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Aggregation of Halofluorescein Dyes

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SUMMARY

The formation constants and absorption spectra of the dimer and trimer of the halogenated derivatives of fluorescein dianion, eosin and erythrosin, are evaluated. The geometric structures of the monomers in the aggregates are determined by means of the exciton theory. The results are compared with those previously obtained of the fluorescein dianion, clarifying some aspects related to the association processes.

1. INTRODUCTION

The spectroscopic characteristics of the dianionic form of fluorescein and those of its halogenated derivatives, eosin and erythrosin, change when the dye concentration increases. This behaviour alters their properties as photosensitizers,[1,2] and as fluorescence quantum yield standards,[3] also weakening the output power of their lasers,[4,5] among other side effects.

The deformation of absorption spectra and the decrease of emission quantum yields and times in concentrated solutions of these xanthene derivatives have been attributed to dimer formation.[6–10] Recently, the evaluation of the absorptivities of very concentrated solutions of fluorescein dianion ($>10^{-1}$ M) has allowed the characterization of its trimer.[9,10]

In this work, the influence of the halogenation of fluorescein dianion on the aggregation properties is studied. The formation constants, absorption spectra and geometric structures of the dimers and trimers of

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eosin and erythrosin are determined. The differences between the parameters that characterize the dimerization and trimerization of the halogenated derivatives and those of the fluorescein dianion allow the interpretation of some aspects related to the association forces of these dyes.

2. EXPERIMENTAL

The eosin and erythrosin used were from Fluka (for microscopy). They were twice recrystallized in ethanol and dried in a vacuum oven. Other chemicals were Suprapur grade from Merck. Spectra were measured in aqueous solutions at pH 12 (NH_4OH), containing 0·01 M KCl. The concentration range was from 5×10^{-6} to 10^{-1} M.

The absorption spectra were recorded in a Perkin-Elmer model EPS-3T. For concentrated solutions, a RIIC cell model BC-14 of variable optical path was used to record the spectra. The absorptivity of these solutions in the visible region of the spectrum was determined from the absorption variations produced by known changes of optical path.

3. RESULTS AND DISCUSSION

The shape of the visible absorption spectrum of the fluorescein dianion and that of its halogenated derivatives eosin and erythrosin (Fig. 1)

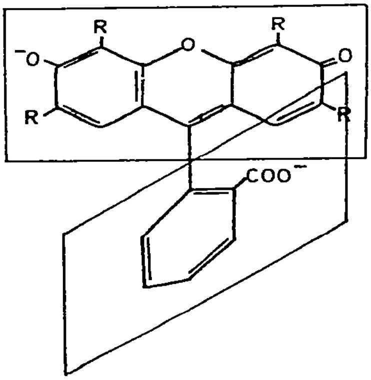

Fig. 1. Molecular structure of the dianionic form of fluorescein (Fl, R = H), eosin ($FlBr_4$, R = Br) and erythrosin (FlI_4, R = I).

changes when dye concentration increases. In Fig. 2 the absorption spectra of erythrosin dianion at different concentrations are plotted. The first variations are attributed to dimer formation ($M + M \rightleftharpoons D$), the equilibrium constant of which is given by

$$K_d = 1 - x/2cx^2 \qquad (1)$$

where x is the monomer molar fraction at a concentration c. The absorptivity of this concentration at frequency ν is

$$\bar{\varepsilon}_\nu = \varepsilon_\nu^m x + \varepsilon_\nu^d (1 - x) \qquad (2)$$

where ε_ν^d is half of the dimer absorptivity and can be taken as the absorptivity of the monomeric units in the dimer. The spectrum of diluted solution ($x \simeq 1$) is the monomer spectrum, $\bar{\varepsilon}_\nu = \varepsilon_\nu^m$.

From the changes produced in the absorption spectrum by the increase

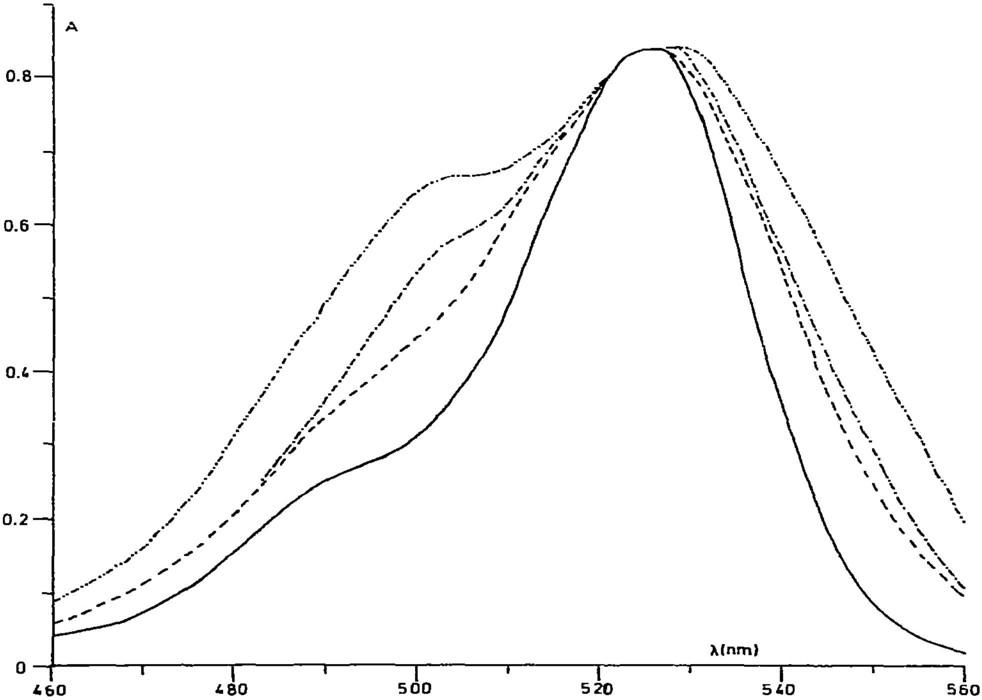

Fig. 2. Absorption spectrum of erythrosin dianion at different concentrations: 5×10^{-6} (——), 3×10^{-3} (– – – –), 10^{-2} (—·—·) and 10^{-1} M (—··—··). Conditions: pH 12: 0·01 M-KCl; 20 °C; normalized to 526 nm.

of dye concentration, the dimerization constant can be evaluated.[9] Table 1 shows the average values obtained at 20 °C. The halogenation of fluorescein dianion supposes an increase in aggregation (in this work, the term 'halogenation' may indicate replacement of the halogen substituted in the fluorescein dianion by one of higher atomic number). The small value obtained for the dimerization constant of these dyes with respect to that of the other xanthene dyes[11] is due mainly to the electrostatic repulsion between the dianions.

TABLE 1

Formation Constants at 20 °C (K_d), Hypsochromism (H_d) and Excitonic Parameters (U, θ, R_I and R_{II}) of the Dimers of Fluorescein (Fl),[9] Eosin ($FlBr_4$) and Erythrosin (FlI_4) Dianions (pH 12, 0·01 M-KCl)

Dye	K_d	H_d	U (*cm*$^{-1}$)	θ (*degrees*)	R_I (*Å*)	R_{II} (*Å*)
Fl	5·0 ± 0·5	0·84	717	76	4·6	8·3
$FlBr_4$	115 ± 3	0·79	743	71	5·3	8·4
FlI_4	127 ± 3	0·77	752	69	5·5	8·5
Estimated error	—	±0·02	±40	±1	±0·4	±0·4

The absorption spectra of the dimers, calculated by means of eqn (2), are represented in Fig. 3. The dimer spectra are composed by two monomer bands with their maxima at greater and smaller energies than the corresponding monomer maximum (Fig. 3b). The hypsochromism values indicating the ratio between the oscillator strengths of dimer and monomer bands are shown in Table 1.

In order to know the geometric disposition of monomers in the dimer, the absorption spectrum of this aggregate is interpreted by means of the exciton theory.[12] The resonance interaction, U, between the chromophore part of the monomers (xanthene group) is half of the energy difference between the maxima of the dimer absorption bands. The angle between the chromophore groups of the dimer is given by

$$\tan^2\frac{\theta}{2}=\frac{f_1}{f_2} \qquad (3)$$

where f_1 and f_2 are the oscillator strengths of the long-wave and the short-wave dimer bands. The distance R between the monomers depends on the

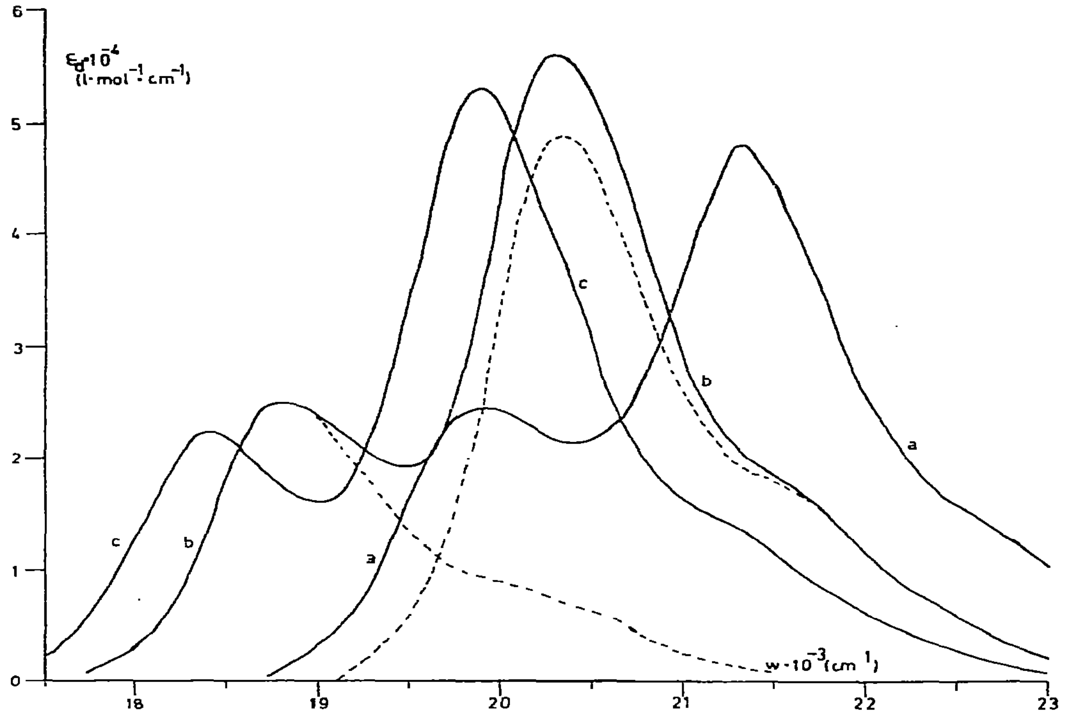

Fig. 3. Dimer absorption spectrum of fluorescein (a),[9] eosin (b) and erythrosin (c) dianions (pH 12; 0·01 M-KCl). Broken lines indicate the breakdown of the eosin spectrum into two monomer bands (excitonic splitting).

geometric model considered. In order to explain the dimer band splitting in the case of dye molecules, two models allowing the two transitions are accepted.[13] In model I, the monomers are in parallel planes with a twist angle θ, while in model II they are in the same plane, forming an angle θ. The resonance interactions of these structures are

$$(Model\ I) \qquad U = -\frac{|\vec{M}|^2}{R_{I}^3}\cos\theta \qquad (4a)$$

$$(Model\ II) \qquad U = -\frac{|\vec{M}|^2}{R_{II}^3}(\cos\theta + 3\sin^2\theta/2) \qquad (4b)$$

where $\vec{M}$ is the monomer transition moment.

In Table I the excitonic parameters obtained for the dimers of the dyes are shown. The increase with halogenation of the resonance interaction parallels the increase in the dimerization constant.

Model I is the more reasonable one because the distance between monomers is nearer to that in the crystalline state (~ 4 Å). Therefore, it is considered that the xanthene groups in the dimers are in parallel planes, placed at a distance of R_1 Å, forming an angle θ. This disposition has been proposed previously.[6]

Molecular models indicate that the angle θ between the dipolar moment of the monomers is due to the steric effect between the phenyl groups. The increase of the distance between the monomers in the halogenated dimers permits a better alignment of its xanthene rings. This is the main reason for the observed increase of the excitonic interaction (Table 1).

In concentrated solutions, the trimer appears ($M + D \rightleftharpoons T$). Its equilibrium constant and absorption spectrum can be approximately calculated if it is considered that the trimer hypsochromism, H_t, is equal to H_d^2 (H_d is the hypsochromism of the dimer).[9] Because of the empirical nature of the calculation method and the non-consideration of the activity coefficients in the equations, the results obtained should be regarded as a first approximation in order to obtain some conclusions.

As Table 2 shows, the trimerization constant, K_t, is greater than the dimerization constant, K_d. Besides, the trimerization increases, but the ratio between the trimer and dimer formation constants decreases, with halogenation.

The average visible absorption spectra of the trimers have three bands, as shown in Fig. 4.

The exciton theory has been applied to a trimer in which the monomers are in parallel planes, at a distance of R_1 Å, forming an angle θ.[9] The geometric parameters used are that of the dimer (Table 1). The differences of energy between the trimer spectrum maxima and those obtained from the exciton theory (Table 3) indicate that the geometric model considered

TABLE 2

Ratios of Dimerization (K_d) and Trimerization (K_t) Constants at 20°C of Fluorescein (Fl).[9] Eosin ($FlBr_4$) and Erythrosin (FlI_4) Dianions, Ratios between Excitonic Interactions (U'/U)

Dye	K_t	K_t/K_d	U'/U
Fl	10	2·1	0·46
$FlBr_4$	155	1·4	0·30
FlI_4	150	1·2	0·26

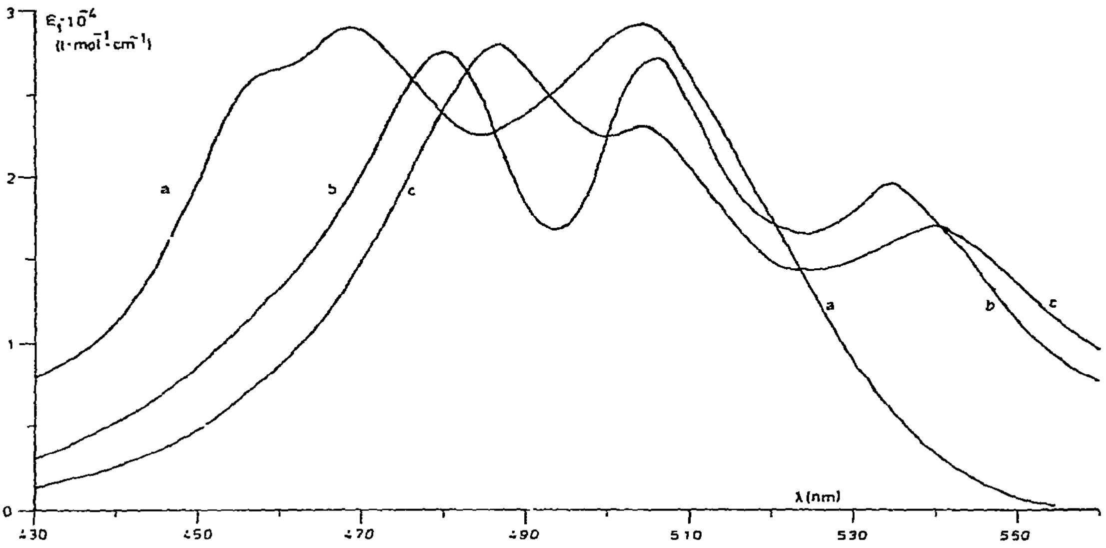

Fig. 4. Trimer absorption spectrum of fluorescein (a),[9] eosin (b) and erythrosin (c) dianion (pH 12, 0·01 M-KCl).

for this aggregate is suitable. So this model explains the increase of the trimerization constant with respect to the dimerization constant (Table 2), because the resonance interaction between non-adjacent monomers,[9] U', has the same sign as the one between adjacent monomers, U (eqn (4))

$$U' = \frac{|\vec{M}|^2}{8R_t^3}\cos 2\theta \tag{5}$$

Also, the decrease with halogenation of the ratio between the trimerization and dimerization constants, K_t/K_d, is due to the decrease of

TABLE 3

Differences (cm^{-1}) between the Trimer Absorption Maxima from the Spectrum (spe) and from the Exciton Theory (exc) of Fluorescein (Fl),[9] Eosin ($FlBr_4$) and Erythrosin (FlI_4) Dianions

	ΔE_{31}^{spe}	ΔE_{32}^{spe}	ΔE_{31}^{exc}	ΔE_{32}^{exc}
Fl	199 2	148 1	205 3	152 0
$FlBr_4$	209 8	107 1	211 3	139 7
FlI_4	210 0	128 3	213 5	136 2

the ratio between the resonance interactions of non-adjacent and adjacent monomers, U'/U (Table 2).

The variations of the parameters that characterize the geometric structures of the aggregates (Table 1) indicate changes in the association forces. The fluorescein aggregates are due mainly to hydrogen bonds between the monomers,[7,9,10] although some contributions of van der Waals forces have also been suggested, mainly in trimer formation.[10] The halogen atoms in the eosin and erythrosin produce a most uniform distribution of the conjugated electronic system of their xanthene groups. This should suggest a larger contribution of dispersion forces in the halogenated aggregates, leading to a better alignment of their monomeric units (Table 1).

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