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Journal of Photochemistry and Photobiology A: Chemistry 89 (1995) 201-207

Journal of
PHOTOCHEMISTRY
AND
PHOTOBIOLOGY
A: CHEMISTRY

Photophysics of 8-anilinonaphthalene-1-sulphonate

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Received 20 October 1994; accepted 7 February 1995

Abstract

The steady state and time-resolved spectra of 8-anilinonaphthalene-1-sulphonate under various conditions of solvent polarity, viscosity and temperature have been investigated. Unlike earlier reports, we have actually observed two emissions (Kosower's non-planar (NP) and charge transfer (CT)) in all solvents. While in low polarity solvents emission from the NP state is dominant, in high polarity solvents emission occurs mainly from the CT state. In the frozen glass at 77 K the CT emission vanishes. An interconversion of the NP to the CT state starts near the melting point of the solvent.

Keywords: 8-Anilinonaphthalene-1-sulphonate; Solvent polarity; Viscosity; Temperature

1. Introduction

Weber and Laurence [1] reported that an intense blue fluorescence of 8-anilinonaphthalene-1-sulphonate (8,1-ANS) is observed in less polar solvents whereas a weak green fluorescence is obtained in aqueous solutions. The extreme sensitivity of the fluorescence parameters to the environment make this molecule suitable for use as a probe in biological investigations [2–4]. The photophysics of this molecule and its derivatives has been extensively studied by Kosower and coworkers in various solvents [5,6]. They suggest that the emitting state in polar solvents is a charge transfer (CT) state formed by conformational change after excitation. The scheme given by them for the electron transfer process in ANS is

$$\begin{tikzcd} S_{0,np} \arrow[r] & S^{*}_{1,np} \arrow[r, shift left, "k_1"] \arrow[d, "k_{np}"] & S^{*}_{1,ct} \arrow[l, shift left, "k_{-1}"] \arrow[r] \arrow[d, "k_{ct}"] & S_{0,np} \\ & S_{0,np} + h\nu & S_{0,ct} + h\nu & \end{tikzcd} \qquad (1)$$

Absorption of light leads to a locally excited state $S^{*}_{1,np}$ (non-planar (NP) geometry) which is then transformed into a CT state $S^{*}_{1,ct}$ (planar configuration). In polar solvents the CT state decays via another electron transfer process, resulting in quenched fluorescence. On the basis of the relative positions of emission maxima with respect to z values (solvent polarity parameter), they suggest that in viscous polar solvents such as glycerol the rate of conversion of the $S_{1,np}$ to the $S_{1,ct}$ state is lowered and emission is observed mainly from $S_{1,np}$. DeToma et al. [7] studied the kinetics of 2,6-ANS as a function of emission wavelength and suggested that the kinetics is not consistent with the two emitting species. The results are supposed to be consistent with the kinetics of solvent relaxation. It has been found [8–10] that in polar solvents the lifetime is more sensitive to the polarity than to the viscosity. However, Sadkowski and Fleming [11] find a small increase in the non-radiative rate and no detectable wavelength shift in ethylene glycol on reducing the viscosity from 19.9 to 3.02 cp by heating to 70 °C. This observation and other arguments are believed to go against Kosower's model of two emitting states.

A study of the photophysics of 8,1-ANS over a wide range of temperature (77–340 K) by steady state and time-resolved spectroscopy is expected to help understand the conformational and environmental effects. Some initial steady state spectroscopic studies gave a surprising indication of two well-resolved emissions not reported explicitly so far. This also necessitated a more detailed and careful reinvestigation of already well-known steady state emission.

2. Experimental details

Glycerol, ethanol, propanol, octanol, 1,4-dioxane and ethylene glycol of spectroscopic grade were used without further purification. Some of the solvents, e.g. ethyl alcohol, were dried over NaOH. The 1,4-dioxane used was distilled twice and doubly distilled water was used for study. 8,1-ANS was obtained as the acid (Aldrich Chemical Co. Ltd), the ammo-

SSDI 1010-6030(95)04057-9

nium salt (Sigma Chemical Co.) and the sodium salt (Kodak).

Various samples of 8,1-ANS were examined during the study. This was necessary because the initial experiment showed two resolved bands in emission with the same excitation spectra. These samples were purified using the method suggested by Sadkowski and Fleming [11]. 8,1-ANS obtained as the ammonium salt was purified by first precipitating the less soluble magnesium salt from an aqueous solution of excess $MgSO_4$ and then recrystallizing from hot water. The final purified sample showed a single spot in thin layer chromatography (TLC). Since all of them gave the same spectrum, we finally chose the sample crystallized with $MgSO_4$. Freshly prepared solutions (8,1-ANS, concentration 5×10^{-5} M) were taken for study. Steady state absorption and emission spectra were recorded using a JASCO V-550 spectrophotometer and FP-777 spectrofluorometer respectively. This spectrofluorometer, when compared with the Perkin–Elmer LS-5 luminescence spectrometer [1], showed a better resolution. Spectra were observed at various temperatures (77–300 K) using the low temperature accessory of the JASCO FP-777.

Decay time measurements were made with the help of an Edinburgh Model 199 fluorescence time domain spectrometer under single-photon-counting conditions [12]. Some of the measurements were done using a mode-locked Nd:YAG synchronously pumped and cavity-dumped dye laser as an excitation source in place of the coaxial gated flash-lamp. Data analysis was done with a PDP 11/2 microcomputer by the reconvolution method using a least-squares-fitting programme. The goodness of fit was estimated by the χ^2 distribution of residuals and standard deviations. Studies have been performed from 77 to 340 K in glycerol and from 80 K to ambient temperature in ethanol, propanol and octanol.

3. Results and discussion

3.1. Steady state spectra

As already mentioned, the steady state spectra in various solvents agree in position with the known spectra, except that each broad band reported in the literature was found to have a structure. The excitation spectra monitored across the entire emission band were exactly the same. This observation, along with the fact that all four samples of 8,1-ANS obtained under different conditions and sources tallied with one another, excluded the possibility of an extraneous impurity; however, the presence of a small quantity of moisture in the solvents could not be ruled out. A separate experiment with dioxane–water mixtures similar to that reported by Kosower and Kanety [5] was performed. Fig. 1 displays the emission spectra of 8,1-ANS in eight solvent mixtures (dioxane–water). The intensity in water falls to about 1/250 of the original value in dioxane. The spectra are normalized to 500 nm. Table 1 gives the peak values of emission maximum along with the data reported by Kosower and Kanety [5]. The $E_T(30)$ values have been taken from Ref. [5]. Since two peaks, one more prominent in dioxane and the other in water, with the same excitation spectrum are observed, we attribute these two peaks to emission from $S^*_{1,np}$ (dioxane) and $S^*_{1,ct}$ (water). As expected, the CT emission is weak and broad. Even in pure dioxane there is a shoulder at about 500 nm and in pure water there is a shoulder at the blue side near 480 nm, indicating the presence of both emissions in each solvent. The trace presence of NP emission in water is more surprising than that of CT emission in dioxane.

Both NP and CT emissions can be investigated in the polarity range $E_T(30) \approx 36$–63 in dioxane–water mixtures. We have plotted the frequency shift of both NP and CT emissions with polarity parameter $E_T(30)$ as measured in

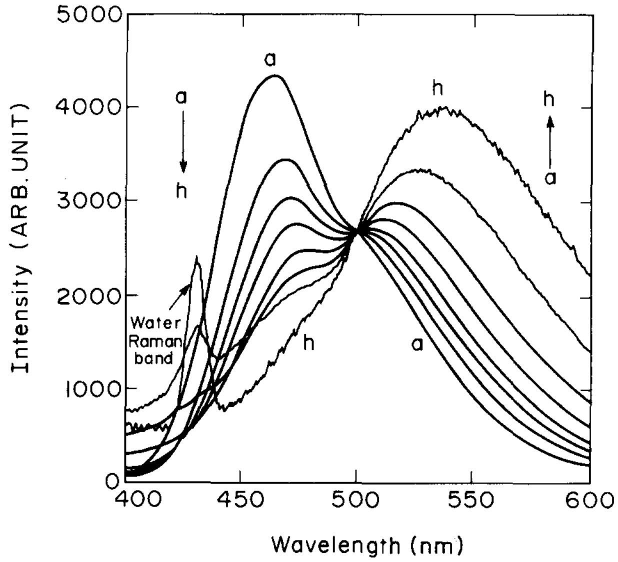

Fig. 1. Fluorescence spectra of 8,1-ANS in dioxane–water mixtures at room temperature. Dioxane contents of solutions used were (a) 100%, (b) 98.1%, (c) 90.5%, (d) 74.9%, (e) 53.0%, (f) 31.1%, (g) 6.9% and (h) 0%.

Table 1
Emission data for 8,1-ANS in dioxane–water mixtures

Dioxane (%)	$E_T(30)$	Em. max. (NP) (nm)	Em. max. (CT) (nm)	Em. max. [a] (nm)
100.0	36.0	463.2	487.0	–
98.9	39.0	468.0	488.0	475.0
98.0	41.1	468.8	499.0	478.0
95.2	43.5	469.8	492.0	482.0
90.5	46.5	471.2	502.0	486.0
84.3	48.0	472.6	505.2	488.0
74.9	50.0	473.0	505.4	491.0
53.0	53.0	477.2	511.0	501.0
31.1	57.0	478.0	517.2	514.0
6.9	61.9	479.0	527.8	530.0
0.0	63.0	480.0	535.6	–

[a] From Ref. [5].

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[1] We thank Professor P. Mohanty of Jawaharlal Nehru University, New Delhi for allowing us to use his Perkin–Elmer luminescence spectrometer.

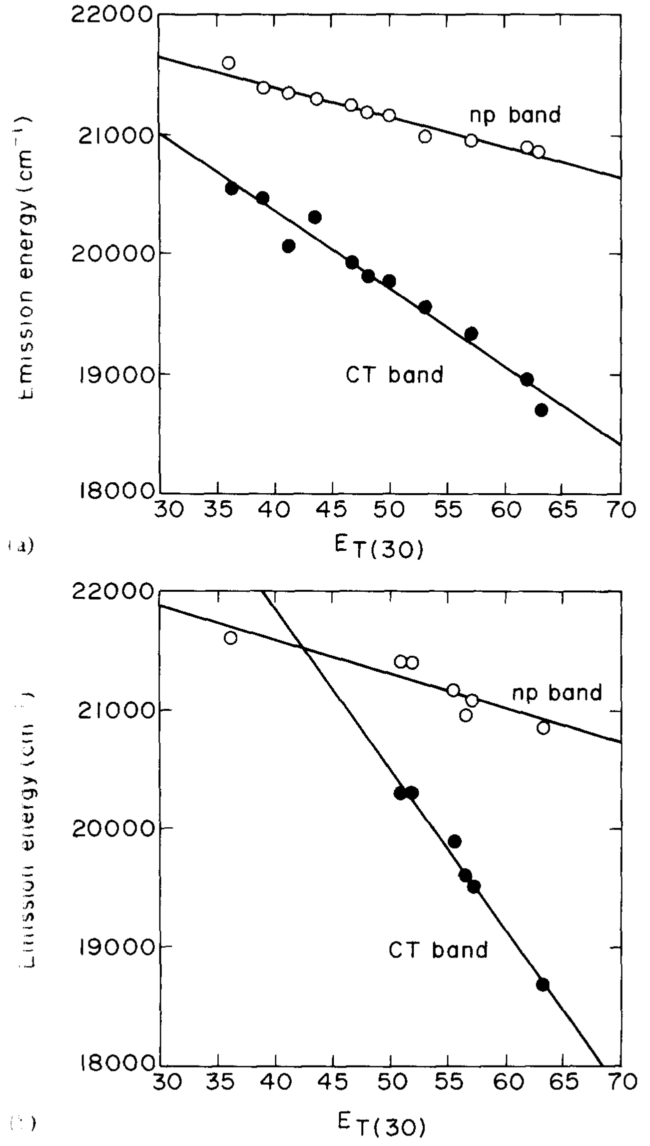

Fig. 2. Plots of emission maximum vs. solvent polarity parameter $E_{T(30)}$ (kcal mol^{-1}) for 8,1-ANS (a) in a series of dioxane–water mixtures and (b) in solvents of various polarities.

solvents of various polarities (Fig. 2(b)) as well as in dioxane–water mixtures (Fig. 2(a)). The NP emission red shifts with a lower slope than the CT emission.

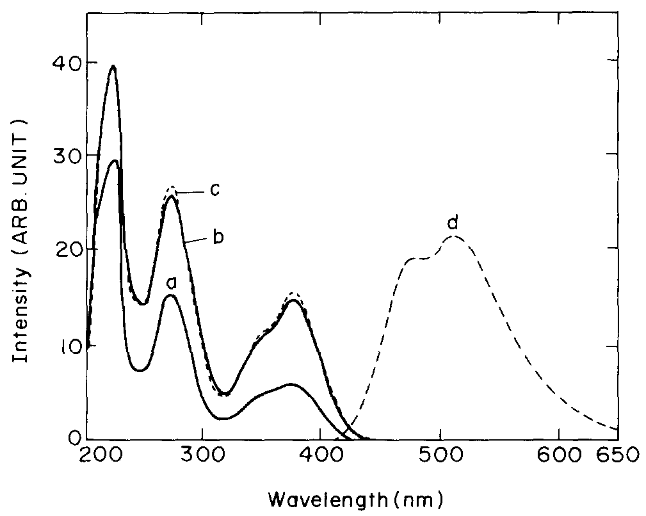

Fig. 3. (a) Absorption, (b, c) excitation (b, monitored at 470 nm; c, monitored at 525 nm) and (d) emission spectra of 8,1-ANS (10^{-5} M) in ethylene glycol at room temperature.

Table 2
Emission data for 8,1-ANS in solvents of various polarities

Solvent	$E_T(30)$	Em. max. (NP) (nm)	Em. max. (CT) (nm)
Dioxane	36.0	463.2	–
Propanol	50.7	467.0	493.0
Ethanol	51.9	467.4	493.0
Methanol	55.5	472.0	502.4
Ethylene glycol	56.3	477.5	510.0
Glycerol	57.0	474.2	513.0
Water	63.1	480.0	535.0

The absorption spectra in all solvents such as ethanol, methanol and ethylene glycol are similar; in water a distinct difference, also observed by Sadkowski and Fleming [11], is seen. Although ethylene glycol does not differ from the other solvents, we show in Fig. 3 the emission, excitation and absorption spectra in ethylene glycol. This case appears somewhat more interesting, because both NP and CT emissions are well resolved and separate excitation spectra can be monitored. As can be seen, the excitation spectra at 470 and 525 nm are identical. The emission and excitation spectra of solid ANS (Mg) salt are similar. Table 2 gives the λ_{max} values of NP and CT emissions in various solvents. The observations recorded above show clearly that there are two excited states (NP and CT) involved in emission which are generated from a single state (NP). It is also known [13] that the longer wavelength edge of emission has a different polarization from the shorter edge.

3.1.1. Spectra at low and intermediate temperatures

The low temperature spectra at 77 K were taken for ethyl alcohol, ethylene glycol and glycerol. In the frozen glass (77 K) the CT emission vanishes. This is as expected, because the charge transfer rate is dependent on viscosity and polarity. In the rigid medium the configurational change is inhibited; the NP state is not converted into the CT state. In ethylene glycol, where at ambient temperature both emissions are substantive, the CT state is completely inhibited at 77 K but appears rather suddenly near the melting point (Fig. 4(A)), which is higher than in ethyl alcohol. Thereafter its intensity increases slightly and the emission shifts towards longer wavelengths. This shift can be rationally understood as being due to solvent relaxation. In ethyl alcohol, where owing to a smaller polarity the NP state is more predominant than the CT state, the temperature variation of emission shows a conspicuous conversion into the CT state around the melting point (Fig. 4(B)). The rate of conversion remains almost constant thereafter up to ambient temperature. Data in Tables 1 and 2 which show a frequency shift in NP and CT emissions

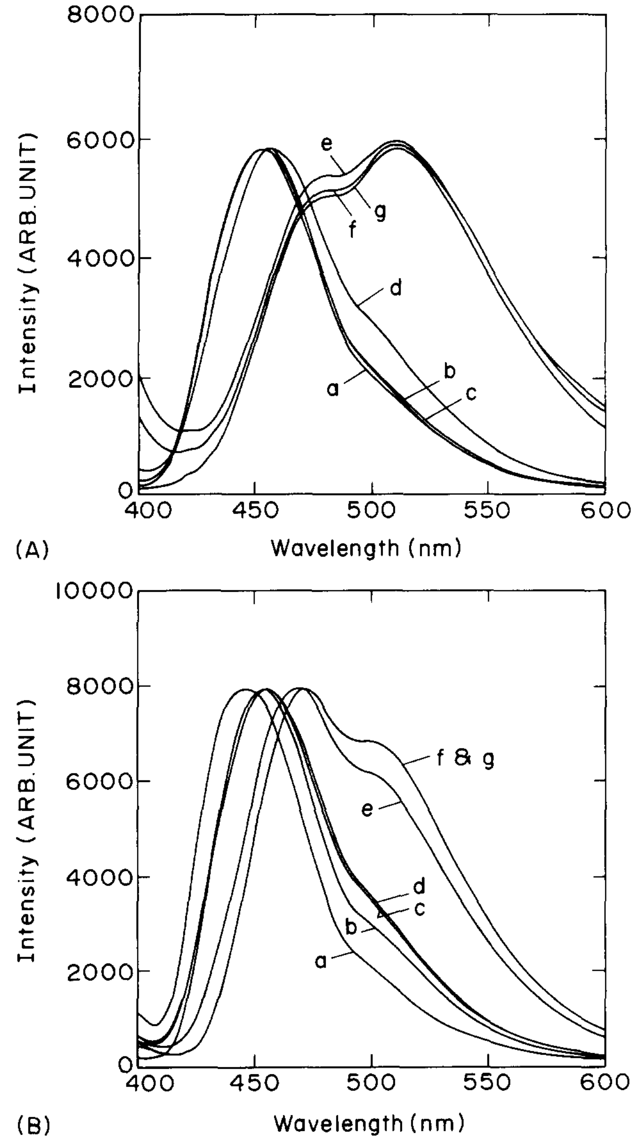

Fig. 4. Emission spectra of 8,1-ANS (A) in ethylene glycol at (a) 77 K, (b) 108 K, (c) 123 K, (d) 253 K, (e) 260 K, (f) 273 K and (g) 298 K and (B) in ethyl alcohol at (a) 77 K, (b) 103 K, (c) 118 K, (d) 133 K, (e) 143 K, (f) 173 K and (g) 203 K.

can be understood on the basis of polarizability changes for the NP emission and dielectric interactions for the CT emission. Similar behaviour is also observed in other solvents. Even in a glycerol–water mixture (1:1) where CT emission is exclusively observed (except for a trace presence of NP emission), the frozen glass shows emission from the NP state only. The relaxation being incomplete in the solid state gives a smaller energy difference between NP and CT emissions.

Fig. 5 displays the ratio of intensities (I_{ct}/I_{np}) vs. temperature in two different solvents. As can be seen, a sudden change takes place near the melting point of the solvent.

3.2. Time-resolved spectra

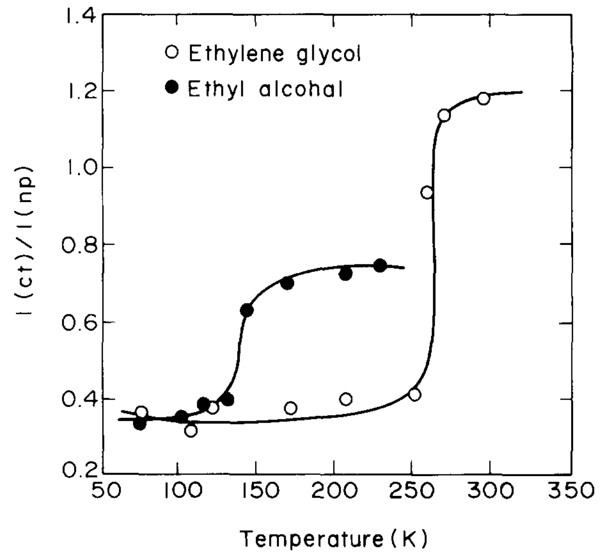

Fig. 5. Temperature variation of ratio of intensities of CT and NP emissions (I_{ct}/I_{np}) for 8,1-ANS in ethylene glycol (○) and ethyl alcohol (●).

8,1-ANS exhibited a large variation in fluorescence decay time in different solvents. It has been reported that in less viscous solvents it exhibits a mono-exponential decay (CT) emission which remains invariant throughout the emission wavelength [8]. However, in viscous polar solvents ($\eta > 8$ cp) the data fit well into a two-state model (Table 3). The fluorescence decay of 8,1-ANS shows a bi-exponential behaviour at the blue edge of the emission profile. Similarly, at the red edge of the emission band the decay is bi-exponential with a rise time. However, at the emission peak the decay is mono-exponential. With increasing polarity of the solvent there is a greater quenching in τ_2 emission, as seen by its low value in glycerol compared with octanol, due to non-radiative quenching, which increases tremendously when $E_T > 58$ in conformity with the results of Sadkowski and Fleming [11]. It is also seen that at the blue edge the amplitude of τ_1 is comparatively larger in high viscosity solvents (more so in glycerol than in octanol).

A two-state model for the photophysics of 8,1-ANS yields Eq. (1). $S^*_{1,np}$ can return to the ground state at a rate k_{np} comprising fluorescence and quenching processes. $S^*_{1,np}$ forms $S^*_{1,ct}$ at rate k_1. $S^*_{1,ct}$ can also fluoresce or return to the ground state by other pathways at the overall rate k_{ct}. The reversible transfer rate from $S^*_{1,ct}$ to $S^*_{1,np}$ is k_{-1}. The standard solutions to the differential rate expression for the decay of $S^*_{1,np}$ and $S^*_{1,ct}$ are given by

$$I_{np}(t) = \alpha_1 \exp\left(-\frac{t}{\tau_1}\right) + \alpha_2 \exp\left(-\frac{t}{\tau_2}\right) \quad (2)$$

$$I_{ct}(t) = \beta\left[\exp\left(-\frac{t}{\tau_2}\right) - \exp\left(-\frac{t}{\tau_1}\right)\right] \quad (3)$$

(if formation of $S^*_{1,ct}$ from $S^*_{1,np}$ is the only excited state process and no direct excitation of CT from the ground state is taking place), where

$$\frac{1}{\tau_1}, \frac{1}{\tau_2} = \tfrac{1}{2}\{(k_{np} + k_1 + k_{ct} + k_{-1}) \pm [(k_{ct} + k_{-1} - k_{np} - k_1)^2 + 4k_1k_{-1}]^{1/2}\}$$

Table 3
Lifetimes and amplitudes of 8,1-ANS (λ_{ex} = 360 nm) [a]

λ_{em} (nm)	Mono-exponential fit		Bi-exponential fit				
	τ (ns)	χ^2	τ_1 (ns)	α_1	τ_2 (ns)	α_2	χ^2
Octanol (10 °C), η = 8 cp, $E_{T(30)}$ = 48							
420	12.13 (0.08)	7.36	0.64 (0.06)	0.157	12.76 (0.04)	0.075	1.15
430	12.34 (0.06)	4.70	0.65 (0.06)	0.129	12.79 (0.03)	0.089	1.01
440	12.42 (0.04)	2.66	0.99 (0.11)	0.059	12.77 (0.03)	0.095	1.01
450	12.35 (0.03)	1.48	1.29 (0.22)	0.027	12.58 (0.03)	0.094	0.95
480	12.57 (0.02)	1.04	5.82 (2.01)	−0.011	12.83 (0.06)	0.145	0.98
510	12.62 (0.03)	1.20	0.47 (0.01)	−0.016	12.53 (0.03)	0.130	1.09
520	12.63 (0.03)	1.23	1.09 (0.39)	−0.019	12.55 (0.03)	0.160	1.13
530	12.54 (0.03)	1.30	1.32 (0.29)	−0.018	12.44 (0.03)	0.160	1.16
Glycerol (20 °C), η = 1490 cp, $E_{T(30)}$ = 57							
430	3.73 (0.08)	19.99	0.68 (0.03)	0.21	4.87 (0.03)	0.06	1.10
450	4.27 (0.06)	10.2	0.79 (0.05)	0.14	4.93 (0.03)	0.08	1.06
480	4.81 (0.03)	2.77	0.93 (0.10)	0.06	5.06 (0.03)	0.11	1.08
510	4.99 (0.02)	1.13					
530	5.13 (0.02)	1.23	0.18 (0.01)	−0.13	5.12 (0.02)	0.14	1.22
540	5.16 (0.02)	1.76	0.79 (0.33)	−0.04	5.04 (0.02)	0.15	1.23

[a] Quantities in parentheses are standard deviations.

I_{np} and I_{ct} are the fluorescence intensities monitored for $S^*_{1,np}$ and $s^*_{1,ct}$ respectively. The fluorescence of $S^*_{1,np}$ shows a bi-exponential decay with lifetimes τ_1 and τ_2, and a rise time corresponding to the lifetime τ_1 will be observed for $S^*_{1,ct}$ fluorescence at the red edge in addition to its decay time τ_2. Our measurements (Table 3) are in partial agreement with this model. From Eq. (3) for a two-state model the amplitudes α_1 and α_2 should be equal (equal to β) at the red edge of the emission profile. However, the anomaly of $\alpha_1 \neq \alpha_2$ may be due to direct excitation of CT emission from the ground state. This may be due to more planar conformers.

3.2.1. *Effect of temperature on decay times*

The decay of 8,1-ANS monitored at 77 K shows a mono-exponential behaviour in all solvents studied. In Table 4 the effect of temperature from 77 to 293 K in glycerol on the lifetime of ANS is given. The decay is mono-exponential between 77 and 218 K. When the glass softens around 228 K, a bi-exponential decay is observed. The mono-exponential decay τ observed at 77 K converts to τ_1 (4.05 ns) and τ_2 (13.69 ns) at the blue edge of the emission. τ_1 has a larger amplitude at the blue edge and a negative amplitude at the red edge. The slower component τ_2 is more towards the red. The sudden decrease in τ_1 at 228 K from 13.6 to 4.05 ns may thus be due to a change from a non-planar to a planar configuration, resulting in the appearance of CT emission. With increasing temperature beyond 228 K both τ_1 and τ_2 decrease, until at 340 K the decay becomes mono-exponential and only CT emission is observed.

In less polar and less viscous solvents such as ethyl alcohol at 77 K a mono-exponential decay is observed which changes to a bi-exponential decay near the glass-softening temperature. The bi-exponential behaviour again changes to mono-exponential around 240 K. This is also true for propanol and

Table 4
Fluorescence decay of 8,1-ANS in glycerol at various temperatures [a]

Temp. (K)	λ_{em} (nm)	Mono-exponential fit		Bi-exponential fit						
		τ (ns)	χ^2	τ_1 (ns)	τ_2 (ns)	α_1	α_2	χ^2	I_1	I_2
80	440	13.61 (0.04)	1.02							
	510	14.66 (0.03)	1.01							
218	440	13.68 (0.03)	1.09							
228	440	11.96 (0.06)	5.41	4.05 (0.16)	13.69 (0.04)	0.04	0.06	0.875	0.16	0.34
	520	16.84 (0.03)	1.02	3.86 (1.08)	16.64 (0.04)	−0.006	0.092	0.926		
253	440	8.87 (0.09)	10.87	2.88	11.08 (0.05)	0.08	0.06	1.069	0.26	0.74
	520	13.42 (0.04)	13.42	2.06 (0.26)	13.03 (0.03)	−0.03	0.13	1.041		
263	440	7.3 (0.05)	14.58	1.94 (0.15)	9.23 (0.03)	0.12	0.09	1.04	0.28	0.71
	510	12.09 (0.06)	1.21	2.67 (0.33)	13.56 (0.04)	−0.017	0.17	1.19		
281	440	4.84 (0.05)	16.2	1.14 (0.11)	6.34 (0.02)	0.16	0.07	1.05	0.29	0.70
	540	6.83 (0.02)	1.65	0.82 (0.10)	6.7 (0.04)	−0.05	0.15	1.11		
303	440	3.22 (0.01)	10.05	0.51 (0.09)	4.01 (0.02)	0.25	0.09	0.99	0.30	0.70
	550	4.15 (0.02)	1.36	0.30 (0.07)	4.11 (0.02)	−0.17	0.18	1.17		
340	440	2.72 (0.03)	1.04							

[a] Quantities in parentheses are standard deviations.

octanol. However, in octanol ($E_{T(30)}=48$ and $\eta=8$ cp) at 283 K a bi-exponential decay is observed.

To rationalize the observations given above, we propose in accordance with Kosower's model that 8,1-ANS has a non-planar configuration in the ground state. In the rigid glass at 77 K the emission is exclusively observed from the NP configuration. As the temperature is raised towards the glass-softening temperature, probably owing to large amplitude participation of the medium, a conformational change takes place from the NP to a planar configuration in the excited state. Henceforth the observed emission consists of both NP and CT emissions (Kosower's NP and CT bands) and the decay follows a bi-exponential behaviour. At higher temperatures the emission comes only from the CT state and a mono-exponential decay appears owing to the conversion of the non-planar to the planar configuration at a fast rate. Several other characteristics of the emission are evident: the CT band is more prominent in polar solvents, is broader and shows larger shifts with polarity than the NP band. The solid specimen of 8,1-ANS (Mg salt) also shows two bands with a smaller separation. In the frozen spectra the CT band vanishes almost completely in all the solvents and reappears rather rapidly rear the melting point — the emission shifts slightly towards longer wavelengths, possibly owing to solvent re-orientation. A detailed study of the two emissions separately was possible in dioxane–water mixtures and variation in the emission frequency of NP and CT states with $E_T(30)$ is possible over the whole $E_T(30)$ range. The results were similar to those of Kosower and Kanety [5].

Acknowledgments

This work was supported by grants from the Department of Science and Technology, India under the National Laser Programme and from the University Grant Commission, New Delhi under the Special Assistant Programme (Department of Special Assistance). A.U. is grateful to Dr. P.B. Bisht for his kind help during the study.

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