FLUORESCENCE SELF-QUENCHING OF HALOFLUORESCEIN DYES

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Summary

The fluorescence quenching of halogenated derivatives (eosin and erythrosin) of fluorescein in aqueous solution was studied over a wide concentration range (5×10^{-6} - 10^{-1} mol dm^{-3}) where aggregates are formed. The rate constants of the quenching produced by the dimers and trimers of the dyes were determined at various temperatures and were compared with those of fluorescein quenching reported previously. The quenching mechanisms are also discussed.

1. Introduction

The dianionic forms of halofluorescein dyes in solution have received considerable attention as photosensitizers [1, 2], quantum counters [3] etc. In recent years they have been used in laser technology as active media for tuning [4, 5]. These applications show the importance of the knowledge of the optical properties of these dyes in solution.

As in other xanthene derivatives [6], an increase in halofluorescein dye concentration produces changes in its absorption and emission spectra that have been attributed to dimer [7 - 10] and trimer [9, 10] formation.

The influence of aggregate formation on the fluorescent characteristics of the fluorescein dianion has been studied previously [11]. In this work the fluorescence behaviour at different concentrations of halofluorescein dyes (eosin and erythrosin) was studied. The rate constants of the observed fluorescence quenching produced by the dimers and the trimers were determined and compared with those of fluorescein quenching.

2. Experimental details

The dyes used (Fluka, for microscopy) were twice recrystallized in ethanol and dried in a vacuum oven. Measurements were carried out in

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aqueous solutions at pH 12 (NH_4OH, Merck Suprapur grade) containing 0.01 mol KCl dm^{-3} (Merck Suprapur grade). The corrected fluorescence and excitation spectra were recorded on a Perkin–Elmer model MPF-3 spectrophotometer. The spectra were registered at 10 °C intervals in the temperature range 20 - 50 °C and at dye concentrations ranging from 5×10^{-6} to 10^{-1} mol dm^{-3}.

In order to decrease the influence on the spectra of the reabsorption and re-emission phenomena, a rectangular cell of optical path length 1 mm was used. The angles between the normal to the cell and the excitation and emission (photomultiplier) directions were 30° and 60° respectively.

3. Results and discussion

The fluorescence spectrum in dilute solutions of fluorescein dianion and its halogenated derivatives eosin and erythrosin is the monomer spectrum (Fig. 1). The fluorescence quantum yield of the monomeric dyes does not depend on the excitation frequency because the corrected excitation spectrum of dilute solutions of the dyes has the same shape as the absorption curve.

The shape of the fluorescence spectrum depends on the dye concentration and on the cell used. With the cell and the geometric arrangement used

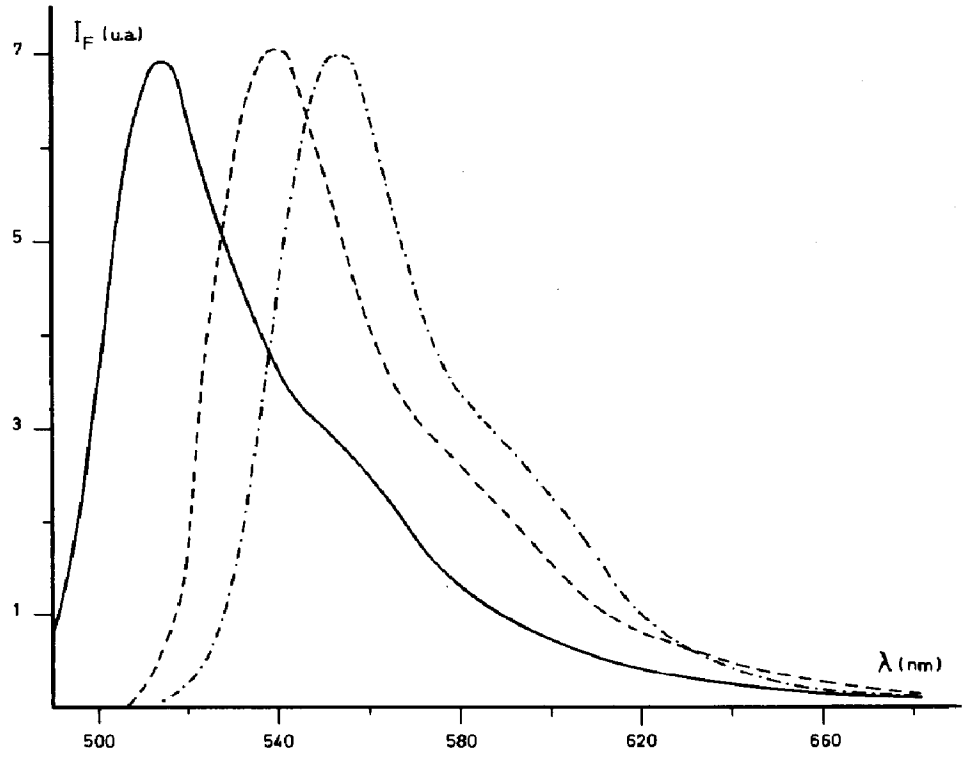

Fig. 1. Corrected fluorescence spectrum of fluorescein (———), eosin (– – –) and erythrosin (— · —) dianions (5×10^{-6} mol dm^{-3}; pH 12; 0.01 mol KCl dm^{-3}; 20 °C).

in this work, an increase in dye concentration produces the following variations (Fig. 2): in dilute solutions (less than 5×10^{-5} mol dm^{-3}) there are no appreciable changes in the spectrum; in the concentration range from 5×10^{-5} to 10^{-3} mol dm^{-3} a shift of the fluorescence maximum to smaller energies is observed; in concentrated solutions (above 2×10^{-3} mol dm^{-3}) the increase in dye concentration produces little change in the emission spectrum.

An increase in the temperature does not appreciably influence the fluorescence spectrum shape, but its intensity may be changed. The variation in fluorescence intensity depends on the dye concentration. In dilute solutions a temperature increment produces a small decrease in the intensity. In concentrations where the dimer is appreciable, the fluorescence intensity increases with temperature and, in very concentrated solutions where the trimer appears, the increase in fluorescence intensity with temperature is smaller.

The changes observed in the emission spectrum in concentrated solutions are due to the reabsorption effect [12]. This is produced by the overlap between the absorption and the emission spectra. The reabsorption decreases the most energetic part of the fluorescence spectrum and increases the other part owing to the re-emission. This implies a shift to smaller

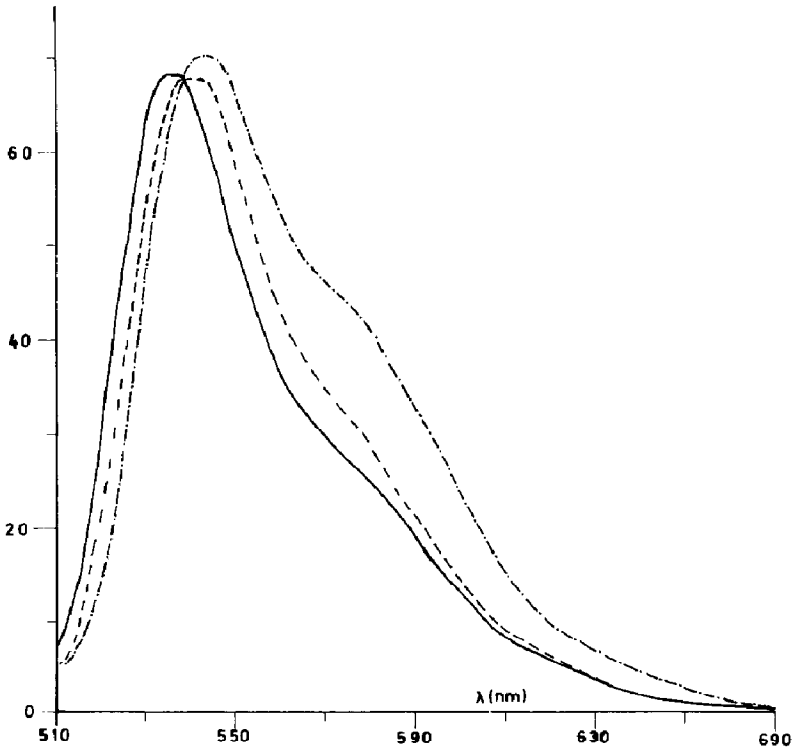

Fig. 2. Corrected fluorescence spectra of eosin dianion at different concentrations (pH 12; 0.01 mol KCl dm^{-3}; 20 °C): ——, 5×10^{-6} mol dm^{-3}; – – –, 1.4×10^{-4} mol dm^{-3}; — · —, 2×10^{-3} mol dm^{-3}.

energies of the fluorescence maximum. The spectra represented in Fig. 2 obey these changes.

In order to study the emission of dye aggregates, an RIIC model BC-14 cell of variable optical path length was used instead of the 1 mm rectangular cell. Unfortunately, the RIIC cell, which can produce less reabsorption than the 1 mm rectangular cell, cannot be used for quantitative measurements because of technical problems. Measurements accomplished with the RIIC cell indicated that the emission quantum yield of eosin and erythrosin dimer at 20 °C is zero. The fluorescence spectra (without reabsorption) of solutions where the concentration of these aggregates is appreciable have the same shape as the monomer fluorescence spectrum. This conclusion could not be reached for the trimer of these dyes because the spectrum (without reabsorption) of very concentrated solutions is very noisy. In any case the fluorescence quantum yield of all aggregates at about 20 °C was taken as zero, and therefore the fluorescence yield of the dyes at any concentration cannot depend on excitation frequency. The excitation spectra of different concentrations of the dyes confirms this conclusion.

The fluorescence quantum yield ϕ is evaluated by comparing the sample fluorescence with that of a standard [3] using the equation

$$\phi = \phi_s \frac{I^a_s}{I^a} \frac{F}{F_s} \tag{1}$$

where I^a is the absorbed radiation intensity and F is the area of the corrected fluorescence spectra. The subscript s indicates the standard substance, which was chosen to be fluorescein at dilute concentrations (5×10^{-6} mol dm^{-3}; pH 12; 20 °C) with a fluorescence quantum yield ϕ^0 of 0.92 [3]. The spectra were corrected [12] by taking into account the reabsorption–re-emission phenomena and the absorption of non-fluorescent molecules (aggregates in this case).

The fluorescence quenching produced by the monomer of the eosin and erythrosin dianions cannot be evaluated. The aggregation of these dyes [9, 10] (Table 1) narrows the concentration interval in which the monomer is the unique species that produces quenching. The small fluorescence decrease produced by the monomer in this concentration interval cannot be

TABLE 1

Dimerization and trimerization constants K_d and K_t at 20 °C (standard concentration, 1 mol dm^{-3}) and the dimer formation enthalpy ΔH_d° of fluorescein (Fl [9]), eosin ($FlBr_4$ [10]) and erythrosin (FlI_4 [10]) dianions

	Fl	*$FlBr_4$*	*FlI_4*
K_d	5.0	115	127
K_t	10	155	150
ΔH_d° (kJ mol^{-1})	−28	−21	−17

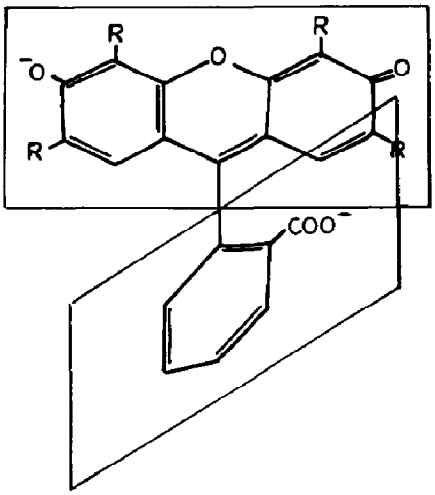

Fig. 3. Molecular structure of fluorescein (Fl, R ≡ H), eosin ($FlBr_4$, R ≡ Br) and erythrosin (FlI_4, R ≡ I) dianions.

detected owing to the experimental error and to the influence of the reabsorption. As eosin and erythrosin have the same structure as fluorescein (Fig. 3), the rate constant $k_{qm} = 1.8 \times 10^{10}$ mol dm^{-3} s^{-1} [11] at 20 °C of the quenching produced by the monomer of this dye was adopted for all the dyes. This assumption does not involve a large error because the quenchings produced by the dimers and the trimers are one magnitude larger.

The small decrease produced by temperature in the emission of dilute solutions of the dyes should be attributed to an increase in the quenching produced by the monomers. The activation energy obtained for the eosin and erythrosin dianions (about 10 kJ mol^{-1}) is similar to that of the fluorescein dianion [11]. This suggests that the quenching mechanisms produced by the monomer of halofluorescein dyes are the same as those of the fluorescein [11]: collisional (through molecular diffusion) and monomer–monomer energy transfer (the dimer provides an energy trap [7, 11]). The collisional quenching, which depends on temperature, does not cause excimer formation [11].

In solutions where the dimer is appreciable, the fluorescence quantum yields obey the Stern–Volmer equation:

$$\frac{\phi^0}{\phi} - 1 = \tau^0 k_{qm}[M] + \tau^0 k_{qd}[D] = \tau^0 k_{qm}[M] + \tau^0 k_{qd} K_d [M]^2 \qquad (2)$$

where k_{qm} and k_{qd} are the rate constants of the quenchings produced by the monomers and the dimers respectively, K_d is the dimerization equilibrium constant (Table 1) and τ^0 is the mean lifetime.

The rate constants of the quenching produced by the dimers at 20 °C, obtained from eqn. (2), are given in Table 2. The values of the mean life used are also given [13]. Table 2 indicates an increase in the quenching produced by the dimer with respect to that produced by the monomer. The

TABLE 2

Rate constant $k_{qd\ 20\ °C}$ of the quenching produced by the dimer at 20 °C, the fluorescence mean lifetime τ^0 of the monomer [13] and the monomer–dimer energy transfer efficiency of fluorescein (Fl [11]), eosin ($FlBr_4$) and erythrosin (FlI_4) (pH 12; 0.01 M KCl)

	Fl	*$FlBr_4$*	*FlI_4*
$k_{qd\ 20\ °C} \times 10^{-11}$ (mol dm^{-3} s^{-1})	3.9 ± 0.3	2.2 ± 0.3	1.4 ± 0.3
τ^0 (ns)	3.6	3.2	3.0
O_{M-D}	1.00	1.06	1.26
$(\phi^0 O_{M-D}/\tau^0) \times 10^{-7}$	26	7	3

halogenation* (Fig. 3) should cause a decrease in the quenching produced by the dimer.

The rate constant of the quenching produced by the dimer was evaluated at different temperatures using eqn. (2) and taking into account the corresponding dimerization constant. This was evaluated using the Van't Hoff equation, the dimer formation constant obtained at 20 °C and the dimerization enthalpy (Table 1). The results indicate that the quenching produced by the dimer of these dyes does not depend appreciably on temperature. This suggests that the quenching is due to monomer–dimer energy transfer. The exciplex formation between excited monomers and dimers should be temperature dependent.

The efficiency of the energy transfer through an interaction of the dipole–dipole type (the Förster formula [14]) depends on the dador emission yield ϕ^0_{Da} (of the monomer in this case), the dador mean lifetime τ^0_{Da}, the distance R between the dador and the acceptor, and the overlap O_{Da-Ac} between the dador emission spectrum and the acceptor absorption spectrum:

$$k_{ET} \propto \frac{\phi^0_{Da}}{\tau^0_{Da} R^6} O_{Da-Ac} \qquad (3)$$

This equation predicts the observed increase in the quenching produced by the dimer with respect to that produced by the monomer. The overlap between the monomer emission spectrum and the dimer absorption spectrum is higher than that between the emission and absorption monomer spectra. The dimer has an absorption band at a lower energy than the monomer [8, 9].

Equation (3) for the energy transfer efficiency also explains the decrease in the quenching produced by the halogenated dimers. Table 2 gives the overlap O_{M-D} between the monomer emission spectra and the dimer absorption spectra (with reference to the fluorescein dianion) and the terms $\phi^0 O_{M-D}/\tau^0$ that are a measure of the energy transfer between the monomers and the dimers. The decrease with halogenation of the monomer–dimer

*In this work the term "halogenation" also indicates an increase in the atomic number of the halogens substituted in the fluorescein dianion.

energy transfer explains the diminution of the quenching produced by the dimer in the halogenated dyes.

The lack of dependence on temperature (between 20 and 50 °C) of the fluorescence quantum yield of the monomers suggests the same behaviour for the lifetime of their first singlet excited states and therefore a similar monomer–dimer energy transfer efficiency at 20 and 50 °C (eqn. (3)).

In very concentrated solutions the quenching is produced not only by the monomer and the dimer but also by the trimer. A tentative scheme for quenching at these concentrations is as follows:

$$\begin{aligned} &M^* + M \rightarrow M + M,\ M + M^* && (k_{qm}) \\ &M^* + D \rightarrow M + D^* && (k_{qd}) \\ &M^* + T \rightarrow M + T^* && (k_{qt}) \end{aligned} \qquad (4)$$

The Stern–Volmer equation is

$$\frac{\phi^0}{\phi} - 1 = \tau^0 k_{qm}[M] + \tau^0 k_{qd} K_d [M]^2 + \tau^0 k_{qt} K_d K_t [M]^3 \qquad (5)$$

where K_d and K_t are the dimerization and trimerization constants (Table 1). The rate constant k_{qt} of the quenching produced by the trimer is obtained from eqn. (5). The values obtained at 20 °C are given in Table 3. The results indicate that this quenching is more effective than that produced by the dimer (Table 2). This is reasonable because the efficiency of the monomer–trimer energy transfer is greater than that of the monomer–dimer transfer. The trimer has an absorption band at a lower energy than the dimer. The decrease with halogenation of the quenching produced by the trimer is explained in the same way as that in the quenching produced by the dimer.

The variations with temperature of the fluorescence intensity can now be explained. The decrease observed in dilute solutions is due to the increase in the quenching produced by the monomer. In solutions where the dimer is appreciable, the increase with temperature of the fluorescence intensity is due to the dimer dissociation. In the most concentrated solution used, the greater stability of the trimer with respect to the dimer [11] explains the smaller increase with temperature of the fluorescence intensity.

TABLE 3

Rate constant k_{qt} of the fluorescence quenching produced by the trimers of fluorescein (Fl [11]), eosin ($FlBr_4$) and erythrosin (FlI_4) at 20 °C (pH 12; 0.01 M KCl)

	Fl	*$FlBr_4$*	*FlI_4*
$k_{qt\ 20\,°C} \times 10^{-11}$ (mol dm^{-3} s^{-1})	17 ± 2	14 ± 2	9 ± 2

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