Picosecond Fluorescence Studies of Xanthene Dyes

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Abstract: Subnanosecond lifetime measurements using picosecond pulses from a mode locked Nd^{3+}/glass laser together with conventional absorption and fluorescence yield methods have been used to study the photophysics of fluorescein and three of its halogenated derivatives (eosin, erythrosin, and rose bengal) in aqueous and simple alcoholic solvents. For each of the dye molecules absorption and fluorescence maxima move towards higher energy ("blue shift") as the solvent changes from *i*-PrOH to H_2O. Fluorescence lifetimes and quantum yields are found to decrease markedly with this solvent change and also with increased halogenation ("heavy-atom effect") of the fluorescein parent. Published triplet yield data confirm that the variations observed in the nonradiative part of the decay rate can be attributed almost wholly to variations in the rate of S_1–T_1 intersystem crossing. A simple and reasonable explanation of the observed effects can be found if for these particular solvent–solute combinations stabilization energies lie in the order $\Delta E(T_1) < \Delta E(S_1) < \Delta E(S_0)$. This idea is consistent with both the increased S_1–S_0 spectral "blue shifts" and the enhanced intersystem crossing rate, arising from a smaller S_1–T_1 energy gap, when these dye molecules are placed in a more aqueous solvent environment. The studies are relevant to the use of these dyes as fluorescent probes in biologically important molecules.

I. Introduction

The fluorescence properties of the xanthene dyes have both theoretical and practical interest. The advent of the dye laser[1] has sparked off renewed interest in the correlation between molecular structure and fluorescence properties, while the use of fluorescent dye probes in structural studies of molecules of biological significance has become an area of considerable activity.[2,3] The usefulness of a fluorescent probe relies on variations in the dye's fluorescent properties with alterations in its environment, solvent polarity being an example. Despite a considerable amount of work, no detailed theory explaining the often very dramatic effects of environment and structure on fluorescence has emerged. For example, the effect of solvent and macromolecular environment on the fluorescence lifetime and quantum yield of perhaps the most widely used fluorescent probe, ANS^- (1-anilinonaphthalene-8-sulfonate), is not well understood although various mechanisms for the fluorescence quenching in polar solvents have been proposed.[4-6] Until a better understanding of the environmental factors affecting the fluorescence of dye molecules has been achieved, detailed conclusions based on fluorescent probe studies must be treated with caution.

Fluorescein and its halogenated derivatives provide an excellent model series for studies of this kind, for not only do the degree and type of halogenation greatly alter the fluorescence yield, but the photophysical properties of these dyes are also very dependent on the nature of the solvent. We have studied absorption and emission spectra, fluorescence lifetimes, and fluorescence yields of eosin, erythrosin, and rose bengal (Figure 1) in a series of alcohols and in aqueous solution. The data provide a clear picture of the changes in nonradiative decay rate that occur in these molecules as a result of changes in the solvent environment.

II. Experimental Section

(a) Chemicals. Eosin (BDH) was purified by recrystallization from acidic solution and rose bengal (BDH) was purified by chromatography on an alumina–talc column. The erythrosin sample (BDH) was used as supplied. Spectroscopic grade (Merck) methanol, ethanol, 2-propanol, and triply distilled water were used as solvents. All the aqueous solutions were buffered to pH 9.2. Dye concentrations of 10^{-4} M were used for the fluorescence lifetime measurements and 10^{-6} M for fluorescence yield measurements.

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(b) Absorption Spectra. Absorption spectra were obtained using 10^{-4} M solutions (1 mm path length) on a Cary 17 spectrophotometer.

(c) Fluorescence Spectra and Quantum Yields. Fluorescence spectra and quantum yield measurements were obtained on a Perkin-Elmer MPF 3 spectrofluorimeter with a corrected spectrum attachment. An excitation and emission bandpass of 4 nm was used for all measurements. A fluorescein solution (10^{-6} M in 0.01 M NaOH) was used as a standard in the determination of absolute quantum yields, assuming an absolute quantum yield of 0.90 under the above conditions.[7] Fluorescence quantum yields for each solution were evaluated by taking ratios of integrated fluorescence intensities and applying a linear correction for optical density at the exciting wavelength and a quadratic correction for refractive index differences.[8] Absolute yields were then calculated from the fluorescein standard. The relative error in the quantum yield determination is estimated to be 10%.

(d) Fluorescence Lifetime Measurements. A full description of the experimental apparatus will be given in another publication.[9] The fluorescence lifetimes were obtained by excitation of the various solutions contained in 10 mm × 10 mm × 40 mm quartz cells with a single pulse (7 ps fwhm) selected from the pulse train of a frequency doubled, mode-locked Nd^{3+}/glass laser. No difference in lifetime was detected in N_2-saturated compared with air-saturated solutions. The fluorescence decay observed at right angles to the direction of the exciting beam was monitored with an Electro-Photonics Photochron II streak camera/optical multichannel analyzer (Princeton Applied Research) combination. The digitized fluorescence decay curves were transferred to a NOVA 2/10 computer and stored on a disk for analysis. The use of right angle detection geometry minimizes the possibility of shortening the observed fluorescence lifetimes by stimulated emission.[10] In the present case those effects should be unimportant as the fluorescence yields are generally low. In addition, rather low (<50 MW/cm^2) excitation powers were employed.

Recent studies[11] have shown that the rotational reorientation times of the fluorescein derivatives are comparable with their fluorescence lifetimes in the solvents used here. This means that, since the exciting light is polarized, polarized detection must be used if accurate fluorescence lifetimes are to be obtained. In the previous study[11] of rotational diffusion of eosin and rose bengal in several solvents, fluorescence lifetimes were obtained by monitoring the fluorescence decay through an analyzer/polarizer set parallel to the polarization of the exciting pulse [$I_\parallel(t)$] and independently with the polarizer set at right angles to this [$I_\perp(t)$]. The true fluorescence decay is then given by

$$K(t) = I_\parallel(t) + 2I_\perp(t)$$

In the present study, polarization bias has been removed by orienting the analyzer at 54°44′ to the direction of $I_\parallel$.[11,12] The superposition of the components of $I_\parallel$ and $I_\perp$ passed by the analyzer in this case yields $K(t)$.

In the lifetime measurements, the effect of reabsorption was minimized by aligning the exciting beam as closely as possible to the

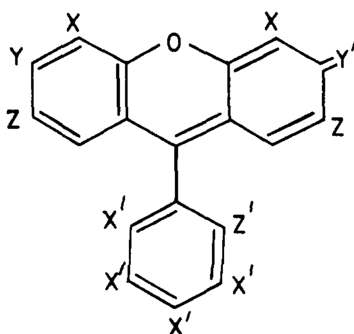


Figure 1. Structural formulas of fluorescein dye derivatives. Fluorescein (dianion): X = Z = H; Y = O^-; X' = H; Y' = O; Z' = CO_2^-. Eosin (dianion): X = Z = Br; Y = O^-; X' = H; Y' = O; Z' = CO_2^-. Erythrosin (dianion): X = Z = I; Y = O^-; X' = H; Y' = O; Z' = CO_2^-. Rose bengal (dianion): X = Z = I; Y = O^-; X' = Cl; Y' = O; Z' = CO_2^-.

viewing window of the sample cell. With the exception of erythrosin in H_2O, which appeared to decompose slightly after prolonged irradiation, no photochemical deterioration of any of the dye solutions during the course of the experiments was noticed. All fluorescence lifetime measurements were carried out at 20 °C.

The fluorescence lifetimes τ_f were obtained from the decay curves by an iterative fitting procedure which involved simultaneous adjustment of the three parameters A, B, and τ in the relation

$$I(t) = A \exp(-t/\tau) + B$$

Here A represents an intensity parameter, B is a baseline parameter, τ is the calculated lifetime, and the data are weighted according to the inverse of the variance of each point. At least five decay curves were obtained for each dye/solvent system, and in each case a good fit to a single exponential was found. The values presented represent a mean of a number of individual measurements, while the reported errors signify the 95% confidence limit ($\pm 2\sigma$). Lifetime and rate constant errors are usually around 10% but in some instances may be higher. In the present study this is mainly due to noise, but some error may be introduced by stray signals in the streak camera[9-11] and by the fact that an error of only 1° in the rotation of the analyzer/polarizer can give rise to about a 10% admixture of either $I_{\parallel}(t)$ or $I_{\perp}(t)$ into $K(t)$.[36] To attempt to avoid errors of the latter kind, an accurate rotary translation stage (Aerotech Model ATS301R) was used for adjustment of the analyzer/polarizer. Figure 2 shows the fluorescence decay of erythrosin in methanol recorded using a single laser shot.

III. Results and Discussion

(a) Absorption and Emission Spectra. The chromophore in the molecules of interest is the xanthene ring (Figure 1). Molecular models show that the phenyl group is sterically hindered and cannot lie in the plane of the xanthene ring. The wavelengths of the absorption and emission maxima for the three dyes in the various solvents are given in Table I. The absorption maxima in ethanol agree well with the values reported by Seybold et al.[13]

The spectra of all three dyes are very similar. The absorption spectra are composed of an intense band corresponding to the allowed origin, a broad shoulder at 0,0 + ~1300 cm^{-1}, most likely associated with 1 ← 0 transitions in the totally symmetric ring breathing mode, and a weaker shoulder at 0,0 + ~2700 cm^{-1}, corresponding to 2 ← 0 transitions. A shift of both absorption and emission maxima toward lower energy is observed as the degree of halogenation is increased. A good mirror image relation between absorption and emission spectra exists for all the spectra. The above findings are consistent with a strongly allowed transition with small geometry change between ground and excited states.

The spectra show that in all cases the predominant species present is the dianion. At the concentrations used in this work no evidence was found for dimer formation in any of the solvents. Förster and König[14] report that dimerization of fluorescein and eosin is unimportant for concentrations less than 10^{-3} M.

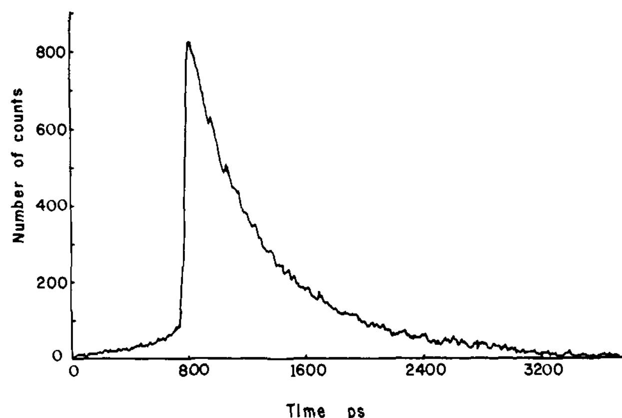


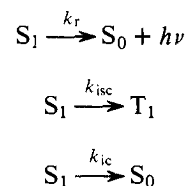
Figure 2. Fluorescence decay of erythrosin (10^{-4} M) in methanol, recorded with a single laser shot.

Table I. Absorption and Emission Maxima (nm)

	Eosin		Erythrosin		Rose bengal	
Solvent	λ_{max}^{abs}	λ_{max}^{fl}	λ_{max}^{abs}	λ_{max}^{fl}	λ_{max}^{abs}	λ_{max}^{fl}
H_2O	515	538	521	534	548	566
MeOH	522	542	526	545	556	571
EtOH	527	550	532	551	558	573
i-PrOH	529	551	532	557	561	576

The absorption and emission bandshapes are very similar in the different solvents. However, the vibronic bands become narrower and better resolved in the higher alcohol solutions. This effect is most pronounced in the rose bengal solutions. For each of the dyes studied a "blue shift" is observed for both absorption and emission as the solvent approaches the aqueous limit. A similar effect has been observed in fluorescein and 6-hydroxy-9-phenylfluoron (HPF) solutions. [In HPF the carboxylic acid group on the phenyl ring is replaced by H; otherwise the structure is identical with fluorescein (Figure 1).] The "blue shift" can be interpreted in terms of a stronger interaction between solvent and ground state dye than between solvent and excited state dye.[15]

(b) Fluorescence Lifetimes and Quantum Yields. We discuss our results in terms of the following intramolecular processes:



$$S_1 \xrightarrow{k_r} S_0 + h\nu$$

$$S_1 \xrightarrow{k_{isc}} T_1$$

$$S_1 \xrightarrow{k_{ic}} S_0$$

Unless the triplet yield (ϕ_T) is known from an independent measurement, the total nonradiative decay rate (k_{nr}) cannot be separated into a sum of intersystem crossing (k_{isc}) and internal conversion (k_{ic}) rate constants. The experimental observables, fluorescence quantum yield (ϕ_f) and fluorescence lifetime (τ_f), expressed in terms of the above nonradiative and radiative (k_r) rate constants, are

$$\phi_f = \frac{k_r}{k_r + k_{isc} + k_{ic}}$$

$$\tau_f = \phi_f / k_r$$

Table II gives the results of the quantum yield and fluorescence lifetime determinations. The ϕ_f values for fluorescein

Table II. Fluorescence Quantum Yields (ϕ_f) and Fluorescence Lifetimes (τ_f, ps)

Solvent	Fluorescein		Eosin		Erythrosin		Rose bengal	
	ϕ_f	τ_f	ϕ_f	τ_f	ϕ_f	τ_f	ϕ_f	τ_f
H_2O	0.92		0.20	1425 ± 140	0.02	115 ± 20	0.018	95 ± 15
MeOH	1.00		0.60	3280 ± 275	0.08	500 ± 60	0.08	655 ± 85
EtOH	0.97		0.69	3620 ± 225	0.08	565 ± 45	0.11	820 ± 95
i-PrOH			0.76	3715 ± 320	0.10	660 ± 60	0.14	1015 ± 110

Table III. Radiative (k_r) and Nonradiative (k_{nr}) Decay Constants ($\times 10^{-8}$ s^{-1})

Solvent	Fluorescein		Eosin		Erythrosin		Rose bengal	
	k_r	k_{nr}	k_r	k_{nr}	k_r	k_{nr}	k_r	k_{nr}
H_2O	1.61	0.14	1.40	5.61	1.74	85.2	1.89	103
MeOH	1.61	0.00	1.83	1.22	1.60	18.4	1.22	14.0
EtOH	1.61	0.05	1.91	0.86	1.42	16.3	1.34	10.9
i-PrOH	1.61		2.05	0.65	1.52	13.6	1.38	8.47

from ref 15 are included for comparison. In all cases the experimental fluorescence decay curves are a good fit to a single exponential decay. The fluorescence lifetimes for eosin and erythrosin in water using the electro-optical Kerr shutter method have been reported previosuly by Porter, Reid, and Tredwell.[16] Our result for erythrosin is in agreement with their value (110 ps) but for eosin their value (900 ps) is considerably shorter than ours (1425 ps), possibly casting doubt on the accuracy of the electro-optical shutter method in this time regime.[16]

Table III gives derived values for the radiative rate constant and the total nonradiative decay constant using τ_f and ϕ_f data in Table II. The values of k_{nr} for fluorescein can be estimated from the quantum yield data of Martin[15] by assuming k_r to have a value similar to that of its halogenated derivatives. The k_r used for these estimates was taken simply to be the average of all other k_r entries in Table III. The value of k_r obtained in this way is around 30% lower than the value obtained by Martin[15] by integration of the absorption spectrum. However, since Martin finds k_r to be essentially constant in the solvents H_2O (2.5×10^8), MeOH (2.44×10^8), and EtOH (2.56×10^8), the conclusions on the variation of k_{nr} with solvent are unaffected.

Two trends are apparent from our experimental results. The nonradiative rate is very sensitive to (i) halogen substitution of the xanthene ring and (ii) the nature of the solvent.

(i) Effect of Halogen Substitution. In a given solvent the nonradiative rate increases dramatically in the series fluorescein, eosin, erythrosin. The nonradiative rate of rose bengal is similar to that of erythrosin. In contrast, the radiative rate is very similar in all the dyes. The triplet yield measurements of Bowers and Porter[17] of these molecules in aqueous solution can be used to estimate the internal conversion yield (ϕ_{IC}) in this solvent. Using ϕ_f (Table II) and these triplet yield values, the relationship $\phi_{IC} = 1 - (\phi_f + \phi_T)$ gives $\phi_{IC} = 0.03$ (fluorescein) (ref 15 gives $\phi_{IC} = 0.05$), $\phi_{IC} = 0.1$ (eosin), $\phi_{IC} \sim 0$ (erythrosin). Internal conversion is, therefore, an unimportant decay mechanism for these molecules in aqueous solutions, so one must conclude that intersystem crossing is responsible for the large changes in k_{nr} in the halogenated derivatives of fluorescein.

It is well known[18] that heavy atoms present either intramolecularly or in the solvent can increase spin-orbit coupling between singlet and triplet states. The series Fl (fluorescein), Br_4Fl (eosin), I_4Fl (erythrosin), and I_4Cl_4Fl (rose bengal) provides a dramatic example; the intersystem crossing rate increases by a factor of ~600 from Fl to I_4Fl in aqueous solution.

The results of Foster and Dudley[19] on the phosphorescence yields of these molecules have been interpreted by several authors[13,20] as contradicting the above ideas. However, as Foster and Dudley themselves point out, their result ϕ_p(eosin) $\simeq \phi_p$(erythrosin) can also be explained by an increase in the $T_1 \rightarrow S_0$ intersystem crossing rate in the iodo derivative. The interpretation of phosphorescence yields alone is, therefore, not straightforward, as radiative and nonradiative decay rates involving T_1 are expected to be sensitive to heavy-atom substitution.[18]

In a study of the photoreduction of the fluorescein dyes by allyl thiourea, Adelman and Oster[21] reported low triplet yields in eosin and erythrosin. As discussed by Bowers and Porter,[17] their results seem to be in error, probably because the photoreduction kinetics are more complex than was originally thought.[17] More recent studies by Nemoto et al.[22,23] using sensitized photoreduction and photosensitized delayed fluorescence[24] confirm the work of Bowers and Porter. We have carried out qualitative measurements of triplet–triplet absorption on a picosecond flash photolysis apparatus similar to that described by Magde and Windsor,[25] and these results also show ϕ_T increasing in the series fluorescein, eosin, erythrosin. The triplet yield of rose bengal is similar to that of erythrosin.

(ii) Effect of Solvent. The second trend is equally marked. In each of the molecules the nonradiative rate increases rapidly as the solvent is changed from *i*-PrOH to H_2O. The effect is most marked in rose bengal where k_{nr} increases by a factor of 12 between 2-propanol and water. In eosin, erythrosin, and rose bengal, the nonradiative rate increases continuously from the higher alcohols through to aqueous solutions. For fluorescein in methanol, Martin[15] gives $\phi_f = 1.00$, so k_{nr} must be small when no heavy atoms are present. The radiative rate (within experimental error) is unaffected by the solvent in all the cases we have investigated. Unfortunately, data on the variation of ϕ_T with solvent are rather sparse. Eosin is the only substituted fluorescein for which triplet yields are available in different solvents. Table IV gives the yields and rate constants for intersystem crossing and internal conversion in aqueous and ethanolic solutions of eosin using triplet yields from four different sources.

These data show that the intersystem crossing rate constant is responsible for the decrease in the fluorescence lifetime between ethanolic and aqueous solutions. It also seems very reasonable that the increases in k_{nr} in erythrosin [$\phi_{IC}(H_2O) \sim 0$] and rose bengal are to be attributed to an increase in k_{isc}. Our value of ϕ_F for eosin in ethanol is in good agreement with the values of 0.68 and 0.67 given by Seybold et al.[13] This

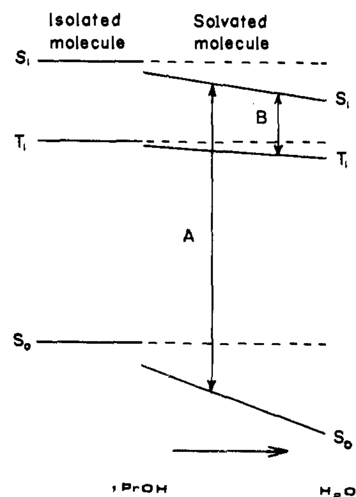


Figure 3. Schematic energy level diagram showing how the S_0-S_1 spectral "blue shift" (A) increases while the S_1-T_1 energy gap (B) decreases when the solvent is changed from *i*-PrOH to H_2O. The isolated molecule levels in this figure have been lowered considerably from what is believed to be their positions in order to show the relative solvent shifts more clearly.

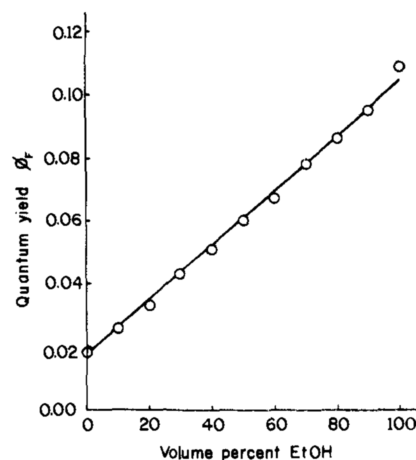


Figure 4. Fluorescence quantum yield of rose bengal in water-ethanol mixtures.

suggests that the value of Fisher et al.,[27] $\phi_T = 0.64$, must be too large.

Previous work in this laboratory[11] has shown that in the solvents used in this work, the fluorescein derivatives rotate as if their volume were at least double that of the free molecule. This volume increase is most likely caused by solvent attachment, as was suggested long ago by Marinesco.[28] In other words, there must be a reasonably strong interaction between the dye molecules and a number of solvent molecules. In view of this result and the spectral shift data in Table I, one can now speculate about the cause of the variation in the nonradiative rates in the different solvents.

The work of Parker and Hatchard[29] on so-called E-type (eosin type) delayed fluorescence has shown that in these molecules the S_1 T_1 energy gap is small [$\sim$3500 cm^{-1} (10 kcal/mol) for eosin in ethanol] and that the major intersystem crossing path is from the vibrationally relaxed S_1 state to the T_1 state. Theoretically,[30] the intersystem crossing rate (k_{isc}) is expected to be quite sensitive to the singlet-triplet energy gap, and therefore solvents which decrease this energy gap are expected to increase the intersystem crossing rate. The results reported here imply that the S_1-T_1 energy gap is reduced as the solvent approaches the aqueous limit. An explanation consistent with this conclusion and the observed spectral shifts is that the solvent stabilization energy (ΔE) in a given solvent decreases in the order $\Delta E(S_0) > \Delta E(S_1) > \Delta E(T_1)$, and in addition that it increases in the order $\Delta E(i\text{-PrOH}) < \Delta E(\text{EtOH}) < \Delta E(\text{MeOH}) < \Delta E(H_2O)$. Since the solvent shifts range over only about 14 nm, the interaction energies need not vary by more than a few times kT (room temperature) for the series of solvents used in this work. The energy level shifts are shown schematically in Figure 3.

To see whether or not the solvent effect is caused by a very specific interaction between the solute and water molecules, rather than by a more general solvent-solute interaction, lifetimes and yields of rose bengal were measured in a series of ethanol-water mixtures. The results for the quantum yield measurements are shown in Figure 4. It can be seen that the quantum yield varies smoothly from 100% EtOH to 100% H_2O. Similar results were obtained for the lifetime measurements. This is consistent with the idea that the solvent interaction energies need not vary over a wide range.

The lifetimes and yields of rose bengal were identical within experimental error in both H_2O and D_2O solutions, in contrast with the results of Martin and Lindqvist for HPF.[31] In their

Table IV. Intersystem Crossing and Internal Conversion Rate Constants and Yields for Eosin

Solvent	ϕ_f	ϕ_T	ϕ_{ic}	k_{isc}	k_{ic}
H_2O	0.20	0.71[a]	0.09	4.98×10^8	0.56×10^8
		0.76[b]	0.04	5.33×10^8	0.28×10^8
EtOH	0.69	0.41[c]		1.13×10^8	
		0.64[d]		1.76×10^8	

[a] Bowers and Porter, ref 17. [b] Soep et al., ref 26. [c] Nemoto et al., ref 22. [d] Fisher et al., ref 27.

study of solvent effects on nonradiative processes in HPF and fluorescein, Martin and Lindqvist[31] and Martin[15] concluded that in weaker hydrogen bonding solvents the internal conversion rate in these molecules is enhanced. The large variations in ϕ_f for HPF in H_2O/D_2O, MeOH/MeOD, EtOH/EtOD ($\phi_f(H) < \phi_f(D)$ in all cases) were taken as evidence for the involvement of the O-H stretching vibration of the solvent molecules in the nonradiative process. Such effects are not evident in our work, and we, therefore, conclude that solvent vibrations do not participate directly in the nonradiative process. Since HPF does not contain heavy atoms, one would not expect rapid intersystem crossing in this molecule. It is surprising that in water or alcohol solutions[15] HPF (fast $S_1 \rightarrow S_0$ internal conversion) behaves so differently from the almost identical molecule fluorescein (slow $S_1 \rightarrow S_0$ internal conversion).

Another possible explanation for the change in k_{isc} with solvent is that structural changes in the dye molecules are produced by the different solvents. This seems to be ruled out by the similarity of the absorption and emission spectra and the rather small spectral shifts in the different solvents. Excited state proton transfer reactions leading to nonfluorescent forms of the dye[32] seem also to be a less probable explanation because of the following observations: (a) $\phi_f + \phi_T \simeq 1$, (b) no variation was observed in the fluorescence yield of rose bengal in aqueous solution as the pH was varied from 7 to 12, (c) Kasche and Lindqvist[33] and Fisher et al.[27] find that the transient species seen in microsecond flash photolysis studies of aqueous eosin are unaffected in both yields and lifetimes by pH change from 5 to 12, and (d) the pK of the excited dianion of fluorescein is very similar in both ground and excited states.[34] See also similar arguements by Martin.[15] Thus, we conclude that the most plausible mechanism for the increase in intersystem crossing rate is a decrease in the S_1-T_1 energy gap. Again, because of the rather small solvent shifts, we do not believe this change in energy gap is very large for the solvent series studied,

perhaps no more than 1-2 kcal/mol. However, this is a relatively large fraction of the total gap energy (~10 kcal/mol).

A similar mechanism to the one above has been proposed by Brand and Gohlke[4] to explain the rapid decrease of ANS^- fluorescence quantum yield and lifetime in polar solvents. These authors propose that the dipole moment of the triplet state is less than that of the S_1 state and, thus, polar solvents will decrease the S_1-T_1 energy gap. Other explanations have been proposed, but the situation is more complex than that with the fluorescein derivatives, as both k_r and k_{nr} in ANS^- vary sharply with solvent. Penzer[5] has proposed that variations in the degree of coplanarity of the anilino and naphthalene rings of ANS^- in different solvents may account for the decrease in yield in the polar solvents, where the two ring systems may be normal to each other. On the other hand, Kosower and Tanizawa[6] invoke two different excited states, noncoplanar and charge transfer species, although again the solvent dependence of ϕ_f in their theory arises from changes in the singlet-triplet energy gap. It is evident that more work is required on the environmental effects on the fluorescence of this important and interesting molecule before its photophysics is fully understood.

IV. Discussion

Picosecond spectroscopy has made it possible to measure fluorescence lifetimes of molecules in solution, even when quantum yields are low. This virtue allows the four important quantities fluorescence lifetime, fluorescence yield, triplet yield, and integrated absorption cross section to be independently assessed in such cases. Solvent-solute interactions may change the rates of nonradiative as well as radiative processes. If the radiative rate is changed or some other more complicated effect arises because of interaction between the dissolved molecule and its environment, it is essential that all four of the above measurements be made in order to sort out reliably the various pathways that the excited molecule travels and to understand fully the effect of solvent on these pathways. These effects and their understanding are particularly germane to the interpretation of biological fluorescent probe experiments.

This paper has presented a very simple example of this type of analysis, where both intermolecular and intramolecular changes in the "environment" of the molecular electronic transition have been made. Intramolecularly, halogen substitution was found to decrease the fluorescence lifetimes and concomitantly the fluorescence quantum yields, while causing no appreciable change in the integrated absorption cross sections. Literature values of triplet yield measurements were then used to show that virtually the entire nonradiative process from the lowest excited singlet of the systems studied could be accounted for in terms of intersystem crossing to the triplet. All the various data are consistent with this pathway, showing that nothing complicated is taking place.

Solvent effects, insofar as they have been studied here, also indicate nothing very complicated or unusual for these systems. The rate of the intersystem crossing process described above is enhanced as one proceeds through the series *i*-PrOH, EtOH, MeOH, H_2O. Because of the "blue shifts" in the $S_1 \leftarrow S_0$ absorption and emission spectra in this series of solvents, the most obvious and simple explanation for the intersystem crossing enhancement is a decrease in the S_1, T_1 energy gap within the solvent series.

These explanations of the intra- and intermolecular environmental effects are totally consistent with views expressed earlier by Martin.[15] The additional pathway of $S_1 \rightarrow S_0$ internal conversion was not important in our experiments. Martin[15] has shown this to be so for fluorescein in the solvents we have used, and, on the basis of the work here, apparently it is also the case for the halogenated fluoresceins. (Eosin in dimethylformamide might be expected to have an important internal conversion pathway.) Martin would attribute the $S_1 \leftarrow S_0$ "blue shifts" to increasing hydrogen bond donating power in the solvent series *i*-PrOH, EtOH, MeOH, H_2O, plus the fact that the S_0 state is more stabilized by this interaction than the S_1 state. Our results would then indicate that the S_1 state is more stabilized than the T_1 state by hydrogen bonding to the solvent. The smooth, linear change in fluorescence lifetime and quantum yield with proportionate change in EtOH concentration in a mixed EtOH-H_2O solvent system indicates, however, that the solvent-solute interaction is not strongly dominant for H_2O. If it were, one would expect gross changes in the above two experimental values with small additions of H_2O to EtOH; then a leveling off or saturation effect would be reached when all "active sites" of the solute molecules are "filled" with H_2O molecules. This picture seems to apply to ANS^- and TNS^- (unpublished work in our laboratory) but not to the fluorescein derivatives.

Picosecond fluorescence depolarization experiments[11] for the solvent-solute systems studied here seem to indicate that solvent attachment increases by about a factor of 3-4 the hydrodynamic volume of the solute molecule to be used in rotational diffusion equations. This effect is not strongly dependent on solvent for the series *i*-PrOH-H_2O. All these data taken together suggest that interactions are strong but not very solvent dependent within the water-alcohol series. The fact that solvent attachment does not seem important for rotating cations, such as rhodamine 6G,[35] implies that the hydrogen-bonding interactions are not strong in those cases. Perhaps local electrostatic repulsion between the positive charge and the positive end of the solvent dipole is responsible. More will be said about this and about a much greater range of solvents in future work.

Acknowledgments. We thank Dr. W. Sawyer of the Biochemistry Department, Melbourne University, for allowing us the use of his fluorimeter. We are especially grateful to Mr. Ray Robbins for writing the single exponential fitting program. We also thank Mr. Ian Harrowfield for advice and help with some of the work reported here. The support of the Australian Research Grants Committee, the Robert A. Welch Foundation, and the U.S. Army Research Office (Grant No. DAAG29-76-G-0289) is also gratefully acknowledged.

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(36) Impurities in the dye samples can also give rise to errors. For these time scales and concentrations the errors are usually not caused by diffusion or excitation transfer but by the contribution impurities make to the fluorescence data. For example, if the impurity is not so highly halogenated as the pure compound, it will very likely have a longer fluorescence lifetime and a higher quantum yield. The higher quantum yield will cause the impurity to contribute disproportionately to the decay curve. An apparently longer lifetime will thus be measured than for the pure compound. [We acknowledge Dr. Ken Spears, Northwestern University, for a discussion about this point.] While precautions were taken (section IIa) to avoid impurity effects, there is always a possibility that some error can arise from this source because of the very impure state of commercially available fluorescein derivatives. The lifetimes reported in this paper are generally shorter than those reported in our earlier work.[11] We do not know whether this is because we have gained more familiarity with the impurity problems in these molecules and are now better qualified to avoid them or whether the more complex analysis in the earlier work together with noisy data led to greater errors than expected.

Photoelectron Spectra of Psychotropic Drugs. 1. Phenethylamines, Tryptamines, and LSD

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Abstract: A number of correlations between calculated highest occupied molecular orbital (HOMO) energies and psychotropic activity have been reported in the literature. In order to determine whether any correlation between experimental ionization potential and drug activity exists, as well as to learn more about the electronic structures of these systems, the photoelectron spectra of phenethylamine and ten substituted derivatives or analogues, of tryptamine and seven derivatives or analogues, and of LSD have been measured. Photoelectron spectral data are given for phenethylamine, *N*-methylphenethylamine, *N,N*-dimethylphenethylamine, 4-hydroxyphenethylamine, 4-methoxyphenethylamine, 3,4-dimethoxyphenethylamine, mescaline, *N*-methylmescaline, γ-phenylpropylamine, amphetamine, methamphetamine, tryptamine, *N*-methyltryptamine, *N,N*-dimethyltryptamine, gramine, 5-methyltryptamine, 5-methoxytryptamine, 5-methoxy-*N,N*-dimethyltryptamine, and lysergic acid diethylamide (LSD). Model compounds, 3,4-dimethoxytoluene and 3,4,5-trimethoxytoluene, have also been studied. The changes in ionization potentials in these series have been interpreted in terms of the influence of substitutions on the molecular orbital energies of these molecules. Although only limited biological data are available, it is shown that not only the first IP, which is not always affected much by substituents, but also the second IP, must be taken into account in order to correlate activity with ionization potentials. The use of this average is justified theoretically, in terms of perturbation, or charge transfer, models of reactivity, as well as empirically.

In 1959, Karreman, Isenberg and Szent-Györgi observed that a number of drugs, including LSD, have unusually high energy highest occupied molecular orbitals (HOMO's), according to approximate calculations.[2] Since that time, a number of correlations between the HOMO energy of a molecule, calculated by various approximate techniques, and the pharmacological activity of the molecule, measured in a variety of ways, have been found.[3] These correlations have been interpreted as evidence for the importance of electron donation or charge transfer from the molecule to an acceptor moiety at the active site. In some tests, correlations between calculated HOMO energies and activities have not been found.[3b]

Phenethylamines with one or more donor substituents on the benzene ring, and tryptamines with simple or elaborate (e.g., LSD) substituents, are the classes of molecules for which correlations between HOMO energies and activities have been most frequently postulated. Psychotomimetic or hallucinogenic activity in man, or some more or less suitable animal model for hallucinogenicity, is the type of activity with which the electron-donor property of these drugs has been correlated.[4]

The ability of the drug to act as an electron donor is not, of course, the only feature required for hallucinogenicity. An aminoethyl side chain or alkylamino group able to assume a molecular location relative to the aromatic ring similar to that found in LSD is optimal for activity,[5] and N-alkylation helps protect the side chain against deactivation by monoamine oxidase.[6] It has also been proposed that the protonated amino side-chain hydrogen bonds to a phosphate moiety of an ATP residue, and the aryl moiety then acts as a donor in an electron donor-acceptor complex elsewhere in the active site.[7,8] Whatever the details of drug-receptor interaction, even a casual inspection of the structures of hallucinogens leaves no doubt that the electron donor ability of the aromatic portion of the molecule must be important in conferring hallucinogenic properties on certain phenethylamines and tryptamines.

Correlations between HOMO energies and activities are, of necessity, based on calculated orbital energies, because orbital energies are merely artifacts, if extraordinarily useful ones, of the Hartree–Fock–Roothaan formalism. The physical property of a molecule which correlates most closely with its HOMO energy is its lowest ionization potential (IP). Koopmans' theorem states that the negatives of the orbital energies of a molecule are equal to the ionization potentials of the molecule:[9]

$$-\epsilon_i^{\mathrm{SCF}} = \mathrm{IP}_i$$

Although this approximation is known to suffer from severe limitations, it is common practice to discuss IP's in terms of