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Fluorescence anisotropy of ketocyanine dyes in pure and mixed binary solvents

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Abstract

Fluorescence anisotropies (r) of two ketocyanine dyes have been determined in neat alcohols, nitriles and their binary mixtures. For a particular class of solvents the values of r is given by Perrin's equation. The lifetimes (τ_f) of the solutes in the S_1 state have been determined from a study of variation of r with the viscosity of the solvent. The calculated τ_f values for the alcohols are higher than that of the nitriles and the results agree well with those obtained by independent measurements in picosecond domain. In alcohol+nitrile binary mixture the solute exhibits preferential solvation by alcohol molecules. The limiting fluorescence anisotropies of the solutes in all the solvents come as ~0.38 indicating that absorption and emission dipole moments are parallel. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Ketocyanine dye; Binary solvent; Fluorescence anisotropies

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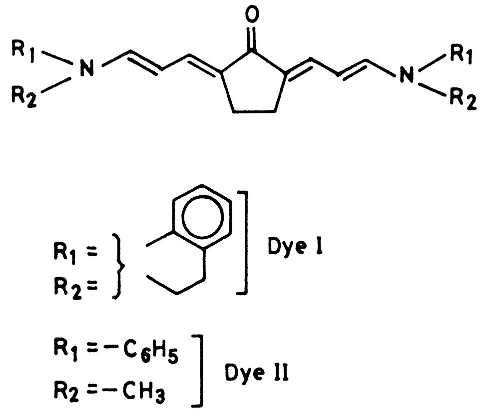
1. Introduction

Fluorophores often act as reporter molecules giving information about the micro-environment around the solute [1]. Different parameters characterising fluorescence may be used for such studies. They include band maxima (λ_{em}), quantum yield (ϕ_f), lifetime (τ_f) in the excited state and the fluorescence anisotropy (r). Of these the last one primarily contains information about the orientational and rotational motion of the fluorophores [2]. Recently we have studied the solvation characteristics in various media using the fluorescence parameters λ_{em}, ϕ_f and τ_f of ketocyanine dyes (I{$C_{27}H_{26}N_2O$} and II{$C_{25}H_{26}N_2O$}) [3–6]. It has been observed that these parameters provide information regarding dye-solvent interaction. The objective of the present work is to investigate the rotational characteristics of the dyes in a solvent cage. The dependence of the rotational characteristics on the micro-environment around the dye has also been looked in to. For this we have measured the fluorescence anisotropies of dye I in neat alcohols (methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, pentan-1-ol, octan-1-ol, cyclohexanol), nitriles (acetonitrile, propionitrile, butyronitrile and benzonitrile) and their binary mixtures (ethanol+octan-1-ol, ethanol+cyclohexanol, acetonitrile+benzonitrile, and acetonitrile+octan-1-ol). Some data have also been taken for the dye II in octan-1-ol+ethanol mixture.

2. Experimental



Ketocyanine dyes (I & II)

The ketocyanine dyes (I and II) were synthesised as described in the literature [7]. Indoline (for dye I), *N*-methylaniline (for dye II), 1,1,3,3 tetramethoxypropane and cyclopentanone were purchased from Sigma chemicals (USA) and used as received. The purity of the prepared compounds was checked by IR spectral data [IR peaks

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obtained in KBr disc: 1610, 1570, 1490 cm^{-1}, m.pt. 280°C {dec}(for dye I) and 1608, 1570, and 1490 cm^{-1}, m.pt. 230°C {dec}(for dye II) and absorption and fluorescence data [λ_{em} 622 nm in ethanol for dye I and 589 nm in ethanol for dye II]. All the solvents were purified and dried by standard procedures and were distilled from CaH_2 immediately before use to ensure the absence of peroxides and oxidizing agents. Mixed binary solvents and solutions were prepared by carefully mixing the components so as to minimize contamination by moisture. Freshly prepared solutions were used for each measurement and the concentration were chosen to give absorbance value less than 0.08. This was done to avoid solute-solute interaction. The fluorescence and excitation spectra were taken on a Hitachi F-4500 spectrofluorimeter, equipped with polarisers and thermostatic cell holder. A constant temperature bath (Heto-Holten: temperature range 243–373 K) was used for the determination of steady state anisotropy values at different constant temperatures. The emission anisotropy at a wavelength λ was calculated from [2]

$$r_{em}(\lambda) = [I_{VV}(\lambda) - (G(\lambda)I_{VH}(\lambda)]/[I_{VV}(\lambda)(2.G(\lambda)I_{VH}(\lambda)], \qquad (1)$$

where $I(\lambda)$ denotes the fluorescence intensity at the wavelength λ and the first and second subscripts H and V respectively refer to the setting of excitation and emission polarisers. $G(\lambda)$ is an instrumental factor representing the polarisation characteristics of the photometric system and is given by

$$G(\lambda) = I_{HV}(\lambda)/I_{HH}(\lambda). \qquad (2)$$

The subscripts in the expression represent the same meaning. Excitation anisotropies (r_{ex}) were calculated similarly. Both the anisotropy values are within ±0.005. Viscosities of some pure solvents were obtained from literature [8,9], the same for others and for mixed binary solvents were determined in the laboratory using a viscosimeter having a high flow-time. The viscosimeter was calibrated using high purity ethanol and benzene at the working temperature. The average of four sets of flow time for each liquid was taken for the purpose of calculation of viscosities.

Dimroth Reichardt empirical solvent polarity parameter $E_T(30)$ values [10] for the neat solvents were taken from the literature. Values for the mixed binary solvents were determined from the wavelength of maximum absorption (λ_{max}) of the indicator dye, viz, N-phenylpyridinium betaine in the solvent according to the relation

$$E_T(30)/\text{kcal mol}^{-1} = 28590/(\lambda_{max}/\text{nm}). \qquad (3)$$

3. Results and discussion

Representative plots showing absorption and emission band along with excitation and emission anisotropy are given in Fig. 1. The figure shows nearly constant values of anisotropy over the entire range of excitation and emission band. This reveals the presence of one direction of the transition dipole moment within the electronic transition (with the vibronic progression) involving S_0 and S_1 states [11]. Values of anisotropy for dyes in pure solvents and also for mixed binary solvents are listed in the Tables 1 and 2. The reported values are at the band maximum. Fig. 2 shows a plot of $r_{(em)}$ as a function of the empirical solvent polarity parameter $E_T(30)$ for pure solvents. It appears that the alcohols and nitriles form separate groups. Basically, anisotropy of emission at a time "t^*" after excitation is an angular correlation function that describes the mutual orientation of the absorption and emission dipole moments at the time of

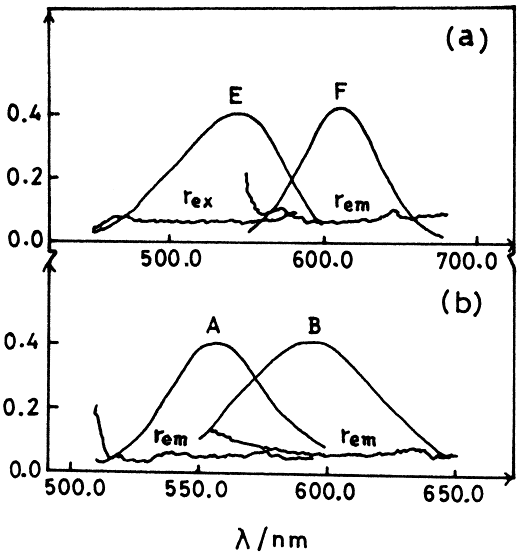

Fig. 1. (a) Excitation (E) and fluorescence (F) band of dye I in ethanol along with anisotropies (r_{ex}=excitation anisotropy, r_{em}=emission anisotropy). (b) Fluorescence band of dye I in acetonitrile (A) and 1:1 mixture of acetonitrile+octan-1-ol (B) along with the anisotropies.

Table 1
Fluoresence anisotropy (r) values along with other solvent parameters at 300 K

Sl. No.	Solvents	$E_T(30)$ (kcal mol^{-1})	r_{em}	η (mPas)
1	Methanol	55.4	0.03	0.54
2	Ethanol	51.9	0.08 (0.08)	1.05
3	Propan-1-ol	50.7	0.09	1.85
4	Propan-2-ol	48.4	0.11	1.92
5	Butan-1-ol	50.2	0.12	2.39
6	Pentan-1-ol	49.1	0.18	3.07
7	Octan-1-ol	48.3	0.23 (0.21)	6.33
8	Cyclohexanol	46.9	0.29	49.9[a]
9	Acetonitrile	45.6	0.05	0.33
10	Propionitrile	43.7	0.07	0.42
11	Butyronitrile	43.1	0.08	0.53
12	Benzonitrile	41.5	0.13	1.18

Data in pure solvents for dye I. Numbers within bracket indicate values for dye II.
[a] Extrapolated value [9].

Table 2
Fluoresence anisotropy (r) values along with other solvent parameters at 300 K

Solvent mixtures	Mole fraction (x_1)	$E_T(30)$ (kcal mol^{-1})	r_{em}	η (mPas)
Ethanol(1)+octan-1-ol	0.2	48.92	0.19 (0.20)	5.15
	0.4	49.52	0.16 (0.17)	3.78
	0.6	50.25	0.14 (0.13)	2.73
	0.8	51.05	0.10 (0.10)	1.90
Ethanol(1)+cyclohexanol	0.2	–	0.24	15.54
	0.4	–	0.21	6.73
	0.6	–	0.14	3.55
	0.8	–	0.10	2.05
Acetonitrile(1)+benzonitrile	0.2	43.12	0.12	0.89
	0.4	43.48	0.08	0.71
	0.6	44.20	0.07	0.55
	0.8	44.95	0.08	0.43
Acetonitrile(1)+octan-1-ol	0.25	–	0.14	2.83
	0.56	–	0.08	1.29
	0.75	–	0.07	0.74
	0.88	–	0.07	0.51
	0.96	–	0.05	0.40

Data for mixed binary solvents. Numbers within bracket indicate values for dye II.

excitation (t=0) and emission (t=t^*) [12,13]. Under continuous illumination, the measured steady state anisotropy gives information about the rotational rate of the solute molecule [1]. The higher anisotropy values for alcohols indicate that the rotational rate of the solute in the alcohol cage is slower than that in the nitrile cage, presumably due to stronger dye-solvent interaction in the former case. It may be pointed out in this context that our previous results also indicated a strong hydrogen-bonding interaction of the excited state of a ketocyanine dye with alcohols [3–6]. In a series of similar solvents the anisotropy value increases as the molecular weight increases. This is expected in view of the increased viscosity of the solvent as the number of carbon atoms increases. In the case of isotropic rotations the measured value of anisotropy (r) is related to the viscosity of the medium by the Perrin relationship [14,15] as follows:

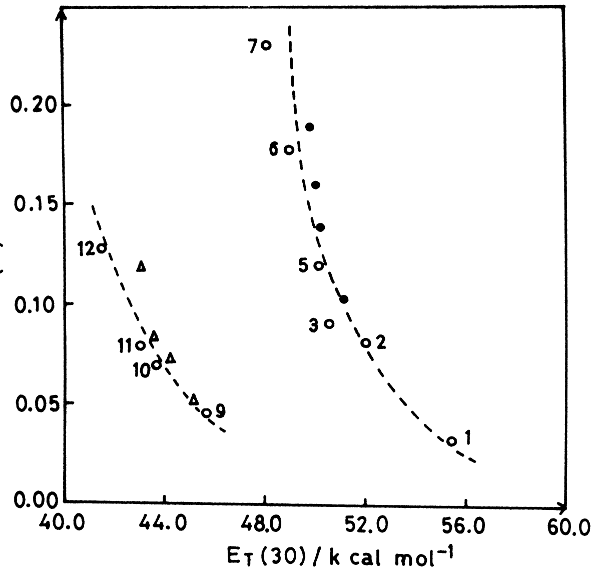

Fig. 2. Plots of r for dye I versus $E_T(30)$ in neat and mixed binary solvents. The numbers refer to the serial numbers of solvents in the Table 1. (●)=mixed alcohols (ethanol + octan-1-ol), (△)=mixed nitriles (acetonitrile+benzonitrile).

$$r = r_0/(1 + \tau_f/\phi), \tag{4}$$

where r_0 refers to the anisotropy value in the limiting condition characterised by the absence of rotational diffusion, τ_f is the lifetime of the probe molecule, ϕ is the rotational correlation time of the fluorophore and is given by

$$\phi = \frac{\eta V}{RT}, \tag{5}$$

where η is the solvent viscosity, V is the molar volume of the fluorophore, R and T are respectively the gas constant and temperature in the Kelvin scale. On rearranging and using the value of ϕ the Eq. (4) takes the following form

$$(1/r) = (1/r_0) + (RT\tau_f/r_0\eta V). \tag{6}$$

Thus a plot of $(1/r)$ versus $(1/\eta)$ at a particular temperature would give a straight line. Fig. 3 shows such plots for pure alcohols and nitriles. Two separate lines can clearly be distinguished for two groups of solvents. Two straight lines for the two classes of solvents indicate that apart from the viscosity dependence, the anisotropies are also dependent on the solvation interactions between the cage and the fluorophore. For a class of solvent where solvation interaction is almost constant, a linear relationship, as expected from the Eq. (6), is obtained. The slope of the straight line plot give

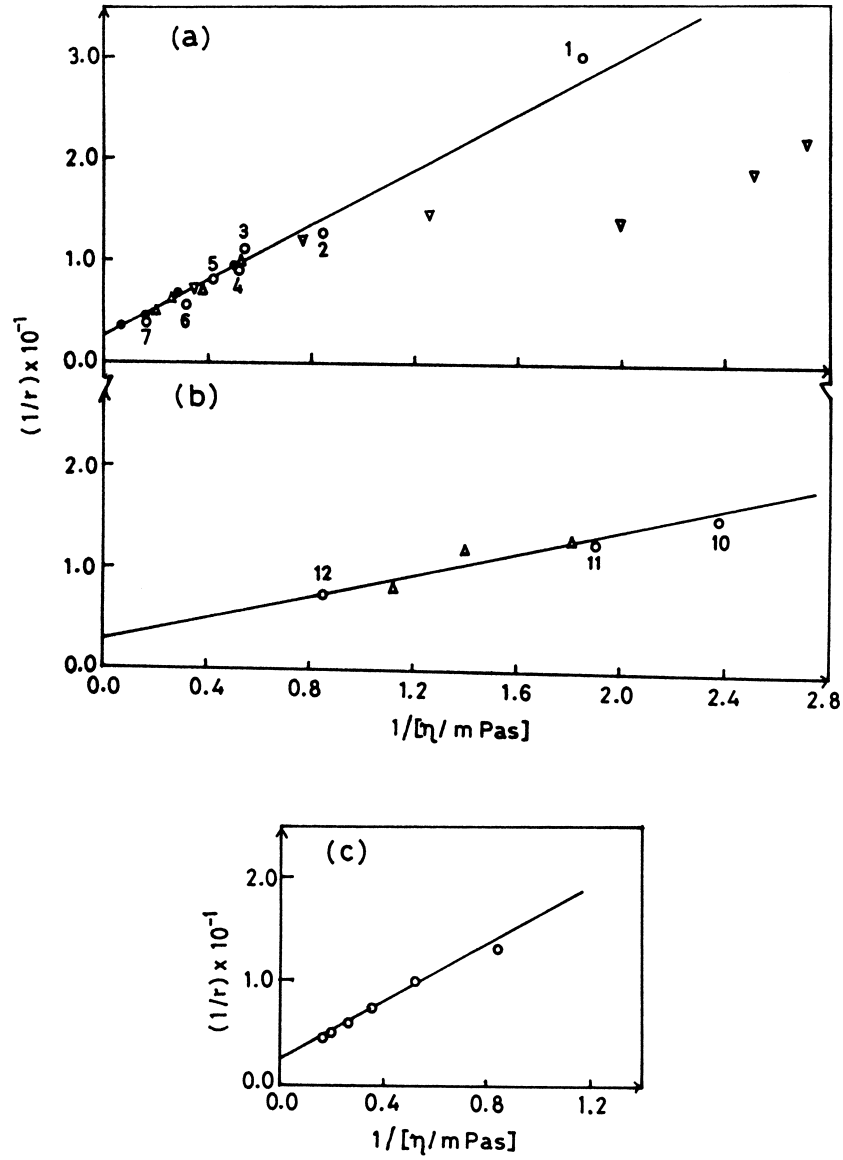

Fig. 3. Plots of $1/r$ versus $1/\eta$ for dye I (a and b) and dye II (c). (a) The numbers refer to the serial number of solvents in the Table 1. (●)=(ethanol+cyclohexanol), (△)=(ethanol+octan-1-ol), (▽)=(acetonitrile+octan-1-ol). (b) Plot of data points for pure nitriles (○) and mixed binary solvents containing nitriles (△). (c) Plots for dye II in ethanol+octan-1-ol mixture.

the value of $(RT\tau_f/r_0V)$ at the experimental temperature and the intercept gives $(1/r_0)$. Assuming the solute molecule to be an oblate ellipsoid rotor V is calculated to be 544×10^{-3} dm^3 mol^{-1} for dye I. The same value of V for dye II has been assumed. The lifetime of the probe molecule as calculated from the slope and the intercept in alcohol solvents is found to be ~2 ns. For nitrile solvents a value of 1.06 ns was calculated. It is interesting to note that the values of τ_f as obtained from the measurement in the picosecond domain are 1680 and 1020 ps for ethanol and acetonitrile respectively [6] for the dye I. The agreement with the values calculated from the anisotropy studies is thus satisfactory. The higher value in the alcohol solvents is due to specific hydrogen bond interaction with the S_1 state of the fluorophore. Similar plot for dye II gives a value of 1.0 ns for τ_f in alcohol solvents. The value obtained from picosecond studies for this dye was found to be ~1500 ps.

The limiting values of anisotropy for both class of solvents, e.g. alcohols and nitriles comes as ~0.38 for both the dyes. Since the limiting value is close to its theoretical maximum of 0.4, this strongly suggests that the absorption and emission dipole-moments are parallel [16] for the ketocyanine dyes.

Lifetime of the fluorophore in the S_1 state can also be obtained from a plot of $1/r$ versus T/η for a particular solvent. Fig. 4. shows such plots for pure octan-1-ol and ethanol. Here also linear plots are obtained. τ_f values obtained from such plots are 1.40 ns for ethanol and 1.0 ns for octan-1-ol. The value of the limiting anisotropy as obtained by this procedure for the solvents are also close to 0.40.

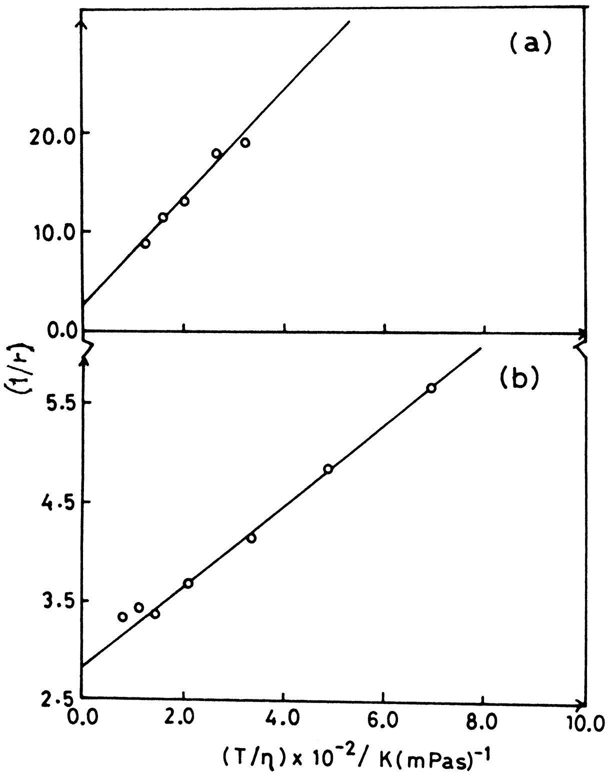

Fig. 4. Plot of $1/r$ versus T/η for dye I in (a) ethanol and (b) octan-1-ol.

Data points for mixed binary alcohol solvents (octan-1-ol+ethanol and cyclohexanol+ethanol) fall on the same straight line for alcohols. Similarly data points for acetonitrile+benzonitrile system fall on the line for the nitriles. This is intelligible in the light of similar solvent-probe and solvent-solvent interaction in alcohol and in nitrile solvents. Data points for mixed binary solvents containing an alcohol and a nitrile (octan-1-ol+acetonitrile) do not fall on line for alcohols except for the octan-1-ol rich region. This indicates that in the octan-1-ol rich region the micro-environment of the solute is almost alcoholic. As the mole fraction of acetonitrile is increased the solute still prefers octan-1-ol in the micro-environment and the anisotropy value does not decrease much. The local mole fraction of octan-1-ol (x_1^L) may be calculated from the relation [17,18]

$$x_1^L = (P_{12} - P_2)/(P_1 - P_2), \quad (7)$$

where P is a property of the solute, in this case $(1/r)$, and the subscripts 1, 2 and 12 refer to octan-1-ol, acetonitrile and their binary mixture respectively. The values of x_1^L are shown in the Fig. 5 as a function of x_1. It appears that $x_1^L > x_1$ over the entire range of solvent composition indicating a preferential solvation of the solute in the S_1 state by octan-1-ol. This is intelligible in view of hydrogen bonding interaction between the carbonyl oxygen in the S_1 state and octan-1-ol. Results of our previous studies also indicate a preferential solvation of the dye in alcohol-nitrile binary mixture [3–6].

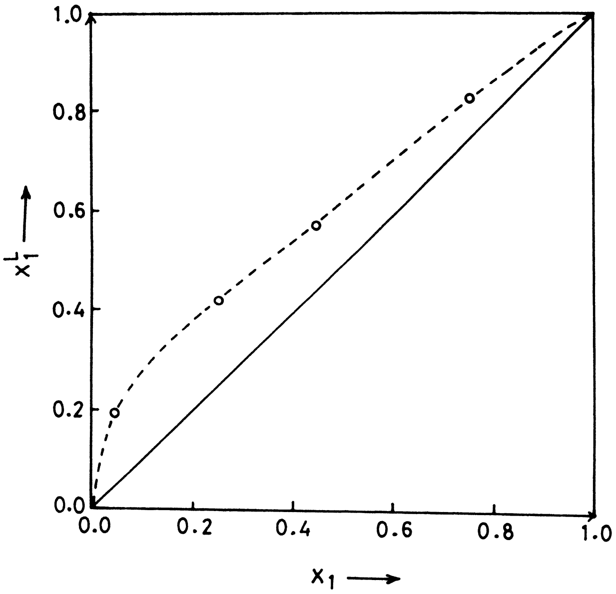

Fig. 5. Plot of local mole fraction of octan-1-ol (x_1^L) versus the bulk mole fraction (x_1) in octan-1-ol+acetonitrile mixed binary solvents. The solid line represents the ideal line.

4. Conclusion

1. Lifetime (τ_f) in the S_1 state of a ketocyanine dye can be obtained from steady state fluorescence anisotropy study of the dye in different solvents. The values agree well with those obtained from studies in picosecond domain.
2. Different τ_f values are obtained for different classes of solvents. τ_f values for alcohol solvents are higher indicating a strong hydrogen bonding interaction between the S_1 state of the solute and an alcohol.
3. In octan-1-ol+acetonitrile binary mixture the solute (S_1 state) is preferentially solvated by the octan-1-ol and the local mole fraction of octan-1-ol may also be calculated.
4. The limiting value of anisotropy is very close to 0.4, the theoretical maximum for all the solvents.

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