.04	25 250	1595	0-1595 (R)	b_{1g} (ω)

[a] Data taken from ref 12 and 20.

Lifetime studies employed a 347.1-nm ruby laser photolysis apparatus, using the principles of fast kinetic spectroscopy. Detailed description of this setup and other experimental details have been described elsewhere.[17]

Pyrene (Kodak) was passed through silica gel in cyclohexane solution and then recovered. All solvents employed were spectrograde. The detergents used were all from commercial sources and these were recrystallized at least twice from ethanol/ether mixtures before use. The data for solvent dielectric constants (ϵ) and dipole moments (D) listed in Table II are taken from ref 18 and 19.

Results and Discussion

I. Pyrene Monomer Fluorescence in Homogeneous Solvents. Pyrene is one of the few condensed aromatic hydrocarbons which shows significant fine structure (vibronic bands) in its monomer fluorescence spectra in solution phase. In the absence of any solvent interactions with the solute (either individually or collectively), the relative intensities of these vibronic bands in the fluorescence spectrum are governed, as in UV absorption spectra, by the relative positions of the potential energy surfaces of the excited singlet states relative to the ground state singlet and by the Franck-Condon principle. Figure 1 presents representative monomer fluorescence spectra for pyrene (in very dilute solutions, pyrene concn = 2 μM, so that complications due to excimers do not arise) in four different solvents. As was noted earlier by Nakajima,[13-16] these spectra clearly show that the vibrational fine structure intensities undergo significant perturbations on going from nonpolar solvents such as *n*-hexane to polar solvents with high permanent dipoles such as acetonitrile. For the sake of convenience in subsequent discussions, the *five* predominant peaks are numbered as I-V and variations in the intensities of the various bands are discussed with respect to the 0-0 band (peak I). Table I summarizes the relative positions of the principal vibronic bands in the pyrene monomer fluorescence together with tentative assignments.[20] The table lists 16 vibronic bands (including the *five* bands referred to above, and seen clearly at room temperature studies) which have been identified in studies at 4 K and for which specific assignments have been made.

In order to gain a better insight into the exact solute-solvent interactions involved in the perturbation of the vibronic band intensities, we have examined at room temperature (25 °C) the relative peak intensities in a wide variety of solvents. Solvents of increasing dielectric constant and dipole moment in the gas phase were selected for study. Table II summarizes the

Table II. Solvent Dependence of Vibronic Band Intensities in Pyrene Monomer Fluorescence

Solvent	Solvent[a] D (Debye)	Solvent[a] ϵ	Rel peak intensities I	II	III	IV	V
		Simple Polar Solvents					
$(CH_3)_2S{=}O$	3.96	46.68	1.00	0.44	0.53	0.54	0.72
CH_3CN	3.92	37.50	1.00	0.48	0.57	0.60	0.78
$HCON(CH_3)_2$	3.82	36.70	1.00	0.46	0.55	0.58	0.76
$NCONH(CH_3)$	3.83	182.0	1.00	0.53	0.65	0.64	0.81
CH_3COCH_3	2.88	20.7	1.00	0.55	0.68	0.74	0.89
$(CH_2OH)_2$	2.28	37.70	1.00	0.50	0.61	0.62	0.79
H_2O	1.85	78.54	1.00	0.60	0.63	0.71	0.85
$CH_3COOC_2H_5$	1.78	6.02	1.00	0.53	0.69	0.65	0.83
HCOOH	1.41	58.5	1.00	0.52	0.61	0.68	0.82
CH_3COOH (Gl)	1.74	6.15	1.00	0.59	0.77	0.82	0.95
C_4H_8O (THF)	1.63	7.58	1.00	0.63	0.83	0.83	0.98
CH_3OH	1.70	32.7	1.00	0.55	0.75	0.69	0.88
CH_3CH_2OH	1.69	24.55	1.00	0.69	0.91	0.88	1.06
$CH_3CH_2CH_2OH$	1.68	20.3	1.00	0.65	0.92	0.80	0.96
1-BuOH	1.66	17.5	1.00	0.68	0.98	0.83	0.99
1-Pentalol	1.65	13.90	1.00	0.74	1.07	0.95	1.10
$CH_3CH(OH)CH_3$	1.05	19.9	1.00	1.21	1.10	0.99	1.23
$(C_2H_5)_2O$	1.15	4.34	1.00	0.69	1.02	0.86	1.03
CH_2Cl_2	1.60	8.93	1.00	0.56	0.73	0.71	0.87
$CHCl_3$	1.01	4.80	1.00	0.60	0.78	0.73	0.88
1-Chlorobutane	2.05	7.39	1.00	0.64	0.92	0.81	0.98
1-Cyanobutane	4.12	20.30	1.00	0.58	0.74	0.75	0.93
		Aromatic Solvents					
Benzyl alcohol	1.71	13.1	1.00	0.63	0.82	0.82	0.95
Benzene	0	2.28	1.00	0.65	0.88	0.82	0.96
Toluene	0.36	2.37	1.00	0.63	0.90	0.79	0.94
Chlorobenzene	1.65	5.62	1.00	0.69	0.92	0.82	0.99
Cumene		2.38	1.00	0.68	0.98	0.84	0.98
p-Xylene	0	2.27	1.00	0.74	1.00	0.93	1.00
		Hydrocarbon Solvents					
n-Hexane		1.89	1.00	1.03	1.65	1.23	1.32
Cyclohexane		2.02	1.00	1.04	1.68	1.25	1.39
Isooctane			1.00	1.04	1.68	1.23	1.36
Dodecane		2.01	1.00	1.05	1.67	1.30	1.39
Dimethylbutane			1.00	1.05	1.74	1.32	1.41
2-Methylhexane			1.00	1.05	1.73	1.29	1.40
3-Methylpentane			1.00	1.07	1.77	1.30	1.40
Squalene			1.00	1.06	1.74	1.30	1.41
Methylcyclohexane		2.02	1.00	1.14	1.80	1.43	1.57
$(CH_3)_4Si$	0		1.00	1.14	1.71	1.34	1.54
		Saturated Fluorocarbon Solvents					
Perfluoromethylcyclohexane			1.00	1.17	2.00	1.43	1.54

[a] Data for solvent dipole moment (D) and dielectric constant (ϵ) are taken from ref 18 and 19.

relative peak intensities for the five aforementioned principal vibronic bands in various solvents. The intensities were normalized with reference to the 0-0 band (peak I). For reference this table also includes the solvent bulk dielectric constant and the dipole moment in the gas phase. Since the peak III shows maximum variations in intensity relative to the 0-0 band, henceforth the relative intensity of peak III to peak I, referred to hereafter as the 3/1 ratio, will be used to discuss the environmental effects on pyrene monomer fluorescence.

Examination of the data presented in Table II leads to several interesting results.

(i) In hydrocarbon solvents of very low dielectric constant ($\epsilon \leq 2$), the relative peak intensities show minimum variations (3/1 ratio 1.65–1.75) for a wide variation in hydrocarbon configuration, viz. chain length, branching, cyclic vs. acyclic, as well as the presence of unsaturated double bonds.

(ii) The solvents examined in this study can be broadly classified into three groups: (a) simple polar solvents with 3/1 ratio in the 0.50–0.80 range; (b) aromatic solvents (3/1 ratio 0.80–1.00); and (c) hydrocarbon solvents (3/1 ratio 1.65–1.75). In each group of solvents, the peak ratio decreases with increasing dipole moment of the solvent. For any two given solvents with the same dipole moment, the one with the higher dielectric constant has the smaller ratio.

(iii) The maximum value for the 3/1 ratio was observed in perfluorinated saturated hydrocarbons. For example, in perfluoromethylcyclohexane the 3/1 ratio is 2.00.

(iv) The peak ratios are quite reproducible (±0.02) and within this error range are independent of the excitation wavelength and concentration of pyrene (including concentration ranges where excimers are present).

(v) While significant variations occur in the vibronic band intensities, the position of the various vibronic bands varies by $<\pm 2$ nm (experimental uncertainty ±2 nm) in all the solvents examined.

Several mechanisms for the interactions of the excited states

of molecules with surrounding solvent molecules have been proposed. These include H bonding, electromagnetic interactions between the dipole moment of the solute with that of the polar solvent, and the reorientation of the solvent molecules around the excited state dipole of the solute (solvent relaxation).[21]

Nakajima, in earlier studies utilizing 25 different solvents,[13-16] attempted to correlate the intensity enhancements of the 0-0 band in the absorption and fluorescence of pyrene to various models. Correlations were attempted against: (i) the solvent dielectric constant ϵ; (ii) Grunwald's parameter $[(\epsilon - 1)/(2\epsilon + 1)]$; (iii) Kosower's Z value; (iv) the square of the relative quantity relating to dispersion energy $(\bar{\alpha}_s/r^6)^2$, where $\bar{\alpha}$ is the mean polarizability of the solvent and r the mean intermolecular distance between the solute and solvent; (v) a factor taking into account the dispersion term, ionization potentials of the solute (I_p) and the solvent (I_s), and an induction term arising from interaction between the solvent permanent dipole and the solute induced dipole:

$$f = \left\{ \left(\frac{3}{2} \frac{I_p I_s}{I_p + I_s} \bar{\alpha}_s + \bar{\mu}^2 \right) \Big/ \bar{\gamma}^6 \right\}^2$$

(here $\bar{\mu}^2$ refers to the mean square of the solvent dipole moment); (vi) a factor that takes into account the polarizability of the solvent dielectric by the permanent dipole moment D of the solvent

$$f' = \left(\frac{\epsilon - 1}{2\epsilon + 1} - \chi \frac{n^2 - 1}{2n^2 + 1} \right)$$

where χ is a variable parameter and n the refractive index of the solvent. No significant correlation was found with any one of the above parameters.

The data presented in Table II can be rationalized in terms of specific interactions between the excited singlet state of pyrene with the solvent molecules. The trend in the data suggests some specific dipole-dipole interactions as a dominant mechanism rather than a universal interaction due to the collective influence of the solvent as a dielectric medium. The mechanisms involving the latter path are fairly well understood and are explained in terms of the static dielectric constant ϵ and the refractive index n of the solvent.[21,22] Earlier studies have reported on the "blurring" of the vibrational fine structures in fluorescence spectra on going from a nonpolar to a polar solvent. The loss in vibrational structure has been interpreted as due to specific solvent-solvent interactions, while the change in the Franck-Condon envelope is believed to be due to environmental modifications of the molecular potential energy surfaces. It is instructive in this context to note that Lawson, Hirayama, and Lipsky[23] have observed that the fluorescence and absorption spectra of benzene in perfluorohexane display the same fine structures as in the vapor phase. They also failed to see any solvent induced frequency shifts. Photophysical studies suggest that saturated fluorocarbon solvents show minimal specific solvent-solute interactions in solution phase at room temperature. The sharpness of the five vibronic frequencies as well as the 3/1 ratio increases on going through the series, simple polar solvents, aromatic solvents, hydrocarbons, and onto saturated perfluorocarbon solvents. These effects strongly suggest specific solute-solvent dipole-dipole interaction mechanisms which are minimal with perfluoro solvents. The strong dipole character of excited singlet states of pyrene is also manifested in the ease with which pyrene forms exciplexes with a wide variety of solutes as well as the ease of excimer formation.

The ground state of the pyrene molecule (point group D_{2h}) is totally symmetrical (A_{1g} state). The first and second electronic states have been assigned $^1B_{3u}$ and $^1B_{2u}$ and are polarized along the short and long axis of the molecule, respectively.[6] The first singlet absorption $^1B_{3u} \leftarrow {}^1A_g$, although not symmetry forbidden, is very weak. The S_1 fluorescence of pyrene ($S_1 \rightarrow S_0$) shows mixed polarization and this has been interpreted as due to mixing of the first electronic state S_1 (short axis polarized) with the nearest second electronic state S_2 (long axis polarized).[12,24] The breakdown of symmetry between the absorption and fluorescence spectra of pyrene due to coupling between S_1 and S_2 states has been noted.[25] The fluorescence spectrum of pyrene shows vibronic bands (cf. Table I) corresponding to allowed b_{1g} vibrations and forbidden a_g vibrations, including the 0-0 band. It has been shown earlier[26] that in a wide variety of aromatic hydrocarbons forbidden vibronic bands in weak electronic transitions show marked intensity enhancements under the influence of solvent polarity. This effect was first observed in the "Ham" bands of benzene. In all cases the intensity enhancements of forbidden vibronic bands roughly parallel the solvent polarity. In the present case of pyrene, peak III (0-737 cm^{-1} band of b_{1g} vibration), which is strong and allowed, shows minimal intensity variation with polarity. The 0-0 band (peak I) and other forbidden vibronic bands of a_g vibration type show significant intensity enhancements in polar solvents. Thus the intensity ratio of peak III/peak I serves as a measure of solvent polarity. The intensity enhancements in the 0-0 band and other a_g vibrations are derived from the nearby second electronic state B_{2u} through coupling with b_{1g} vibrations. In the case of pyrene, the data presented in Table II indicate that specific solute-solvent interactions which mix the allowed and forbidden solute transitions are important, although environmental effects of solvent as a whole in reducing the symmetry of the solute in the presence of surrounding solvent molecules cannot be neglected. Although the existence of the Ham effect, viz., intensity enhancements of forbidden vibronic bands in weak electronic transitions, is known,[26] no theory is available for quantitative verification of the intensity enhancements due to solvents, reported here. Qualitatively, the peak ratio 3/1 can be used as a measure of the extent of interaction between the solvent dipoles and the excited singlet states of pyrene. When there is minimal coupling, the peak ratio is 2.00 (as in perfluoromethylcyclohexane). However, efficient dipolar coupling, with solvents such as acetonitrile or dimethyl sulfoxide, causes a drop in the ratio to as low as 0.5. It is interesting to note that according to the vapor phase fluorescence spectrum of pyrene, published in ref 13, the 3/1 ratio is about 2.45, in excellent agreement with our predictions.

II. Pyrene Fluorescence in Micellar Media. The distinct solvent dependence of vibronic fine structure intensities in pyrene monomer fluorescence can be profitably employed in fluorescence probe studies of bioaggregates. Pyrene is a strongly hydrophobic probe and its solubility in water is very low (2-3 μM). In the presence of micelles and other macromolecular systems, pyrene is preferentially solubilized in the interior hydrophobic regions of these aggregates. In recent years, pyrene has been successfully employed as a fluorescence probe in the study of micellar[4] and membrane-like systems of phospholipid dispersions.[5] These studies focus on the use of the dynamics of quenching of pyrene monomer fluorescence and on excimer formation processes. This section provides a demonstration of the use of vibronic band structure intensity in pyrene monomer fluorescence as a probe in studies of micellar systems.

Figure 2 presents typical fluorescence spectra of pyrene in aqueous sodium lauryl sulfate (NaLS) solutions above and below the critical micelle concentration (cmc). Below cmc, there are no micelles present and the pyrene fluorescence spectrum corresponds to that in water with a 3/1 ratio ~0.66. (Pyrene 3/1 ratio in water is 0.64.) However, as the detergent concentration increases above the cmc pyrene is solubilized in the hydrophobic interior as illustrated by the increased 3/1 ratio. Figure 3 shows variations of the 3/1 ratio of vibronic

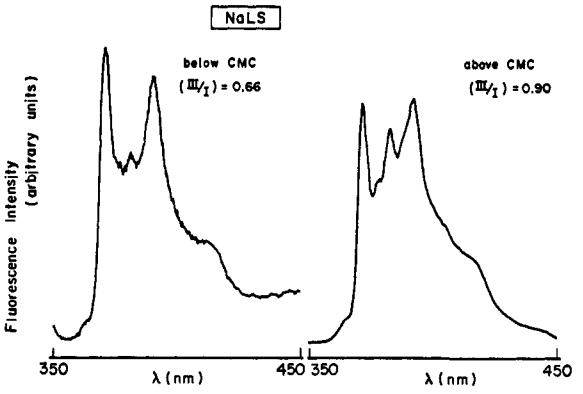

Figure 2. Pyrene monomer fluorescence in aqueous sodium lauryl sulfate (NaLS) solutions, at NaLS concentrations below and above the cmc.

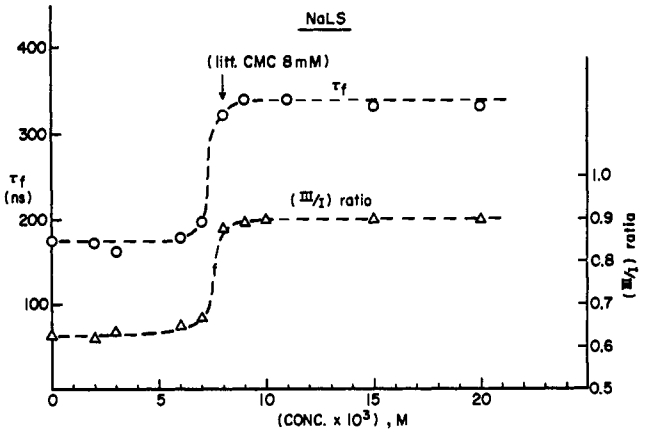

Figure 3. Variation of pyrene monomer fluorescence lifetime (τ_f) and 3/1 ratio in aqueous sodium lauryl sulfate (NaLS) solutions as a function of NaLS concentration.

Table III. Determination of Cmc by Vibronic Band Intensity Variations in Pyrene Monomer Fluorescence

Micellar system[a]	Cmc (pyrene)	Lit. cmc
Cationic		
CTAB	8.0×10^{-4}	9.2×10^{-4}
DeTAB	6.0×10^{-2}	6.5×10^{-2}
DAC	1.5×10^{-2}	1.5×10^{-2}
Anionic		
NaLS	8.0×10^{-3}	8.2×10^{-3}
NaDS	3.5×10^{-2}	3.3×10^{-2}
NaL	2.4×10^{-2}	2.4×10^{-2}
Nonionic		
Triton X-100	2.4×10^{-4}	2.6×10^{-4}
Igepal CO-630	5.5×10^{-5}	4.6×10^{-5}

[a] Abbreviations used: CTAB, cetyltrimethylammonium bromide; DeTAB, decyltrimethylammonium bromide; DAC, dodecyltrimethylammonium chloride; NaLS, sodium lauryl sulfate; NaDS, sodium dodecyl sulfate; NaL, sodium laurate; Triton X-100, *p*-di-*tert*-butylphenoxy polyethyleneoxy(9.5) ether; Igepal Co-630, *p*-nonylphenoxy polyethyleneoxy(9.0) ether.

band intensities of 2 μM pyrene as a function of NaLS concentration. The curves show a sharp increase in the 3/1 ratio at cmc. Since both the fluorescence lifetime as well as the 3/1 ratio in the vibronic band intensities are functions of the environment around the probe, both τ_f and the 3/1 ratio show sharp breaks at the cmc. This indicates the onset of micellization at this concentration. Table III summarizes typical cmc data determined for a variety of ionic and nonionic detergents using pyrene 3/1 ratio as a probe. There is excellent agreement between the cmc's determined by this method with the literature cmc values,[27] indicating the reliability of the present technique. Similar studies in this laboratory[28] have shown that cmc values for lysolecithin micelles can also be accurately determined by this method. The cmc values for lysolecithins are of the order of micromolar, and light scattering data give cmc values over a wide range. The method is very sensitive in monitoring subtle changes in the environment around the probe. The method has also been successfully applied[28] to study structural features of phospholipid dispersions such as phase transitions, effects of additives on lipid phase fluidity, and on mixed micelles. Mast and Haynes[29] recently demonstrated the use of total fluorescence intensity as a probe to determine cmc of micellar systems. In studies of membrane model systems and other macromolecules, the determination of the exact concentration of probe incorporated into the system is very difficult. Hence methods based on total monomer fluorescence intensity are of limited utility.

It is noted that the 3/1 ratio in NaLS micellar solutions (at concentrations above cmc) reaches a steady value of 0.88 ± 0.03. Table IV summarizes the relative fluorescence intensities for various peaks of pyrene in various micellar solutions. For comparison the table also includes data on the zeta potential (ζ) as well as the dielectric constant (ϵ) for the micelle-water interface, taken from the literature.[31-35] As is shown for NaLS, above cmc the ratio (3/1) remains fairly constant and independent of the probe as well as the detergent concentration. It has been observed that the pyrene 3/1 ratio is quite dependent on the nature of the surfactant head group, but is independent of the surfactant concentration, length of the hydrocarbon chain, or presence of external additives such as *n*-

Table IV. Summary of Relative Band Intensities in Pyrene Fluorescence in Various Micellar Solutions

Micellar system[a]	Relative band intensities I	II	III	IV	V	ζ,[b] mV	ϵ[c]
NaL	1.00	0.74	0.96	0.93	1.06		39
DAC	1.00	0.68	0.95	0.82	0.98	125	26
NaLS	1.00	0.65	0.88	0.77	0.92	150	45
Brij 35	1.00	0.65	0.85	0.69	0.85		
Triton X-100	1.00	0.60	0.76	0.72	0.89		≤15
Igepal CO-630	1.00	0.59	0.77	0.75	0.88		≤12
DeTAB	1.00	0.63	0.78	0.82	0.94		28
CTAB	1.00	0.58	0.77	0.75	0.91	75	16
CTAC	1.00	0.58	0.74	0.71	0.87		18
DTAC	1.00	0.57	0.73	0.69	0.85		24

[a] For abbreviations see footnote of Table III; DTAC, dodecyltrimethylammonium chloride. [b] Zeta potential for the micelle–water interface; data taken from ref 31–34. [c] Dielectric constant (ϵ) for the micelle–water interface; estimated using pyrene-3-aldehyde as a fluorescence probe (ref 35).

hexane or electrolytes such as NaCl or NaBr. There is increasing evidence in the literature[30] that inner cores of spherical micelles are hydrocarbon-like. It is somewhat surprising that the 3/1 ratio remains in the range of 0.70–1.00 for various micellar systems, while pyrene in pure hydrocarbon solvents has a 3/1 ratio of 1.65. Various factors such as NMR chemical shift data, the strong hydrophobic character of pyrene, the long fluorescence lifetime of 450 ns in NaLS micelles (a value similar to those observed in cyclohexane), as well as the quenching studies with bound counter ions and externally added quenchers[4,36,37] suggest solubilization of pyrene in the interior core of the micelle. Also, the high electric fields of several kilovolts per centimeter present in the stern layer of the micelle and the observed 3/1 ratio for pyrene in various micellar systems rules our solubilization of pyrene at the micellar surface. In accord with these observations the 3/1 ratio remains constant for variations in the nature of the counter ions (of different hydrophobicity) and for the addition of salts such as NaCl which are known to be absorbed at the micellar surface. Hence the observed 3/1 ratios can also be interpreted as a measure of the compactness of the head group structures and the extent of surface charge. An alternative explanation can also be sought in terms of the extent of water penetration into these micellar systems. There have been suggestions that water can enter the micelles and extend up to four carbons from the head group.[30] In micelles with compact head groups such as sodium laurate (NaL), lauryl ammonium chloride (LAC), and sodium lauryl sulfate, the 3/1 ratios are higher, indicating a smaller water penetration in these micelles compared to micelles with larger head groups such as CTAB and nonionics such as Brij 35.

References and Notes

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