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SOLVENT EFFECTS ON FLUORESCENCE OF INTRAMOLECULAR HETEROEXCIMER SYSTEM AND ITS USE FOR INVESTIGATING POLARITY OF MICROSCOPIC ENVIRONMENT

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Intramolecular heteroexcimer emission from pyrene-$(CH_2)_3$-N,N-dimethylaniline was very sensitive toward solvent polarity and was used for estimating the microscopic polarity of micelles, liposomes, and microemulsions. Liposomes show polarity change as the phase transitions.

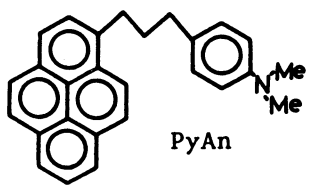
Intramolecular excimer and heteroexcimer systems have widely been studied to elucidate the structural requirement for the complex formation as well as the charge-transfer process in the excited state. From these studies, it has been found that the intramolecular heteroexcimer emission is very sensitive toward solvent polarity and viscosity.[1-6] These aspects suggest that the heteroexcimer emission can be used as a probe for investigating microscopic properties of the environment. Indeed the intramolecular excimer system has been utilized to investigate the dynamic behavior of polymer and/or lipid bilayer membranes.[7,8] Of course, the same polarity and viscosity dependence is also observed in the intermolecular heteroexcimer systems. As clearly indicated by Georgescauld et al.,[8] the most outstanding advantage of the intramolecular system is that the concentration of the probe can significantly be lowered so that the probe does not perturb the microenvironment.

The present study deals with the solvent effects on the heteroexcimer emission from N,N-dimethyl-4-[3-(1-pyrenyl)propyl]aniline (PyAn) and the use of PyAn as a probe to investigate microscopic polarity of hydrophobic interiors of micelles, liposomes, and microemulsions.

Figure 1 shows the plot of the energy of the heteroexcimer emission band ($h\nu_{HE}$) against the solvent polarity parameter ($f(\varepsilon,n)$);

$$f(\varepsilon,n) = 2(\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1) \qquad (1)$$

where ε and n are the dielectric constant and the refractive index of the solvent, respectively.[2] All experiments were undertaken at 25°C unless otherwise noted and the sample was bubbled with N_2 gas before measurement. The solvent effects were studied by using non-aqueous solvents as well as mixed solvents of MeOH-H_2O, pyridine-H_2O, and tetrahydrofuran (THF)-H_2O. At the range of ε between 1.8 (n-hexane) and 32.6 (MeOH), a good linear relationship was observed between $h\nu_{HE}$ and $f(\varepsilon,n)$ in both



PyAn

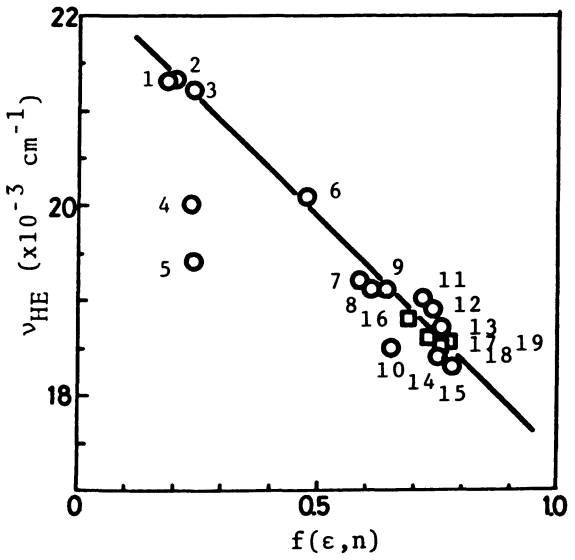


Fig. 1. Plot of ν_{HE} vs. f(ε,n). [PyAn] = 5×10^{-6} M, 1: n-hexane, 2: cyclohexane, 3: decalin, 4: benzene, 5: 1,4-dioxane, 6: diisopropyl ether, 7: ethyl acetate, 8: THF, 9: dichloromethane, 10: pyridine, 11: n-butyl alcohol, 12: isopropyl alcohol, 13: EtOH, 14: acetone, 15: MeOH, 16: 90%(w/w) THF-H_2O, 17: 80% THF-H_2O, 18: 70% THF-H_2O, 19: 60% THF-H_2O.

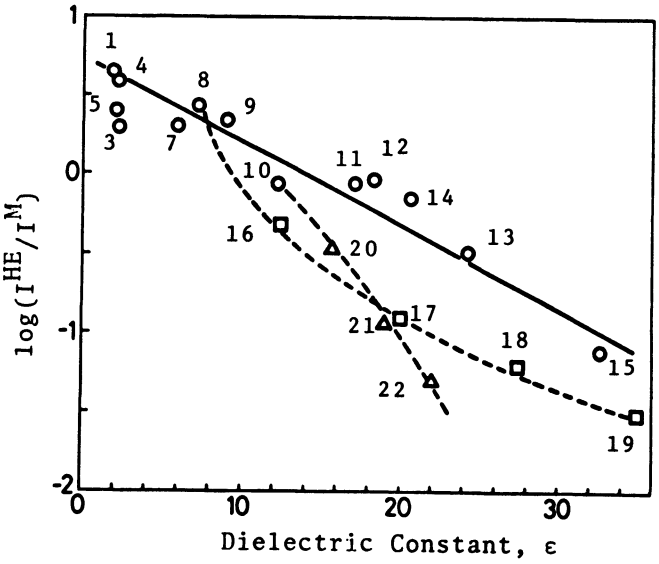


Fig. 2. Plots of $\log(I^{HE}/I^{M})$ vs. ε. 20: 95%(w/w) pyridine-H_2O, 21: 90% pyridine-H_2O, 22: 85% pyridine-H_2O.

non-aqueous and aqueous THF solutions. This result is in good agreement with that obtained by Masaki et al..[2] The plots for benzene, pyridine, and 1,4-dioxane, however, are deviated from the straight line. The deviation in the cases of benzene and pyridine may be ascribed to the π-π interaction between the heteroexcimer and solvents. No heteroexcimer emission was observed at the range of 40<ε<53 (MeOH-H_2O and DMSO). In aqueous methanolic solution of ε>53, a structureless broad emission band appeared again at around 480 nm when the concentration of PyAn was considerably high (5×10^{-6} M). Lowering PyAn concentration reduced the ratio of the emission intensity of the broad band to the monomer one, suggesting that intermolecular association of PyAn occurs because of its low solubility.

Figure 2 shows the plots of the ratio of the heteroexcimer emission intensity to the monomer one (I^{HE}/I^{M}) against ε. The monomer emission scarecely shifts on varying the solvent polarity. The emission intensity of the band at the shortest wavelength was chosen as I^{M}. A fairly good linear relationship was observed between $\log(I^{HE}/I^{M})$ and ε in non-aqueous solvents. Since not only solvent polarity but also solvent viscosity affects emission quantum yield of heteroexcimer, the linear relationship shown in Fig. 2 may be acceptable for the cases in the relatively fluid media. The point is the effect of H_2O in organic solvents. As shown in Fig. 2, H_2O in pyridine and in THF markedly quenches the heteroexcimer emission. Although the mechanism for the quenching by H_2O is not clear, it seems that a specific solvation by H_2O enhances the radiationless decay of the heteroexcimer.

Table I. Estimation of Microscopic Polarity of Micelles, Liposomes, and Microemulsions.[a)]

System	λ_{HE}, nm	I^{HE}/I^{M}	f(ε,n)	ε n = 1.3	n = 1.5
DPPC (5×10^{-3} M) liposome	483	0.19	0.33	2.4	2.9
DMPC (5×10^{-3} M) liposome	495	0.13	0.43	3.2	3.9
SDS (0.1 M) micelle	-	-	-	>40	
DTAC (0.1 M) micelle	-	-	-	>40	
Triton X-100 (2%) micelle	515	0.04	0.59	5.5	7.9
Microemulsion[b)]	518	0.10	0.61	6.0	8.9

a) All measurements were carried out at 25°C under N_2 atmosphere. [PyAn] = 5×10^{-6} M.

b) SDS(7.3%)-n-amyl alcohol(10%)-n-hexadecane(2.7%)-H_2O(80%).

The straight line in the plot of $h\nu_{HE}$ vs. f(ε,n) may be used as a calibration curve for estimating microscopic polarity of hydrophobic interiors of micelles, liposomes, and microemulsions. The results are listed in Table I. In the case of the surfactant micelles, only PyAn in Triton X-100 micelles exhibited the heteroexcimer emission. The present result indicates that ε of the region where no heteroexcimer emission is observed is >40. The micellar interiors of sodium dodecyl sulfate (SDS) and dodecyltrimethylammonium chloride (DTAC), therefore, seems considerably polar. This conclusion is consistent with that obtained by Menger et al.[9)] and Waka et al..[10)] The considerably weak heteroexcimer emission were observed in dimyristoyl- (DMPC, heteroexcimer emission maximum, λ_{HE}: 495 nm) and dipalmitoylphosphatidylcholine (DPPC, λ_{HE} 483 nm) sonicated liposomes. Since we do not have any information about the n-value of liposome, the ε-values of the DMPC and DPPC liposomal interiors cannot strictly be estimated. Then we chose 1.3 and 1.5 as the n-values and calculated the ε-values by using eq. 1. The ε-values of lipid bilayers are about 2.5∿4.0. These values are somewhat smaller than that estimated from the intermolecular heteroexcimer system ($10.4<\varepsilon<12.3$).[10)] Since large amounts of electron donor needs to observe the intermolecular heteroexcimer emission, coexisting electron donor may perturb the membrane structure. Both inter- and intramolecular heteroexcimer systems indicate that the interior of the lipid bilayer is considerably apolar as compared with the surfactant micelles.

The I^{HE}/I^{M}-values of the heteroexcimer systems investigated herein were significantly small as compared with those expected from λ_{HE}. It can be assumed that the reorientation of PyAn to form a sandwich configuration is restricted in the ordered molecular assemblies such as liposomes. It has been well known that the mobility of a molecule in lipid bilayer abruptly increases on varying the bilayer phase from gel to liquid crystalline. Then we studied the temperature effect on the I^{HE}/I^{M}-values and λ_{HE}. The results are shown in Fig. 3 and 4. The gradual increase of the I^{HE}/I^{M}-values was observed at 20∿40°C. The I^{HE}/I^{M}-values sharply increased at 40∿45°C. These two temperature ranges correspond to the pretransition (26°C) and transition temperatures (T_c, 41°C) of DPPC liposomes.[11)]

Interestingly, the shift of λ_{HE} was also accompanied by the phase transition of the DPPC liposomes (Fig. 4). At the temperature well below T_c (10∿15°C), λ_{HE} appeared at 480 nm (estimated ε: 2.3∿2.7). The emission maximum shifted to longer

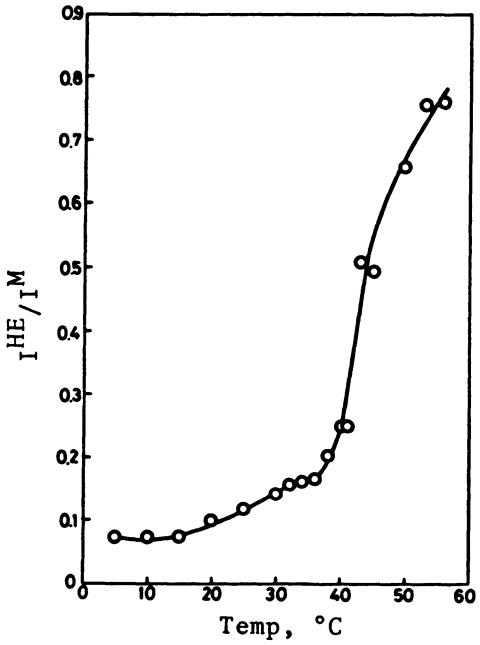


Fig. 3. Variation of I^{HE}/I^{M} as a function of temperature for PyAn incorporated into DPPC liposomes. [PyAn] = 5×10^{-6}M, [DPPC] = 5×10^{-3}M.

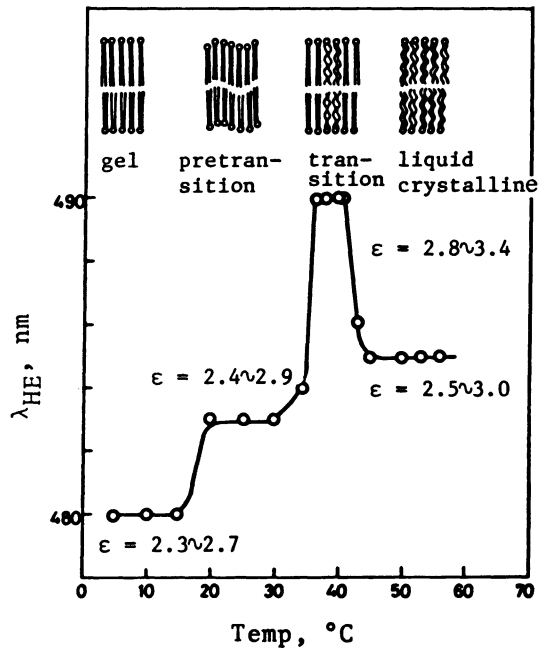


Fig. 4. Variation of λ_{HE} as a function of temperature for PyAn incorporated into DPPC liposomes.

wavelength in the pretransition (483 nm, ε = 2.4∿2.9) and transition state (490 nm, ε = 2.8∿3.4). In the liquid crystalline state (>45°C), λ_{HE} shifted again to shorter wavelength (485 nm, ε = 2.5∿3.0). The lipid bilayer in the transition state consists of the lipid assemblies in both the gel and liquid crystalline states. It has been found that the permeability of such phase separated lipid bilayer membrane is much higher than that the bilayer membranes in the gel and liquid crystalline states.[12] The H_2O penetration into the lipid bilayer in the transition state seems to cause the increase of the polarity of the lipid bilayer.

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