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FLUORESCENCE QUANTUM YIELD EVALUATION: CORRECTIONS FOR RE-ABSORPTION AND RE-EMISSION

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Summary

A practical method of correcting for the influence of re-absorption and re-emission processes on fluorescence spectra is proposed. The fluorescence quantum yield of the fluorescein dianion is evaluated at various concentrations and is compared with the results obtained with other more complicated methods.

1. Introduction

The determination of the fluorescence quantum yields of molecules in solution is necessary in order to study radiationless transitions (internal conversion and intersystem crossing), fluorescence quenching, transfer of excitation energy etc.

Because of secondary processes (*i.e.* re-absorption and re-emission), methods of emission yield evaluation have many problems when they are applied to optically dense solutions. Some correction methods [1 - 3] for re-absorption and re-emission effects are tedious to work with. Other methods [4, 5] need additional measurements and do not correct the fluorescence spectra but only the fluorescence quantum yield.

The re-absorption treatment proposed in this work allows an adequate and simplified correction of fluorescence spectra at every frequency (this is necessary when there are two or more species). The method is applied to the geometrical arrangement shown in Fig. 1. A practical method for re-emission correction is also proposed.

The fluorescence quantum yield of the fluorescein dianion is evaluated in the concentration range from 5×10^{-6} to 3×10^{-1} M and the results are compared with literature data.

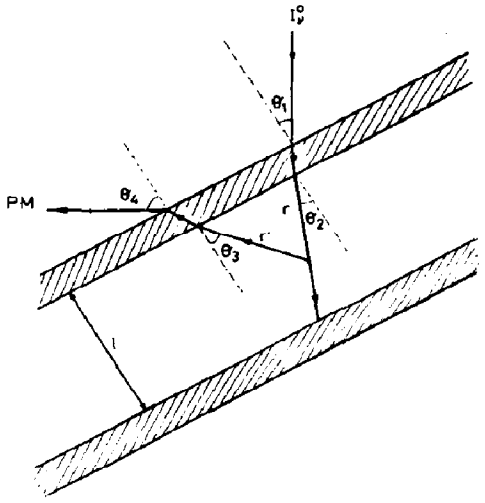

Fig. 1. The optical path of excitation and emission radiation in a 1 mm rectangular cell.

2. Experimental

The fluorescein used was obtained from Fluka (for microscopy). It was twice recrystallized from ethanol and was dried in a vacuum oven. Other chemicals were of Merck Suprapur grade quality. Spectra were measured in aqueous solutions at pH 12 (NH_4OH) and containing 0.01 M KCl. The corrected fluorescence spectra were recorded at 20 °C in a Perkin–Elmer model MPF-3 spectrophotometer.

3. Results and discussion

To make a comparative study the correction methods proposed for evaluation of the true fluorescence quantum yield are applied to the fluorescein dianion, because this system has been previously used with other correction methods [2 - 4]. Dianion aggregates appear in concentrated solutions of fluorescein. In the concentration interval between 5×10^{-6} and 3×10^{-1} M the monomer coexists with the dimer and trimer. The dimerization and trimerization equilibrium constants at 20 °C are 5 and 10 respectively [6].

The fluorescence quantum yield Φ can be evaluated by comparing the fluorescence of the sample with that of a standard [7] using

$$\Phi = \Phi_s \frac{I_s^a}{I^a} \frac{F}{F_s} \qquad (1)$$

where I^a is the radiation intensity absorbed and F is the area of the corrected fluorescence spectrum. The subscript s indicates the standard; the standard chosen is fluorescein at dilute concentrations (5×10^{-6} M, pH 12, 20 °C) with a fluorescence quantum yield of 0.92 [8, 9].

Effects which have not been taken into consideration in eqn. (1) appear in optically dense solutions, *e.g.* re-absorption–re-emission, refraction of the emitted radiation, inactive absorption (in fluorescence) of other molecules (aggregates in this case), shifts in the absorption region etc.

The existence of re-absorption and re-emission phenomena in fluorescein can be demonstrated if molecules of the dye solution in a rectangular cell with a 1 mm optical path are excited. The emission spectrum is recorded. Then the same emission spectrum, but after being crossed through another similar cell with the same solution, is recorded. The radiation detected is that transmitted by the second cell and its possible emission due to absorption of the radiation emitted by the first cell. The maximum of the second spectrum is shifted to lower energies since the high energy part of the spectrum is decreased because of overlap between the absorption and emission spectra (re-absorption). The low energy region of the spectrum is increased because of re-emission. The shift in the maximum of the fluorescence is not due to the fluorescein aggregates because they have no emission at 20 °C [6].

The influence on the spectra of the re-absorption and re-emission processes was studied with cells of various geometries: rectangular cells with 1 cm, 1 mm and approximately 0.1 mm optical paths, a triangular cell and an RIIC cell (model BC-14) of variable optical path. The RIIC cell and the approximately 0.1 mm rectangular cell, both placed in positions similar to that of the 1 mm rectangular cell (Fig. 1), produce less re-absorption than the 1 mm cell but it is not possible to make quantitative measurements because the accurate optical path is unknown. However, although the 1 mm rectangular cell produces more re-absorption, there are no problems with shifts in the absorption region. Therefore this is the cell we used in this work.

The rectangular cell with a 1 mm optical path is placed as shown in Fig. 1. The angles between the normal to the cell and the excitation and emission (photomultiplier) directions are $\theta_1 = 30°$ and $\theta_4 = 60°$ respectively. The θ_2 and θ_3 angles are given by $\sin \theta_2 = \sin 30/n$ and $\sin \theta_3 = \sin 60/n$, where n is the refractive index of the solution (considered to be wavelength independent). The optical path in the photomultiplier direction traversed by the radiation emitted at a distance r from the cell wall is

$$r' = r \cos \theta_2 / \cos \theta_3 = rz$$

The maximum value of the excitation optical path is $r_{max} = l/\cos \theta_2$.

Measurements are performed at $\theta_1 = 30°$ because, although at $\theta = 45°$ the sensitivity is maximum, there is a considerable contribution from radiation reflected by the cell wall.

Using this geometrical arrangement the corrections for various processes that contribute to the measurements are studied.

The inactive absorption (in fluorescence) of aggregates is corrected for by multiplying the absorbed intensity by the factor $\epsilon_\nu^m x_m / \bar{\epsilon}_\nu$ deduced from the equation

$$\bar{\epsilon}_\nu = \epsilon_\nu^m x_m + \epsilon_\nu^d x_d + \epsilon_\nu^t x_t \tag{2}$$

where $\bar{\epsilon}_\nu$, ϵ_ν^m, ϵ_ν^d and ϵ_ν^t are the solution, monomer, dimer and trimer extinction coefficients at frequency ν, and x_m, x_d and x_t are the monomer, dimer and trimer mole fractions. $\bar{\epsilon}_\nu$ is evaluated using an RIIC cell of variable optical path [6] and the monomer mole fraction x_m is calculated from the dimerization and trimerization constants (5 and 10 respectively [6]). Therefore the excitation intensity absorbed by the monomer is given by

$$I_{ex}^a(m) = I_{ex}^\circ \{1 - \exp(-2.3\,\bar{\epsilon}_{ex}\, cl/\cos\theta_2)\}\ \frac{\epsilon_{ex}^m x_m}{\bar{\epsilon}_{ex}} \tag{3}$$

where $l/\cos\theta_2$ is the optical path of the excitation radiation into the cell (Fig. 1).

The refractive index of the solution influences the excitation and emission optical paths (Fig. 1). Moreover, the emission emerging from the cell is refracted. The correction factor [10] is

$$\frac{\cos\theta}{n(n^2 - \sin^2\theta)^{1/2}} \tag{4}$$

where θ is the observation angle (60° in this work, see Fig. 1).

The shift in the absorption region (dilution effect) is corrected for [3] using

$$\left(\frac{l'}{2n\cos\theta} + d\right)\Big/ d \tag{5}$$

where $l' = l/\cos\theta_2$, θ is the observation angle (60°) and d is the distance from the face of the cell to the observing aperture (cm). Since $l = 0.1$ cm and $d \approx 10$ cm, this factor is close to unity.

The emission measurements of the fluorescein dianion are most influenced by re-absorption and re-emission processes when the geometrical arrangement shown in Fig. 1 is used. The method proposed for the re-absorption correction is a general method applied to this arrangement.

The intensity of the excitation radiation (ex) absorbed by the monomer in a layer dr at a distance r from the cell face (Fig. 1) is given by

$$-dI_{ex}^a(m) = I_{ex}^\circ \{\exp(-2.3\,\bar{\epsilon}_{ex}\, cr)\}\ 2.3\bar{\epsilon}_{ex}\, c\,dr\ \frac{\epsilon_{ex}^m x_m}{\bar{\epsilon}_{ex}} \tag{6}$$

The fluorescence emitted by this layer at frequency ν in the photomultiplier direction (solid angle Ω) is

$$dF'(\nu) = K \frac{\Omega}{4\pi} dI^{a}_{ex}(m)\Phi A(\nu) \quad (7)$$

where $A(\nu)$ is the Einstein coefficient of spontaneous emission for the transition of frequency ν. Therefore $A(\nu)$ is proportional to the fluorescence intensity in dilute solutions (*i.e.* when there is no re-absorption). The emission quantum yield Φ of the monomer (the aggregates do not emit) is independent of the excitation frequency [6, 8]. K is a geometrical factor.

The radiation emitted by the layer goes through the solution via an optical path r' (Fig. 1) and emerges from the cell in the photomultiplier direction. Therefore the radiation arriving at the detector is

$$dF(\nu) = dF'(\nu) \exp(-2.3\bar{\epsilon}_{\nu} cr') \quad (8)$$

where the last term takes account of the re-absorption. Substituting eqns. (6) and (7) into eqn. (8) and considering that $r' = r \cos\theta_2/\cos\theta_3 = rz$ (Fig. 1), eqn. (8) can be integrated between $r = 0$ and $r_{max} = l/\cos\theta_2$ in order to obtain the fluorescence that arrives at the photomultiplier (obs):

$$F^{obs}_{(\nu)} = K \frac{\Omega}{4\pi} I^{\circ}_{ex} \Phi A(\nu) \frac{\epsilon^{m}_{ex} x_m}{\bar{\epsilon}_{ex} + \bar{\epsilon}_{\nu} z} [1 - \exp\{-2.3(\bar{\epsilon}_{ex} + \bar{\epsilon}_{\nu} z) cl/\cos\theta_2\}] \quad (9)$$

When there is no re-absorption ($\bar{\epsilon}_{ex} \gg \bar{\epsilon}_0 z$), eqn. (9) is transformed into

$$F(\nu) = K \frac{\Omega}{4\pi} I^{\circ}_{ex} \Phi A(\nu) \frac{\epsilon^{m}_{ex} x_m}{\bar{\epsilon}_{ex}} \{1 - \exp(-2.3\bar{\epsilon}_{ex} cl/\cos\theta_2)\} \quad (10)$$

i.e. into the conventional expression for the fluorescence distribution. The ratio between the extinction coefficients takes into account the inactive absorption (in fluorescence) of aggregates.

Therefore to obtain the fluorescence without re-absorption it is necessary to multiply the observed fluorescence (obs) by the factor

$$\frac{\bar{\epsilon}_{ex} + \bar{\epsilon}_{\nu} z}{\bar{\epsilon}_{ex}} \frac{1 - \exp(-2.3\bar{\epsilon}_{ex} cl/\cos\theta_2)}{1 - \exp\{-2.3(\bar{\epsilon}_{ex} + \bar{\epsilon}_{\nu} z) cl/\cos\theta_2\}} \quad (11)$$

The correction factor (11) for re-absorption depends on the cell geometry $l/\cos\theta_2$, the excitation penetration $\bar{\epsilon}_{ex}$ and the overlap $\bar{\epsilon}_{\nu}$ between the absorption and emission spectra.

Factor (11) decreases when the excitation extinction coefficient increases (*i.e.* when there is less penetration). Re-absorption is minimum when the absorption maximum of the monomer is used in the excitation.

The series expansion of exponentials shows that the correction factor for re-absorption is unity in sufficiently dilute solutions. The concentration range where no re-absorption occurs thus depends on the excitation extinction coefficient $\bar{\epsilon}_{ex}$, the cell geometry $l/\cos\theta_2$ and the overlap $\bar{\epsilon}_{\nu}$ between the absorption and emission spectra.

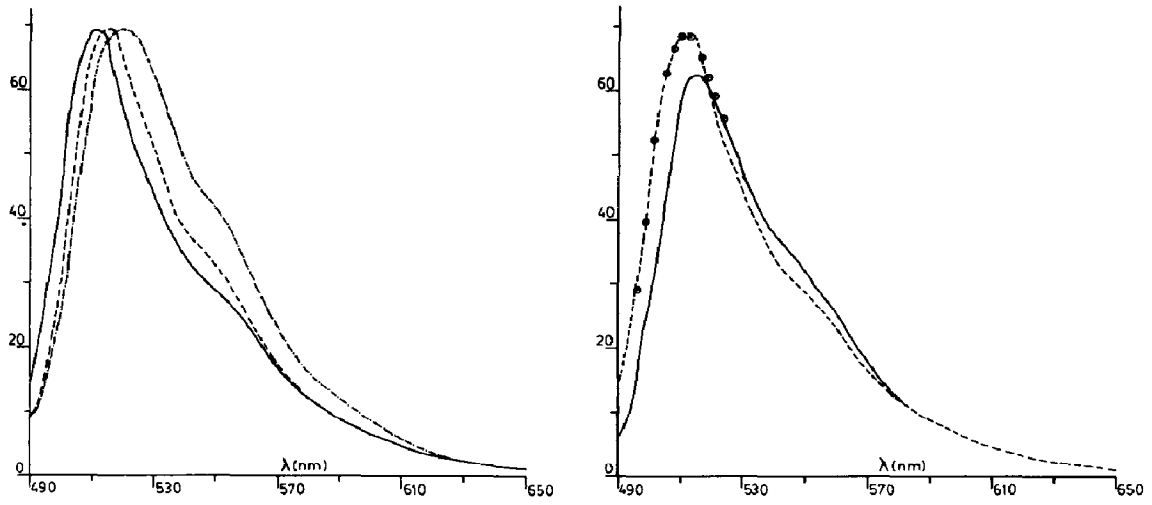

Fig. 2. Fluorescence spectra of fluorescein at various concentrations: ——, 5×10^{-6} M; - - -, 5×10^{-4} M; - - - - -, 10^{-2} M. Conditions: pH 12, [KCl] = 0.01 M, 20 °C, rectangular cell of optical path 1 mm (Fig. 1), excitation wavenumber w_{ex} = 20 833 cm^{-1}.

Fig. 3. A correction for re-absorption (⊙) of the fluorescence spectrum (——) at a concentration of 5×10^{-4} M. The fluorescence spectrum (- - -) at a concentration of 5×10^{-6} M. Conditions: pH 12, [KCl] = 0.01 M, 20 °C, w_{ex} = 20 833 cm^{-1}, 1 mm rectangular cell (Fig. 1).

In concentrated solutions (more concentrated than about 2×10^{-3} M in fluorescein dianion) factor (11) is reduced to its first term (the second term being unity). In general the first term does not depend on concentration and therefore the re-absorption is constant. This is not the case for fluorescein because the formation of aggregates changes the extinction coefficients $\bar{\epsilon}_{ex}$ and $\bar{\epsilon}_{\nu}$. However, although the re-absorption factor changes with concentration in this concentration range, it is a slow process.

The second term of factor (11) dominates in the re-absorption at intermediate concentrations and increases with increasing concentration.

These conclusions explain the changes with concentration of the fluorescence spectrum of fluorescein (Fig. 2). In Fig. 3 the fluorescence spectrum of fluorescein of concentration 5×10^{-4} M is shown corrected for re-absorption. The high energy part of the spectrum corrected for re-absorption is similar to the 5×10^{-6} M spectrum (without re-absorption). The difference in the low energy region of the spectrum is due to re-emission.

Although re-absorption always increases with concentration, re-emission from a given concentration decreases due to the decrease of the emission yield with concentration and also to re-absorption by the aggregates (the aggregate absorption spectra have greater overlap with the fluorescence spectra than the monomer absorption spectrum [6]). Re-emission is lower than 7% with the cell and geometric arrangement used in this work (Fig. 1). Also the geometric factors that contribute to the re-emission [2, 3] are difficult to

evaluate. For these reasons a simple method is proposed to correct the areas of fluorescence spectra.

If solutions have no aggregates, the area of the observed fluorescence [11] is given by

$$F^{obs} = I^{a}_{ex}(m)\Phi\frac{1-a}{1-\Phi a} \tag{12}$$

where a is the probability of self-absorption of one emitted photon. When monomers and dimers exist it is necessary to take into account the proportion y of photons re-absorbed by the monomers, considered to be the ratio of monomer and solution absorptions in the emission region. Then eqn. (12) is transformed into

$$F^{obs} = I^{a}_{ex}(m)\Phi\frac{1-ay}{1-\Phi ay} \tag{13}$$

where ay is the probability of absorption by the monomer of one emitted photon.

The factor y is evaluated from eqn. (2). When there are only monomers and dimers it can be written as

$$\bar{\epsilon}_{0\nu} = \epsilon^{m}_{0\nu}x + \epsilon^{d}_{0\nu}\frac{1-x}{2} \tag{14}$$

where x is the monomer mole fraction and $\bar{\epsilon}_{0\nu}$, $\epsilon^{m}_{0\nu}$ and $\epsilon^{d}_{0\nu}$ are the overlaps between the monomer fluorescence spectrum and the solution, monomer and dimer absorption spectra [6]:

$$\epsilon^{m,d}_{0\nu} = \int_{\nu}\epsilon^{m,d}_{\nu}F_{\nu}d\nu \tag{15}$$

Therefore the proportion of photons re-absorbed by the monomer is

$$y = \frac{x}{x + S(1-x)/2} \tag{16}$$

where $S = \epsilon^{d}_{0\nu}/\epsilon^{m}_{0\nu}$. When the trimer exists the factor y is calculated in the same way but taking eqn. (2) as a whole into account.

When there is no re-absorption, and therefore also no re-emission, eqn. (13) is transformed into

$$F = I^{a}_{ex}(m)\Phi = F^{obs}\frac{1-\Phi ay}{1-ay} \tag{17}$$

giving $F^{obs} \leqslant F \leqslant F^{cor}$, where F^{cor} is the area of the spectrum corrected for

TABLE 1

Fluorescence quantum yields of fluorescein (pH 12, [KCl] = 0.01 M, 20 °C) at various concentrations

Concentration (M)	Φ	*Concentration* (M)	Φ
5.000×10^{-6}	0.92	1.000×10^{-2}	0.397
2.000×10^{-5}	0.920	1.500×10^{-2}	0.245
5.000×10^{-5}	0.918	2.000×10^{-2}	0.167
1.000×10^{-4}	0.914	3.000×10^{-2}	0.095_3
3.000×10^{-4}	0.905	5.000×10^{-2}	0.042_0
5.000×10^{-4}	0.889	7.500×10^{-2}	0.023_0
1.000×10^{-3}	0.868	1.000×10^{-1}	0.019_6
2.000×10^{-3}	0.814	1.500×10^{-1}	0.010_7
3.000×10^{-3}	0.755	2.000×10^{-1}	0.004_2
5.000×10^{-3}	0.637	3.000×10^{-1}	0.003_6

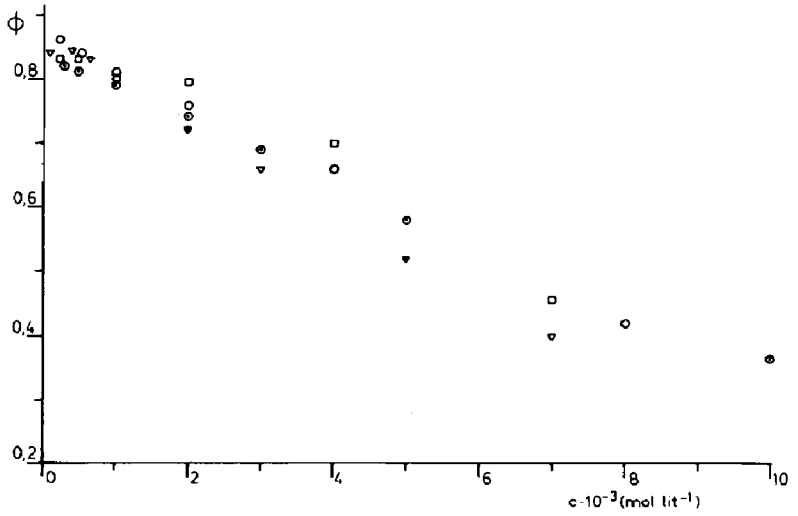

Fig. 4. The fluorescence quantum yield of fluorescein (pH 12) at various concentrations: ○, from ref. 2; ▽, from ref. 3; □, from ref. 5; ⊙, this work.

re-absorption using factor (11). The true fluorescence F, the quantum yield Φ and the absorption probability a of an emitted photon are not known exactly. For this reason an iterative method is used. It is assumed that the ratio $(F^{cor} - F^{obs})/F^{obs}$ is the fraction a of absorbed photons:

$$F^{cor} = F^{obs}(1 + a) \tag{18}$$

The iterative method is carried out in the following way. From the area of F^{obs} and from eqn. (1), a value $\Phi^{(1)}$ is obtained. From this value and using eqn. (17) a fluorescence $F^{(1)}$ is calculated. This $F^{(1)}$ value leads to a new $\Phi^{(2)}$ value again using eqn. (1). This evaluation is successively applied until a constant value of the fluorescence yield Φ is reached.

The fluorescence quantum yield is evaluated using eqn. (1) and taking into account the corrections discussed. The results obtained at 20 °C for the fluorescein dianion are shown in Table 1.

Figure 4 includes data of Budo *et al.* [2] and of Melhuish [3], obtained using their methods. The data of Rohatgi and Singhal [5], with a more simplified method that requires additional measurements to evaluate the reabsorption, are also shown. Finally, the results obtained in this work are given (the values have been multiplied by the factor 0.84/0.92 so that they can be compared with the literature data).

The good agreement between the values for the fluorescence quantum yield of fluorescein at various concentrations obtained in this work and the literature data, and the rapid evaluation of the correction factors make this method a more simple and practical method than those of Budo *et al.* [2], Melhuish [3] and Rohatgi and Singhal [4, 5].

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References

1 J. M. Demas and G. A. Crosby, *J. Phys. Chem.*, *75* (1970) 991.
2 A. Budo, J. Domby and L. Szöllösy, *Acta Phys. Chem., Szeged (N.S.)*, *2* (1965) 18.
3 W. H. Melhuish, *J. Phys. Chem.*, *65* (1961) 229.
4 K. K. Rohatgi and G. S. Singhal, *Anal. Chem.*, *34* (1962) 1702.
5 K. K. Rohatgi and G. S. Singhal, *Photochem. Photobiol.*, *7* (1968) 361.
6 I. Lopez, *Ph.D. Thesis*, University of Basque Country, Spain, 1980.
7 C. A. Parker, *Photoluminescence of Solutions*, Elsevier, New York, 1968.
8 G. Wever and F. W. Teale, *Trans. Faraday Soc.*, *53* (1957) 646.
9 W. R. Dawson and M. W. Windsor, *J. Phys. Chem.*, *72* (1968) 3251.
10 A. Shepp, *J. Chem. Phys.*, *25* (1956) 579.
11 J. B. Birks, *Organic Molecular Photophysics*, Vol. 1, Wiley, New York, 1973.