

Aggregation Phenomena in Xanthene Dyes¹

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The formation of aggregated dye molecules in concentrated aqueous solution was first suggested to explain the rather substantial deviations from Beer's law evidenced by highly colored organic ions such as methylene blue, certain cyanines, and crystal violet. Aggregation of these dyes and others such as the xanthenes² has fundamental consequences in applications as diverse as photographic technology,³ tunable lasers,⁴ fluorescence depolarization diagnostic devices,⁵ and photomedicine.⁶ Since the formation of aggregates modifies the absorption spectrum and photophysical properties of a dye, it affects its ability to emit at a certain wavelength or to act as a photosensitizer. The applications above are solely dependent on one or both of these phenomena.

Absorption and emission spectroscopy are the techniques most widely used and yield the most information about aggregation of complex organic molecules in aqueous solution because spectral shifts, nonconformity with Beer's law, and fluorescence quenching at high concentrations are all indicative of second-order spectroscopic interference between dye chromophores. As an example, the effect of concentration on the absorption spectrum of rhodamine 6G is presented in Figure 1. Analysis of changes in the absorption spectrum with dye concentration allows one to obtain the equilibrium constant for the association process and to derive the absorption and occasionally the emission spectrum of the aggregates. Several mathematical treatments have been applied and developed for this purpose. As we will discuss later, reported equilibrium constants for a given system are in good agreement but discrepancies exist in the spectra derived for the aggregates from different theoretical treatments. These discrepancies are both qualitative and quantitative, and not only the values of molar absorptivities but also the spectral shape depend on the method of calculation employed.

Several other experimental methods are also applied to the study of the association of organic molecules in solution, including diffusion,⁷ conductivity,⁸ light scattering,⁹ and polarography.^{10,11}

The strength of the aggregation between two or more dye molecules depends on the structure of the dye, the solvent, the temperature, and the presence or absence of electrolytes. In general, dyes aggregate more strongly in water than in organic solvents, and more generally

still in solutions of high ionic strength. The presence of alcohol or glycerol in a concentrated aqueous solution of a dye prevents the formation of aggregates. This has been interpreted in terms of the influence of the solvent dielectric constant on the electrostatic repulsion between hydrophobic, yet ionic, dye molecules. However, Arvan and Zaitseva¹² present evidence against this interpretation. Working with a number of dyes in simple and mixed solvents of identical dielectric constants, they show that the chemical nature of the solvent is more important than the dielectric constant in inducing the formation of aggregates. On the basis of these results,¹² the authors propose that the presence of hydroxyl groups in the solvent molecules plays an important role in promoting aggregation. We will comment in detail on the mechanism of aggregation later in this Account.

Various mechanisms have been suggested to explain the forces holding dye ions together in solution. These include additive forces of a van der Waals type,¹³⁻¹⁶ intermolecular hydrogen bonding,¹⁷⁻¹⁹ hydrogen bonding with the solvent,^{12,15,16,18,19} and coordination with metal ions.²⁰⁻²² Each of these will be discussed in relation to specific dye systems later in this Account. In a given dye/solvent system, more than one mechanism may be important. In the case of fluorescein and its halogenated derivatives, the nature of the driving force for aggregation changes with the extent of halogenation. Hydrogen bonding appears to be responsible for the dimerization of fluorescein in protic solvents.²³ However, for its halogenated derivatives, the driving force is a combination of hydrogen bonding and dispersion/hydrophobic forces, the participation of the latter increasing with increasing halogen substitution and with

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Oscar Valdes-Aguilera holds a Ph.D. degree from Brandeis University. Born in Viña del Mar, Chile, he received his predoctoral education in Chile and Brazil. After two years at Wellman Laboratories for Photomedicine, Harvard Medical School, he joined Professor Neckers' laboratory at the Center for Photochemical Sciences in 1986. His research interests are in photochemistry, electron transfer, dye aggregation, and photodynamic therapy.

Douglas C. Neckers is a native of Chautauqua County, New York, and completed his formal education at Bucknell University, Hope College, and the University of Kansas. His teaching career, which now spans nearly a quarter of a century, has been carried out almost entirely in the non-Ph.D.-granting institutions Hope College and Bowling Green State University, where he is now Distinguished Professor. Rose Bengal came into his life in the early 1970s, and the xanthene dyes have occupied his research attention since.

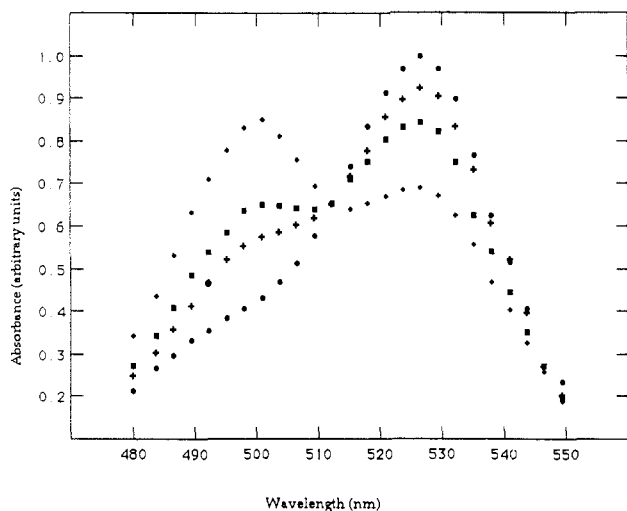


Figure 1. Absorption spectra of rhodamine 6G in aqueous solution at 22 °C. (Adapted from ref 34.) Circles: 7.90×10^{-7} M. Crosses: 7.90×10^{-5} M. Squares: 1.48×10^{-4} M. Diamonds: 7.41×10^{-4} M.

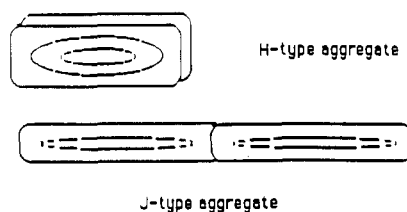


Figure 2. A representation of H-type and J-type aggregates.

the presence of more polarizable halogens.²⁴

Exciton theory^{25,26} predicts that the excited-state levels of the monomer split in two upon dimerization. One level is of lower and the other of higher energy than the monomer excited state. This splitting is a consequence of the two possible arrangements (in-phase and out-of-phase oscillation) of the transition dipoles of the chromophores in the dimer. The interaction energy between the chromophores is a function of the transition moment of the monomer, the angle and distance between the transition dipoles. Transitions from the ground state to either excited state are possible. However, the number of bands actually observed depends on the geometry of the dimer:

(a) For parallel dimers (H-type, Figure 2), the transition to the lower energy excited state is forbidden and the spectrum consists of a single band blue-shifted with respect to the monomer.

(b) For head-to-tail dimers (J-type, Figure 2), the transition to the higher energy excited state is forbidden and the spectrum shows a single band red-shifted with respect to the monomer. The spectrum of a concentrated solution of 1,1'-diethyl-2,2'-cyanine chloride, which forms both types of aggregates simultaneously, is shown in Figure 3.

(c) In dimers having intermediate geometries, both transitions are partially allowed and band splitting is observed. The absorption spectrum shows two bands of similar bandwidth. In this case, the angle between the transition dipoles can be calculated from the os-

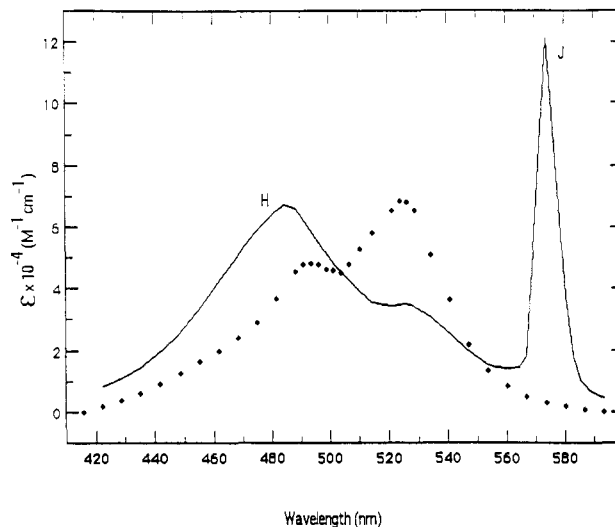
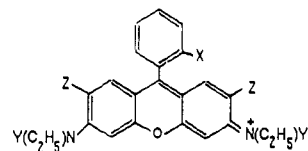


Figure 3. Absorption spectrum of aqueous solutions of 1,1'-diethyl-2,2'-cyanine chloride. Diamonds: 1.3×10^{-5} M; dye exists as monomer. Solid line: 1.4×10^{-2} M; mixture of monomer and aggregates. H and J indicate the positions of the bands for the H-type dimer and J-type aggregate, respectively. Adapted from: West, W.; Carroll, B. H. In *Theory of the Photographic Process*; Mees, C. E. K., Jones, T. H., Eds.; Macmillan: New York, 1966.



X	Y	Z	Dye
COO ⁻	C ₂ H ₅	H	Rhodamine B
CO ₂ C ₂ H ₅	H	CH ₃	Rhodamine 6G

Figure 4. Structures of rhodamine 6G and rhodamine B.

cillator strengths of the component bands.

In this Account we will critically review the aggregation behavior of several xanthene derivatives and comment on structural properties in the dye that affect the thermodynamic parameters of the dimerization process. First we review the literature on the aggregation characteristics of the laser dyes rhodamine B and rhodamine 6G. Association of fluorescein and its halogenated derivatives is reviewed in the next section. We concentrate mainly on quantitative studies of dye aggregation performed by absorption spectroscopy. Band splitting is always observed for the xanthenes, indicating that these aggregates are neither parallel nor linear but have intermediate geometry. Finally we consider the case of Rose Bengal in detail.

Rhodamine 6G

Rhodamine 6G, Figure 4, is frequently used in tunable lasers.²⁷ In fact, an aqueous solution of the dye containing Triton X to prevent aggregation was used to produce the first continuous-wave laser.²⁸ Formation of aggregates decreases the emission quantum yield of rhodamine 6G by a combination of monomer-dimer energy transfer and absorption of radiation by non-fluorescent dimers.²⁹ Since the absorption spectra of

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Table I
Dimerization Equilibrium Constant and Spectral Properties of Rhodamine 6G Dimers in Aqueous Solution^a

K_d , M ⁻¹	λ , nm	$\epsilon \times 10^{-4}$	ϵ_1/ϵ_2	ref
2600	500	16.9	2.02	29
	525	8.36		
2400	498	8.18	3.89	30
	532	2.12		
2570	490	6.50	b	31
	b	b		
6200	498	12.8	1.36	32
	528	9.38		
1695	499	18.6	2.98	34
	531	6.25		

^a For the monomer, $\lambda_{\max} = 527$ nm, $\epsilon_{\max} = 7.58 \times 10^4$ M⁻¹ cm⁻¹.
^b Only one band is derived for the dimer.

solutions of rhodamine is a function of concentration, aggregation of rhodamine 6G has been postulated in both aqueous solution and organic solvents.^{4,29-35} Figure 1 shows the spectrum of the dye at four concentrations in water, and Table I presents values of the dimerization equilibrium constant and spectral parameters of the dimer in aqueous solution.

Although there is general agreement on the values of K_d , the spectrum derived for the dimer of rhodamine 6G from theory depends on the method of calculation. A general rule in the field of xanthene aggregation, this is related to the narrow range of compositions attained within the concentration ranges used in most studies of dye association. Except for one case,²⁹ the studies on rhodamine 6G aggregation have been performed within concentration ranges where the monomer predominates. Since both the equilibrium constant and molar absorptivity of the dimer are treated as variables and their values determined by iteration, it is important to have a good estimate of the dimer molar absorptivity from experimental data because of the complexity of the problem.

The dimerization enthalpy for rhodamine 6G in water has been determined from the effect of temperature on the equilibrium constant. The values obtained, -5.7 kcal/mol³¹ and -6.9 kcal/mol,³² have been interpreted as evidence for hydrogen bonding in dimer formation.

Rhodamine 6G exists as the monomer in ethanol at the concentrations used in dye lasers, 1×10^{-5} - 1×10^{-4} M. For concentrations greater than 5×10^{-3} M, dimers and trimers have been detected.³⁵ Changes in the absorption spectrum in EtOH with dye concentration are less pronounced than those observed in aqueous solution. Therefore a combination of spectrophotometry and vapor pressure osmometry is required to study the aggregation behavior in ethanol. The spectrum of the dimer³⁵ in EtOH is red-shifted with respect to the monomer, indicating that the geometry of the dimer is also different in ethanol from that formed in water, where the absorption maximum is at shorter wavelength than that of the monomer, Table I.

Association of rhodamine 6G in ethanol is considerably weaker than it is in water, the dimerization

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equilibrium constant being 0.27 M⁻¹. The enthalpy and entropy of dimerization are 9.3 kcal/mol and 29.4 eu, respectively. According to Ruiz Ojeda et al.,³⁵ dimerization in ethanol is endothermic. It involves the breaking of two hydrogen bonds between ethanol and the monoethylamino groups of rhodamine 6G and formation of one hydrogen bond between the monoethylamino group. The entropy increase is due to the breakdown of the solvation structure. This interpretation is consistent with the different geometries observed for the dimer in water and ethanol, and also with the larger equilibrium constant, 540 M⁻¹, obtained in dioxane/5% methanol.³⁰

Rhodamine B

Rhodamine B (neutral form, Figure 4) is commonly used as a photosensitizer,³⁶ as a quantum counter,³⁷ and as an active medium in dye lasers.²⁷ Equilibrium studies in aqueous solution³⁸ indicate that the neutral form is predominant for pH > 4.

Aggregation of rhodamine B has been studied by several laboratories,^{14,17,24,34,38-44} and the dimerization equilibrium constant in neutral aqueous solution^{14,24,34,41,43} is $(1.3-1.5) \times 10^3$ M⁻¹. At pH = 12, Lopez Arbeloa and Ruiz Ojeda^{39,40} report a value of 2.1×10^3 M⁻¹. As is the case with rhodamine 6G, there are differences in the dimer absorption spectrum depending on who measures it. However, it is agreed that the absorption maximum of the dimer is blue-shifted with respect to that of the monomer.

The effect of temperature on the association equilibrium constant in aqueous solution has been studied by Lopez Arbeloa and Ruiz Ojeda,⁴⁰ Levschin and Gorskov,⁴² and Rohatgi and Singhal.²⁴ The values of the dimerization enthalpy reported by these different authors are -4.1 kcal/mol, -10 kcal/mol, and -2.8 kcal/mol, respectively. Entropy values are 1.2 eu⁴⁰ and 5.3 eu.²⁴ This disagreement among the reported thermodynamic values precludes a definite conclusion as to the dimerization driving force. The interpretation provided by Rohatgi and Singhal,²⁴ that the dimerization of rhodamine B is driven by hydrophobic forces, is certainly incorrect since if this were the only driving force the exothermicity observed should be not greater than 1 kcal/mol.⁴⁵ Considering this fact, along with the observation that the reaction occurs with a decrease in volume,⁴⁴ it is possible that both hydrogen bonding and hydrophobic forces are important in the dimerization of rhodamine B in aqueous solution.

Levschin and Lonskaya¹⁷ have reported that aggregates are not detectable in ethanol and other organic solvents up to a rhodamine B concentration of 5×10^{-3} M. On the basis of this observation, we calculate a value of 25 M⁻¹ as an upper limit for the equilibrium constant in these solvents. In contrast, Selwyn and Steinfeld³⁴

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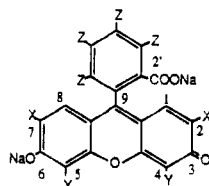
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Compound	X	Y	Z	λ_{\max} (nm in H ₂ O)	λ_{\max} (nm in EtOH)	θ^{\dagger} (EtOH)	θ^{\dagger} (H ₂ O)	ρK_1^{\dagger}	ρK_2
Rose Bengal	I	I	Cl	549.0	557.0	.86	.75	3.72	—
Phloxin	Br	Br	Cl	538.5	547.5	.40	.65	3.69	—
Erythrosin	I	I	H	525.5	532	.69	.63	4.18	—
Eosin	Br	Br	H	517.0	523	.32	.57	3.80	—
	Cl	Cl	H	510.0	520	.05	.05	—	—
	I	H	H	506.5	513	.33	.48	3.75	4.75
	Br	H	H	504	510	.32	.42	—	—
	H	Cl	H	502.5	511	.04	.07	3.69	4.91
Fluorescein	H	H	H	490.0	499	.03	.03	4.38	6.99

[†] Singlet oxygen formation

[†]Data from I. M. Issa, R. M. Issa and M. M. Ghoneim, *Z. Phys. Chem.*, **250**, 161 (1922)

Figure 5. Structures and properties of fluorescein and halo-fluorescein dyes.

find aggregation in ethanol and EPA (ether-pentane-alcohol) to be stronger than in water. The dimerization equilibrium constants in ethanol and EPA are $2.04 \times 10^4 \text{ M}^{-1}$ and $1.61 \times 10^4 \text{ M}^{-1}$, respectively. The association enthalpy and entropy in ethanol are -4.1 kcal/mol and 6.0 eu . The corresponding values in EPA are -0.8 kcal/mol and 1.6 eu . From these results the authors³⁴ concluded that dimerization of rhodamine B in EPA is driven exclusively by dispersion forces whereas hydrogen bonding with the solvent plays an important role in the association in ethanol.

The spectra derived for the dimer of rhodamine B in ethanol and EPA³⁴ show absorption maxima at longer wavelengths as compared to the monomer, indicating that the geometry of the dimer in the organic solvents is different from that obtained in aqueous solution.

Fluorescein and Halofluorescein Dyes

Fluorescein and its derivatives form the foundation almost by themselves for the molecular probe industry so important in medical diagnostics. They have various applications in photochemistry such as quantum counters,³⁷ active media in tunable lasers,²⁷ and photosensitizers.^{46,47} Figure 5 shows the structures of the dyes we consider in this section, as well as a number of their photochemical and photophysical properties.

Several studies have been performed on the association of these dyes by both absorption and emission spectroscopy.^{18,23,24,48-54} Values for the dimerization equilibrium constant reported by the different authors generally agree from group to group, but discrepancies exist in the absorption spectrum derived for the dimer. The existence of trimers and higher aggregates in aqueous solution has also been reported for fluorescein, Eosin, and Erythrosin by Lopez Arbeloa⁵⁰⁻⁵² and by Xu and Neckers for Rose Bengal.⁵⁴

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Table II
Dimerization Equilibrium Constants (M^{-1}) of Fluorescein and Halofluorescein Dyes in Aqueous Solution, Glycerol, and Ethanol at 28°C^a

dye	water	glycerol	ethanol
FL	5	3.9	3.6
FLCl ₂	60		
FLI ₂	83	67	59
FLBr ₄	110	91	77
FLI ₄	140		
FLCl ₄ Br ₄	190		
FLCl ₄ I ₄	250	182	167

^aData from ref 23.

Table III
Thermodynamic Parameters for the Dimerization of Fluorescein and Halofluorescein Dyes in Aqueous Solution at 28°C^a

dye	ΔH° , kcal/mol	ΔS° , eu
FL	-7.6	-22
FLI ₂	-7.9	-17
FLBr ₄	-7.1	-14
FLI ₄	-6.5	-12
FLCl ₄ I ₄	-4.9	-5

^aData for fluorescein from ref 24; other data from ref 23.

Rohatgi and co-workers^{23,24,48,49} have studied the series of halofluorescein dyes in detail, and their values of the dimerization constants in three different solvents are presented in Table II. The tendency to aggregate increases with increasing halogen substitution and also with the presence of more polarizable halogens.

It is worth noting that the dimerization constants for these xanthenes in ethanol and glycerol are similar to those obtained in water. Except for the data shown in Table II and the results reported by Selwyn and Steinfeld,³⁴ it is normally observed that dye aggregation in ethanol and other organic solvents is considerably weaker than in aqueous solution.

As shown by Rohatgi and Mukhopadhyay,²³ a plot of $\log K_d$ versus the sum of atomic polarizabilities of the halogens is linear, with fluorescein being off the line. (In fluorescein, dimerization is controlled by hydrogen bonding, hydrophobic forces being not important; see next paragraph.) The lines obtained in water, ethanol, and glycerol are parallel to each other, indicating that similar forces are involved in all three solvents. In particular, the good correlation with the polarizability of the halogens suggests that hydrophobic forces play an important role in the association process of the halofluorescein derivatives.

The dimerization enthalpy and entropy for fluorescein in aqueous solution are -7.6 kcal/mol and -21.7 eu , respectively. On the basis of these values it has been proposed that association of fluorescein in aqueous solution is mediated by hydrogen bonding with the water molecules.^{24,49}

Perusal of the thermodynamic parameters for the series, Table III, reveals that the nature of the forces responsible for the process changes with progressive halogenation. Lower exothermicity and less negative entropy for the more aggregating dyes suggest increased participation of hydrophobic forces.

The halogen atoms produce a more uniform distribution of the charge in the xanthene, thus adding importance to dispersion forces. Disruption of water structure upon aggregation contributes to a less negative entropy. The increased participation of dispersion

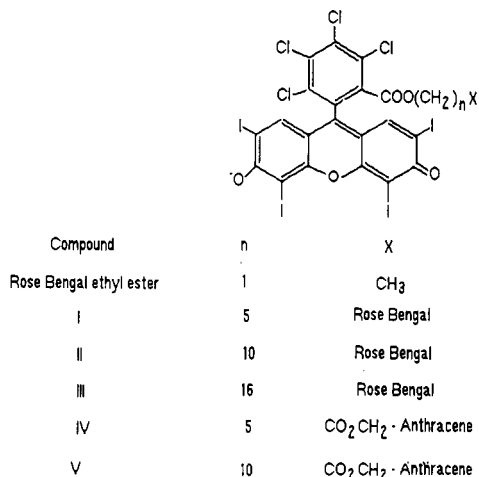


Figure 6. Structures of Rose Bengal derivatives.

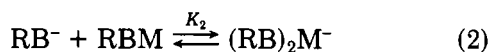
forces should lower the absolute value of the enthalpy because these forces are independent of the temperature.⁴⁵

Rose Bengal Derivatives

Rose Bengal is an efficient singlet oxygen sensitizer in alcohol and in aqueous solution.^{55,56} Several non-polar derivatives of Rose Bengal, easier to purify than the parent dye, have been synthesized and studied in our laboratory.⁵⁷⁻⁵⁹ These derivatives are prepared by esterification of the carboxylate group, resulting in a substance with good solubility in nonpolar solvents and high molar absorptivity in the visible region. As has been shown previously,^{57,59} formation of aggregates modifies the absorption spectrum and decreases the quantum yield of singlet oxygen by approximately 50%.

In the absence of electrolytes, in aqueous solution, Rose Bengal ethyl ester (Figure 6)^{20,21} exists as a monomer up to a concentration of 3×10^{-5} M (solubility problems prevent us from using higher concentrations). Addition of salts modifies the absorption spectrum, and its shape depends on the dye and electrolyte concentrations. As an example of these results, the effect of potassium nitrate, 1.0 M, on the absorption spectrum is presented in Figure 7.

From the analysis of the spectral changes with ionic strength and dye concentration, we proposed a mechanism for the dimerization of Rose Bengal ethyl ester induced by alkali metal cations.^{20,21} The mechanism involves coordination between the metal cation and the xanthene chromophore, eq 1 and 2. We found no ev-



idence for the existence of trimers or higher aggregates under our conditions, and values of K_1 and K_2 , as well as the dimer spectrum, were determined for the five alkali metal cations.

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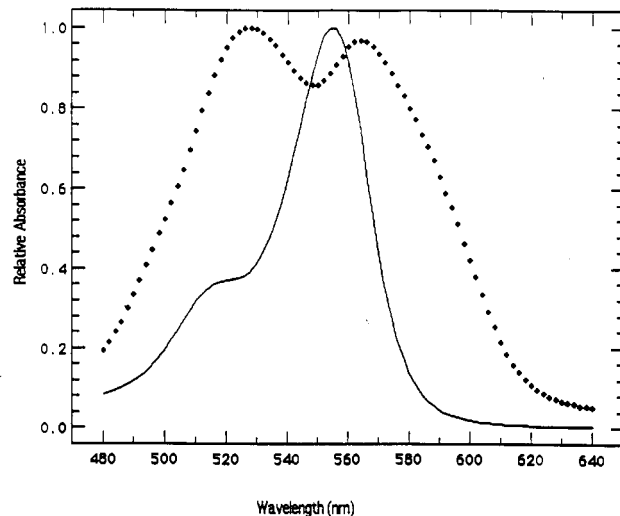


Figure 7. Effect of potassium nitrate on the absorption spectrum of Rose Bengal ethyl ester in aqueous solution. Solid line: no potassium nitrate added; Rose Bengal ethyl ester, 1×10^{-6} – 2×10^{-5} M. Diamonds: Rose Bengal ethyl ester, 8×10^{-6} M; potassium nitrate, 1 M.

Table IV
Values of K_1 at Zero Ionic Strength and High Electrolyte Concentrations for Rose Bengal Ethyl Ester in Aqueous Solution

M ⁺	$K_1(\text{zero})$	$K_1(\text{high})$
Li ⁺	3.37	1.65
Na ⁺	11.2	5.75
K ⁺	141	5.40
Rb ⁺	257	10.4
Cs ⁺	25.3	15.2

Table V
Values of K_1K_2 and K_2 for Rose Bengal Ethyl Ester in Aqueous Solution^a

M ⁺	$K_1K_2 \times 10^{-5}, \text{M}^{-2}$	$K_2 \times 10^{-5}, \text{M}^{-1}$	$\alpha, \text{\AA}^3$
Li ⁺	4.65	2.81	0.033
Na ⁺	19.0	3.30	0.162
K ⁺	30.6	5.67	0.854
Rb ⁺	63.8	6.13	1.411
Cs ⁺	159	10.5	2.483

^a α is the polarizability of the cation.

Table IV presents values of K_1 for the alkali cations. K_1 is a function of both the concentration and the identity of the cation. $K_1(\text{zero})$ is the value obtained by extrapolation to zero ionic strength whereas $K_1(\text{high})$ is the limiting value observed for electrolyte concentrations higher than 0.05 M. A plot of $\log K_1(\text{zero})$ versus the reciprocal of the radius of the solvated cation is linear,²¹ and we conclude that the driving force of reaction 2 is purely electrostatic.

The product K_1K_2 , i.e., the ratio [dimer]/[monomer]² for $[\text{M}^+] = 1$ M, is presented in Table V. The stability of the dimer depends on the cation, increasing by a factor of 34 in going from Li⁺ to Cs⁺. Ours was the first report of such a specific cation effect in aqueous solution.²¹

Values of the equilibrium constant K_2 increase with the polarizability of the cation, Table V. However, the effect is moderate. Increasing the cation polarizability 75 times increases K_2 by a factor of approximately 4. Comparison of Tables IV and V reveals that K_2 is 3–5 orders of magnitude larger than K_1 , and we conclude that the dimerization step is driven mainly by the hydrophobicity of the dye.

Table VI
Spectral Properties of Dimers of Rose Bengal Ethyl Ester Formed in the Presence of Alkali Metal Cations

M ⁺	λ, nm	ε × 10 ⁻⁴ , M ⁻¹ cm ⁻¹
Li ⁺	526	7.86
	576	5.83
Na ⁺	528	6.86
	572	6.21
K ⁺	526	7.02
	572	6.47
Rb ⁺	532	6.98
	570	6.49
Cs ⁺	532	7.62
	570	6.06

Table VII
Spectral Properties of Rose Bengal Derivatives in Water/5% Ethanol

compound	λ, nm	ε × 10 ⁻⁴ , M ⁻¹ cm ⁻¹	ε ₁ /ε ₂
ethyl ester	554	7.65	2.40
	518	3.19	
I	547	7.89	1.71
	515	4.61	
II	546	4.84	1.81
	510	2.67	
III	544	9.88	1.96
	511	5.03	

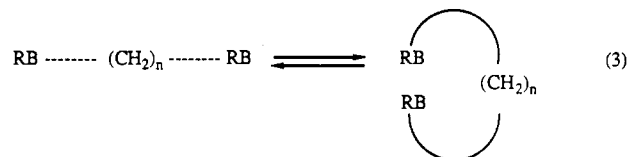
The spectra derived for the dimers formed in the presence of alkali cations^{20,21} are similar to those observed for Rose Bengal and other xanthene dyes in aqueous solution. Table VI summarizes the spectral properties of these dimers. We have shown, by using spectral deconvolution techniques and by applying exciton theory,^{25,26} that the distance and the angle between the monomers in the dimer depend on the identity of the cation.²¹

A different kind of Rose Bengal system we have studied consists of derivatives containing two chromophores separated by methylene chains of different lengths, Figure 6. Comparison of the spectral properties of these derivatives with those of Rose Bengal ethyl ester allowed us to detect the formation of aggregated conformers.^{60,61} As we have already mentioned, aggregates of Rose Bengal ethyl ester are not formed in the absence of electrolytes.

Table VII summarizes the spectral properties of those derivatives containing two Rose Bengal moieties. Compounds I–III obey Beer's law for concentrations less than 8 × 10⁻⁶ M, indicating that the spectral changes observed under these conditions are not due to intermolecular interactions. For concentrations higher than 8 × 10⁻⁶ M, intermolecular aggregates are formed, producing spectral changes similar to those observed for Rose Bengal ethyl ester in the presence of electrolytes.

In ethanol and other polar organic solvents, the spectral shapes of I–III are identical with that of Rose Bengal ethyl ester, except that their molar absorptivities are twice as large when compared to the model. Therefore, we interpret the results shown in Table VII as evidence that derivatives I–III form intramolecular dimers in aqueous solution, eq 3.

The ratio of molar absorptivities in water and ethanol was used as a measure of the stability of the dimers.



The values of ε_{water}/ε_{EtOH} are 0.430, 0.254, and 0.493 for I, II, and III, respectively.⁶⁰ Thus, the stability of the dimers decreases in the order II > I > III. There are two opposing factors determining the stability of the aggregates: the longer the chain, the easier it is to fold and bring the dye moieties together; on the other hand, the probability that the dye moieties occupy regions in space close to each other decreases as the length of the chain increases. Thus, the stability of the cyclic conformation does not increase or decrease monotonically with the length of the methylene chain.

The equilibrium constant for reaction 3 could not be determined by absorption measurements due to spectral overlap. Although the dimers do not emit,⁶⁰ fluorescence measurements in ethanol (where no dimers are detected) indicate that singlet–singlet energy transfer occurs between the Rose Bengal moieties. This observation prevents us from obtaining the concentration of the open conformer in aqueous solution by using emission techniques.

The quantitation problem indicated above does not exist in the case of derivatives IV and V, Figure 6, in which one of the Rose Bengal chromophores has been replaced by anthracene. The spectral changes observed are similar to those obtained with I–III and indicate formation of a nonfluorescent cyclic conformation in aqueous solution.⁶¹

Comparing the fluorescence spectra of IV and V with that of Rose Bengal ethyl ester, we have determined the equilibrium constants for the intramolecular aggregation of IV and V in aqueous solution. The values are 1.78 for IV and 10.1 for V. As has been observed for the compounds containing two Rose Bengal moieties, a more stable cyclic conformation is obtained when the chromophores are separated by 10 methylene groups.

The spectra derived for the cyclic conformation of IV and V indicate that the geometry of the cyclic conformation depends on the length of the methylene chain. Two well-resolved maxima, at 571 and 542 nm, are obtained for IV, with the long-wavelength peak being more intense, whereas the spectrum of V shows an absorption maximum at 543 nm and a shoulder in the 565–580-nm region.⁶¹

Intramolecular energy transfer and excimer formation are influenced by both the chain length and the nature of the solvent. Winnik⁶² has used pyrene end-capped polystyrenes to study the dynamics of chain cyclization. For the long chains employed, pyrene moieties separated by 60–2000 bonds, he finds that the rate constant of excimer formation decreases with increasing chain length.

More closely related to our work is the report by Mugnier et al.⁶³ on the efficiency of intramolecular energy transfer in molecules containing coumarin chromophores linked by a variable number of methylene groups (n = 3, 4, 8, 12). In dimethylformamide the

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efficiency of energy transfer decreases in the order of n values $3 = 4 > 8 > 12$, whereas in propylene glycol the efficiency of energy transfer is independent of the length of the methylene chain. The latter result is similar to what we observe for IV and V in ethanol, namely, that intramolecular quenching of anthracene fluorescence by the Rose Bengal moiety is almost 100% efficient in both derivatives.⁶¹

Concluding Remarks

The field of dye-dye aggregation has received considerable attention in the past, and we expect this situation to continue in the future, especially in regard to dyes used in laser technology. Formation of aggregates reduces the output of the laser by a combination of absorption of radiation by nonfluorescent aggregates

and quenching of the monomer excited state.

No single mechanism for dye association can be advanced, and in most cases, the association is controlled by a combination of forces. In aqueous solution, hydrogen bonding through the solvent appears to be a common feature in the aggregation of xanthene dyes. The relative importance of hydrogen bonding as opposed to hydrophobic interactions depends on the hydrophobicity of the dye, more polarizable substituents enhancing the participation of dispersion forces.

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