

CONCENTRATION DEPOLARIZATION OF THE FLUORESCENCE
OF DYESTUFFS IN VISCOUS SOLUTION

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The phenomenon of concentration depolarization of fluorescence of dyestuffs in viscous (or rigid) media is re-examined theoretically and experimentally. The Jabłoński active sphere and Förster-Ore models are considered along with a new expression developed from the step-wise transfer model of Weber and containing a data-fitted constant. The effect of concentration quenching of fluorescence that occurs at high concentrations of dyestuff is included.

The various expressions are compared with experimental data for fluorescein in 95% (v/v) alkaline-aqueous glycerolic solution. Data-fitting of the course of concentration depolarization to the Förster-Ore model gives the characteristic interaction parameter for transfer as: $R_0 = (45.6 \pm 0.7) \text{ \AA}$, which coincides with that obtained from the absorption and fluorescence spectra of $(45.6 \pm 0.8) \text{ \AA}$.

The applicability of the theoretical models for excitation wavelengths other than that of the '0-0' transition is discussed and greatly increased depolarization demonstrated for wavelengths much shorter than this. The repolarization of fluorescence excited at long wavelengths of absorption in concentration depolarized systems ("red-edge" effect of Weber) was observed and partially explained in terms of these considerations.

The anomalous course of depolarization observed at low concentrations, compared with theoretical predictions, is tentatively ascribed to the existence of extended regions containing, on average, higher concentrations of solute than expected for a random distribution of solute ("super"-solvation shells), supposed to be due to solvent structural changes induced by solvent-solute interaction.

Technical limitations prevented the unequivocal experimental determination of the course of concentration depolarization at the highest concentrations, but it appears that the active sphere approximation is less appropriate than the other two in this range.

Expressions are derived for the correction of raw experimental yield and polarization data for front-face illumination and for excitation by and observation of beams of finite aperture as well as a normalization procedure for comparing polarizations under differently depolarizing reabsorption conditions.

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1. General introduction

(a) Theoretical

The problem of concentration depolarization of the fluorescence of dyestuffs and other complex molecules with broad band spectra has been extensively treated by theorists over the past twenty years or so. The analyses have been based on various models of the luminescence centre and of the effective spatial distribution of the interacting molecules in solution. The inclusion of a specific mechanism for the radiationless transfer of excitation energy between molecules has not always been necessary. Again, while the analyses due, for instance, to Ore [1] and Jabłoński [2, 3] stress the importance of re-transfer of excitation energy to the originally excited molecule, those of Vavilov [4] and Weber [5, 6] assume that no re-transfer occurs. On the other hand, Weber has included the (small) polarization contributed by centres not primarily excited, while Ore and Jabłoński have assumed that it is zero. Critical reviews of the theories and subsequent modifications of them have been given by Eriksen and Ore [7, 8] and by Knox [9]. The added complication of a change in the fluorescence yield at high concentrations, probably due to the formation of non-(or weakly) fluorescent dimers as suggested by Förster [10] and referred to as concentration quenching, can in principle be allowed for in most theories (see e.g. Szalay, Sárkány and Tombácz [11] and Eriksen [12]).

Recent refinements of the active sphere model of Jabłoński have led in general to much increased difficulty in calculation for only small changes in the predicted course of concentration depolarization e.g. the extended shell models of the luminescence centre given by Bojarski [13] and Kowski [14]).

(b) Experimental

Cauchois [15] and later Pheofilov and Sveshnikov [16] performed the first quantitative measurements of concentration depolarization on dyestuffs in glycerolic solutions of high viscosity, the latter authors collecting data over a very wide range of concentration. They observed an approximately linear relationship between the reciprocal of the degree of polarization, p^1 , and the concentration except in the region of high concentration where concentration quenching becomes marked. They eliminated the effect of trivial reabsorption (radiative transfer) by an approximate correction factor based on comparison of the polarization observed for a very thin layer with no appreciable absorption with that for a completely absorbing layer. Much later, Szalay and co-workers [11] repeated these experiments with more refined instrumentation and in alkaline or acid glycerolic solution to eliminate possible effects of the presence of several ionic species of the dyestuffs together in solution. Their empirical results were corrected by the method of Budó and co-workers [18] for the effects

¹ The degree of polarization, p , is defined here as $(I_{||} - I_{\perp}) / (I_{||} + I_{\perp})$ where $I_{||}$ and I_{\perp} are the observed components of intensity (quantum flux) of the emission polarized parallel and perpendicular to the electric vector of plane polarized exciting light and usually observed perpendicularly to the polarization vector and direction of the exciting light beam. In view of the random orientation of absorption moment vectors in solution it may take values $\frac{1}{2} \geq p > -\frac{1}{3}$ (see e. g. Jabłoński [17]).

of secondary fluorescence resulting from trivial reabsorption and applicable to the conditions of their experiments. The final results found good agreement with the simple active sphere model [2, 3] when modified for the effect of concentration quenching. Their results, along with those of Pheofilov and Sveshnikov, were also analysed by Eriksen [12], who obtained good agreement with the Förster-Ore equation [1], again on correction for the yield change at high concentrations.

Various cases of concentration depolarization in solid solutions (polymethylmethacrylate) have been studied, mainly by Kawski and co-workers (see [7, 8]) and found to agree quite well with theory. However, the uncertainty of homogeneity of luminescent centres [19] and of true randomness of distribution in such solutions renders interpretation of the results difficult.

The results of Weber [20] with phenol in propylene glycol at low temperature have been stated to support the linear relationship noted first in [16] and predicted by Weber [5, 6] on the basis of his transfer model. Knox has analysed these results in more detail [9].

Trosper, Park and Sauer [21] have used the theoretical results of both Ore and Weber to analyse the concentration depolarization of monolayers of chlorophyll *a* diluted with lipid, taking into account the two-dimensional arrangement. The technical difficulties of such measurements led to rather large standard errors in these experiments.

2. Theoretical section

(a) Model of Jabłoński [2, 3]

In this model, transfer of excitation energy is assumed to take place only within an initially arbitrary (small) volume, v (the active sphere) containing the primarily excited molecule. The distribution of the number of other molecules in the sphere is given, for a completely random statistical distribution of molecules in solution, by:

$$p_k = \frac{v^{k-1}}{(k-1)!} e^{-v} \quad (1)$$

where p_k is the probability of finding k molecules (one excited) in a volume v for a solution of molar concentration c_M of unexcited molecules (usually effectively the same as the total concentration — see *e.g.* Brand and Witholt [22]), with $v = vN'c_M$ (N' is Avogadro's number per cm^3). With the further assumption that the transfer probability between any pair of molecules within an active sphere is the same, the result, given in its simplest form by Bojarski [23] as:

$$\frac{\bar{\eta}}{\eta_0} = \frac{2(v-1+e^{-v})}{v^2} \quad (2)$$

where $\frac{\bar{\eta}}{\eta_0}$ is the fraction of excitation energy associated with the initially excited molecule, is obtained. On assigning zero polarization to the emission of molecules excited by transfer,

this also represents the fractional anisotropy, $\frac{\bar{r}}{r_0}$. Jabłoński [28] has shown that this is a good approximation for this model.

This result has been modified to:

$$\frac{\bar{\eta}}{\eta_0} = \frac{[(\Phi/\Phi_0)+1][(\Phi/\Phi_0)^{\nu-1}+e^{-\nu}]}{[(\Phi/\Phi_0)^{\nu}]^2} = \frac{\bar{r}}{r_0} \quad (3)$$

by Szalay and co-workers [11] to take account of possible changes in quantum yield (Φ) with concentration, on the assumption that:

$$\Phi/\Phi_0 = \tau/\tau_0$$

where τ is the emission lifetime and the subscripted quantities those obtaining and constant at low concentrations.

(b) Förster-Ore model

Förster [29] originally considered the interaction between neighbouring pairs of molecules only as valid for low concentrations and averaged over a statistical distribution of nearest neighbour distances, again assuming that only initially excited molecules contributed to the polarization. The results did not agree too well with experiment since transfer

² Classically the polarization of luminescence emission has been described by the degree of polarization, p , already defined. This has been replaced by the more useful quantity, emission anisotropy (EA), r , introduced specifically by Jabłoński [24, 25]:

$$r = (I_{||} - I_{\perp}) / (I_{||} + 2I_{\perp})$$

and thus related to the degree of polarization by:

$$r = 2p / (3 - p)$$

The EA has also been used implicitly in the form:

$$\left(\frac{1}{p} - \frac{1}{3} \right)^{-1} = \frac{3}{2} r$$

in the addition law for polarization of Weber [26] and given several different designations. Thus Knox [9] used the term polarization function:

$$g(p) = \left(\frac{1}{p} - \frac{1}{3} \right)^{-1}$$

while Weber and Young [27] called this the emission anisotropy and designated it A . Eriksen and Ore [7] called this auxiliary quantity q , defined by:

$$q = (I_{||} - I_{\perp}) / I_{Av} \quad \text{with} \quad I_{Av} = \frac{2}{3} (I_{||} + 2I_{\perp}),$$

I_{Av} being the fluorescence intensity averaged over all directions of emission. The quantity is useful because of its additive property:

$$\bar{r} = \sum F_i r_i / \sum F_i$$

where F_i is proportional to the total emission intensity (quantum flux) *i. e.* directly proportional to the yield due to species i .

Generally the ratio of the EA to a limiting value is important and in this respect the different usages are immaterial.

from the acceptor to molecules other than the donor was not considered. Ore [1] modified this model by including the approximation that nearest neighbours of the primary acceptor at distances up to the donor-acceptor separation prevented, by themselves accepting the excitation, any re-transfer to the initially excited molecule. The result, averaged for a statistical distribution, is given by:

$$\frac{\bar{\eta}}{\eta_0} = \int_0^{\infty} \{\xi^2(\xi^2 + \gamma^2)/[\xi^2 + \gamma^2]^2 - \gamma^4 \exp(-11\xi/16)\} \exp(-\xi) d\xi \quad (4)$$

where ξ is the average number of molecules in a sphere of radius R for a given concentration c_M and γ the ratio of this concentration to the critical concentration, $c_0 = 3/4\pi N' R_0^3$.

³ R_0 corresponds to the critical transfer distance giving the first order rate constant for transfer between donor, D , and acceptor, A , in the approximation of very weak dipole-dipole interaction (see Förster [30]):

$$\mu_{DA} = \frac{1}{\tau} \left(\frac{R_0}{R_{DA}} \right)^6$$

with R_{DA} the donor-acceptor separation and τ the donor lifetime in absence of transfer. R_0 may be determined from spectral data and properties of the medium:

$$R_0^6 = \frac{9(\ln 10) \Phi \kappa^2}{128\pi^5 n^4 N'} \mathcal{J}(\bar{\nu}) \quad (4')$$

where Φ is the quantum yield in absence of this transfer ($\Phi = \tau/\tau_e$, the ratio of observed and natural lifetimes) κ^2 the orientation factor for dipole-dipole interaction whose average value here is taken as $\frac{2}{3}$ and n the refractive index of the medium. $\mathcal{J}(\bar{\nu})$, the overlap integral, is defined as:

$$\mathcal{J}(\bar{\nu}) = \int_0^{\infty} \varepsilon_m(\bar{\nu}) f(\bar{\nu}) \frac{d\bar{\nu}}{\bar{\nu}^4}$$

or, converted to a wavelength basis, as:

$$\mathcal{J}(\bar{\nu}) = \int_0^{\infty} \varepsilon_m(\lambda) f(\lambda) \lambda^4 d\lambda$$

where ε_m in the molar extinction coefficient, $\bar{\nu}$ the frequency of excitation in wavenumbers and $f(\bar{\nu})$ ($f(\lambda)$) the quantum spectrum of fluorescence normalised to unity:

$$f(\bar{\nu}) d\bar{\nu} = \frac{F(\bar{\nu}) d\bar{\nu}}{\int_0^{\infty} F(\bar{\nu}) d\bar{\nu}} = \frac{F(\lambda) d\lambda}{\int_0^{\infty} F(\lambda) d\lambda} = f(\lambda) d\lambda$$

with $F(\bar{\nu})$ ($F(\lambda)$) the quantum flux per unit frequency interval (wavelength interval) at frequency $\bar{\nu}$ (wavelength λ).

Dependence of transfer efficiency on a rate proportional to R^{-6} has been strongly supported by the work of Stryer and Haugland [31] and Gabor [32] in model systems with fixed donor-acceptor separations, as has the dependence of the transfer rate itself on the value of the overlap integral (see Haugland, Yguerabide and Stryer [33]).

It should be noted that R_0 defined above in terms of spectral properties obtains in the case that an equilibrium thermal (Boltzmann) distribution of energy levels in the first electronic excited state has been attained (see Förster [30, 34]). If transfer would be very fast under this condition (μ of the order of 10^2 to $10^4 \tau^{-1}$), then before-relaxation transfer may occur from higher vibronic levels with even greater efficiency in the case of self-transfer since the effective fluorescence spectrum is shifted to higher energies so that the value of the overlap integral increases as discussed by Guéron, Eisinger and Schulman [35]. Thus the R_0 value defined is generally valid only for excitation of the energy of the pure electronic (zero-zero, '0-0') transition since no vibrational relaxation of the excited state population will occur, the population being already relaxed.

The critical concentration was taken by Förster [36] as that for which

$$\frac{\bar{p}}{p_0} = 0.5. \quad (5)$$

This corresponds to

$$\frac{\bar{r}}{r_0} = 0.46 \quad (5a)$$

when a limiting value of $r_0 \simeq 0.34$ commonly found for dyestuffs is taken and coincides remarkably well with the value of expression (4), 0.47 for $\gamma = 1$.

(c) Stepwise transfer model

The above discussed models, although found to agree reasonably well with data so far accumulated, are both based on quite severe approximations. They consider only a part of the acceptor population, do not take full account of the orientation effects in estimating transfer efficiency and disregard the contribution to the polarization of secondarily excited emitters. The last aspect has been stressed by Weber [5, 6]. He approximates the average transfer chain to a chain of average transfers and writes down, where $\frac{\bar{\eta}}{\eta_0}$ is the average relative yield of fluorescence from initially excited emitters, the fraction f_m of total energy associated with the m 'th acceptor as:

$$f_m = \left(1 - \frac{\bar{\eta}}{\eta_0}\right)^m \left(\frac{\bar{\eta}}{\eta_0}\right). \quad (6)$$

Now, again following Weber [5], the polarization of the fluorescence after m transfers expressed here in terms of the EA is:

$$r_m = r_0 \left(\frac{3}{2} \cos^2 \theta_m - \frac{1}{2}\right) \quad (7)$$

where θ_m is the angle between initial absorption and final emission transition moment vectors,⁴ while:

$$\left(\frac{3}{2} \cos^2 \theta_m - \frac{1}{2}\right) = \prod_{i=1}^m \left(\frac{3}{2} \cos^2 \theta_i - \frac{1}{2}\right). \quad (8)$$

⁴ This expression, Perrin's law of isotropic depolarization [37], is very general for θ as defined, regardless of the origin of the orientation change, *e. g.* it can be used to derive the limiting (fundamental) EA , r_f : putting $r_0 = r_{\text{excitation}} = 1$ for the plane polarized exciting light, then:

$$r_f = \left(\frac{3}{2} \overline{\cos^2 \theta} - \frac{1}{2}\right).$$

Now the absorption probability is proportional also to $\cos^2 \theta$ and with a spatial distribution weighting factor of $\sin \theta d\theta$:

$$\overline{\cos^2 \theta} = \frac{\int_0^{\frac{\pi}{2}} \cos^4 \theta \sin \theta d\theta}{\int_0^{\frac{\pi}{2}} \cos^2 \theta \sin \theta d\theta} = \frac{1/5}{1/3} = \frac{3}{5}$$

so that: $r_f = 0.4$.

For excitation in a transition at right angles to the emission moment vector, $\overline{\cos^2 \theta} = \cos^2 \left(\frac{\pi}{2}\right) = 0$ so that: $r_f\left(\frac{\pi}{2}\right) = -0.5 r_f = -0.2$.

Considering each transfer as average with respect to angular distribution also, (7) and (8) yield

$$r_m = r_0 \left(\frac{3}{2} \overline{\cos^2 \theta} - \frac{1}{2} \right)^m$$

Averaging over the angular dipole-dipole interaction parameter κ^2 (see Ketskeméty [38], Weber [6] and Knox [9]):

$$r_m = r_0 (0.04)^{m \cdot 5} \quad (9a)$$

The observed *EA* of the solution as a whole is then:

$$\bar{r} = \frac{\sum_{m=0}^{\infty} f_m r_m}{\sum_{m=0}^{\infty} f_m} = r_0 \sum_{m=0}^{\infty} (0.04)^m \left(1 - \frac{\bar{\eta}}{\eta_0} \right)^m \left(\frac{\bar{\eta}}{\eta_0} \right) \quad (10)$$

since $\sum_{m=0}^{\infty} f_m = 1$.

Thus

$$\frac{\bar{r}}{r_0} = \frac{\left(\frac{\bar{\eta}}{\eta_0} \right)}{1 - 0.04 \left(1 - \frac{\bar{\eta}}{\eta_0} \right)} = \frac{\left(\frac{\bar{\eta}}{\eta_0} \right)}{0.96 + 0.04 \left(\frac{\bar{\eta}}{\eta_0} \right)} \quad (11)$$

This last equation might be used in further correcting the results of Jabłoński and Ore, but the error committed is never greater than 4% and, in view of the inherent errors already present, not really justified. In the former case, calculations have shown [28] that even a factor of 0.04 in Eq. (9a) is too high (by about 60%) on the model considered there, making the correction even less important.

The determination of $\frac{\bar{\eta}}{\eta_0}$ in terms of the very weak coupling rate constant describing the interaction leading to energy transfer in the case considered⁶ depends on the solution of a coupled system of equations (see Knox [9]) given in one generalized form as:

$$-\frac{d\mathcal{S}(t)}{dt} = \left\{ \frac{1}{\tau_i} \left[1 + \sum_{j=1}^{N-1} \left(\frac{R_{0ij}}{R_{ji}} \right)^6 \right] \mathcal{S}_i(t) - \sum_{j=1}^{N-1} \frac{1}{\tau_j} \left(\frac{R_{0ji}}{R_{ji}} \right)^6 \mathcal{S}_j(t) \right\} \quad (12)$$

⁵ The decrease of *r* at each stage of transfer to 4% of the previous value corresponds to the series for polarization:

$$p = \frac{1}{2}, p_1 = \frac{1}{48}, p_2 = \frac{1}{1048}, \dots$$

given by Galanin [39].

⁶ The validity of considering this interaction for dyestuffs in solution to the exclusion of other interactions, *e. g.* weak dipole-dipole with an R^{-3} distance dependence, has been questioned by Robinson and Frosch [40, 41]. It appears, however, that for the complex molecules dealt with here, very weak interaction passes over directly into strong interaction manifested in absorption and possibly fluorescence spectral changes (see Förster [30], Guéron and co-workers [35]) which are not observed over at least the greater part of the concentration range (see Experimental Section).

for a system of N interacting particles, where $\mathcal{E}_i(t) = \frac{\eta_i(t)}{\eta_{0i}}$, τ_i is the lifetime of fluorescent molecule i in absence of transfer and R_{0ij} is given by Eq. (4') with $\Phi_i = \frac{\tau_i}{\tau_{0i}}$ and due regard to the orientation factor κ_{ij}^2 . The generalization admits of differences between centres but the simplification is usually made that $\tau_i = \tau(c)$ (to allow in an average way merely for concentration quenching) so that, extracting the orientation factors as $R_0^6 = \kappa^2 R_0'^6$:

$$-\frac{d\mathcal{E}_i(t)}{dt} = \frac{1}{\tau} \left\{ \mathcal{E}_i(t) + \sum_{j=1}^{N-1} \kappa_{ij}^2 \left(\frac{R_0'}{R_{ij}} \right)^6 \left[\mathcal{E}_i(t) - \mathcal{E}_j(t) \right] \right\} \quad (13)$$

which still disregards back transfer of excitation from a third or further acceptor directly to the initially excited molecule. Eq. (13) has been solved completely only for an isolated pair of molecules, by Förster [29], and a general solution for a statistically distributed array of interacting molecules in solution has not been found (see Trospen and co-workers [21]).

From a solution of Eq. (13) for $\mathcal{E}_1(t)$ under the initial condition $\mathcal{E}_1(0) = 1$, the required average relative yield from initially excited molecules would then be obtained as an average over the lifetime:

$$\frac{\bar{\eta}_1}{\eta_0} = \frac{1}{\tau} \int_{t=0}^{\infty} e^{-\frac{t}{\tau}} \mathcal{E}_1(t) dt. \quad (14)$$

In view of these difficulties the transfer and re-transfer processes will be regarded here as approximating to a collection of isolated "hopping" events of frequency described by the rate constant for transfer, μ , in the manner of Ore [1]. He considered the transfer probability given by:

$$P = \mu / \left(\frac{1}{\tau} + \mu \right)$$

where μ is the transfer rate constant defined for Eq. (4), so that, for an isolated pair of molecules 1 and 2:

$$\frac{\bar{\eta}_1}{\eta_0} = \frac{1-P}{1-P^2} = \frac{1+\mu\tau}{1+2\mu\tau} \quad (15)$$

which is the result of Förster [29]. Eq. (15) has a form identical with a result to be derived elsewhere [42] on consideration of an extremely simplified model for the distribution of many interacting centres:

$$\frac{\bar{\eta}_1}{\eta_0} = \frac{1-\bar{P}}{1-0.72\bar{P}^2} \quad (16)$$

An approximate solution of Eqs (13) and (14) might therefore be given by:

$$\frac{\bar{\eta}_1}{\eta_0} = \frac{1-\bar{P}}{1-\alpha\bar{P}^2} \quad (17)$$

where α remains a data-fitted constant and $(1-\bar{P})$ is the solution $\mathcal{E}_i(t)$, averaged over the lifetime as in Eq. (14), of Eq. (13) disregarding back transfer:

$$-\frac{d\mathcal{E}_i(t)}{dt} = \left\{ \frac{1}{\tau} \left[1 + \sum_{j=1}^{N-1} \kappa_{ij}^2 \left(\frac{R'_0}{R_{ij}} \right)^6 \right] \right\} \mathcal{E}_i(t) \quad (18)$$

with the well known result given by Förster [43] and others [44, 45]:

$$1-\bar{P} = 1-2qe^{q^2} \int_q^\infty e^{-x^2} dx = 1-\sqrt{\pi}qe^{q^2}[1-\text{erf } q] \quad (19)$$

where

$$q = \frac{2}{3} \pi^{3/2} \bar{\kappa} R'_0{}^3 N' c_M \text{ and } \text{erf } q = \frac{2}{\sqrt{\pi}} \int_0^q e^{-x^2} dx^7$$

The value of $\bar{\kappa}$ applicable here is that first derived by Galanin [44] and later by Maksimov and Rozman [46] and Steinberg [47] of $\bar{\kappa} = 0.690$ and not $\sqrt{(\bar{\kappa}^2)} = \sqrt{2/3}$ which applies to rapidly rotating molecules only.

α in Eq. (17) may be found by substitution of experimental data or by comparison with other theories most reasonably at the half-polarization point $\left(\frac{\bar{r}}{r_0} = 0.46 \right)$. Comparison with the Förster-Ore expression gives $\alpha = 0.595$.

It should be noted that the result of Weber [5, 6] describing the average number of transfers in an average transfer chain, \bar{n} , rewritten in terms of EA as:

$$\bar{\eta} = \frac{25}{24} \left(\frac{r_0}{\bar{r}} - 1 \right) \quad (20)$$

is still valid here but not as simply related to the transfer rate as in his model; here:

$$\bar{n} = \frac{1 - \left(\frac{\bar{\eta}}{\eta_0} \right)}{\left(\frac{\bar{\eta}}{\eta_0} \right)} = \left(\frac{\eta_0}{\bar{\eta}} \right) - 1 \quad (21)$$

with $\frac{\bar{\eta}}{\eta_0}$ determined by Eqs (17) and (19) or, in other approximations, by Eqs (3) or (4). Eq. (21) with assignment of zero polarization to acceptor molecules is equivalent to that given in terms of degree of polarization by Anderson and Weber [48].

⁷ An apparent misprint in the limits of the error integral in Ref. [43] has been carried over into several publications.

3. Experimental section

(a) Preparation of solutions

The concentration depolarization of fluorescein in alkaline glycerol solution was to be studied over four decades of concentration. Fluorescein was supplied as the crystalline disodium salt, uranin (M. W. 376.3) from British Drug Houses Ltd., Poole, Dorset, U.K. and used without further purification. The solvent was 0.1M sodium hydroxide in glycerol containing 5% (*v/v*) water (\equiv 18% M/M). The sodium hydroxide, obtained from "Ciech", Gliwice, Poland, was made up as a 2M aqueous solution and this added to 9.5 parts by volume of glycerol (from the same suppliers) purified by double distillation under reduced pressure [49]. The solvent was rendered homogeneous by heating to *ca* 70° and mixing. Narrow-range pH paper indicated a pH greater than 9.5.

15 ml of 0.1M fluorescein in the aqueous glycerolic solvent was made up by dissolving 0.5645gm of the disodium fluorescein in part of the solvent with heating to *ca* 70° and mixing, then making the volume up to 15 ml at room temperature. Further heating and mixing ensured a homogeneous solution. Dilutions with solvent of 5 to 5, 8 to 2 and 9 to 1 ml followed by the mixing procedure described gave respectively 0.05, 0.02 and 0.01M solutions. These were serially diluted three times with solvent 9 to 1 ml to give a concentration range down to 10^{-5} M. All dilutions were made with a 5ml graduated syringe at room temperature. The solutions were stored at room temperature in the dark and all measurements described below were also made at room temperature (22 to 27°).

(b) Absorption spectra

Absorption spectra of the solutions prepared were determined in a thin layer cell fitted with plane quartz windows, the layer thickness being variable up to 1cm (in practice layer thicknesses up to 1mm were employed) with a pitch of 200 μ m and a practical limit of about 5 μ m layer thickness for purposes of reproducibility. The cell was mounted in a holder fitted to a Zeiss glass prism monochromator with entry and exit slit widths set at 0.05 mm, giving a spectral band width of 0.3 nm at a wavelength of 400 nm to 0.8 nm at 560 nm. No change in the absorption spectra measured occurred on further increasing the resolution. The detector was an EMI 9558B red-sensitive photomultiplier and the extinction read directly from the logarithmic scale of a Zeiss galvanometer. Zero calibration of the cell was made against a 1 mm path length quartz cuvette containing the glycerolic solvent. Balance was obtained by varying the voltage applied to the photomultiplier (3 \times 400 volt batteries in series).

(c) Fluorescence emission spectra

Fluorescence emission spectra, read at 5 nm intervals between wavelengths of 480 and 670 nm, were determined for surface-illuminated samples contained in the thin layer cell. The fluorescence was focussed onto the slit of the Zeiss glass prism monochromator (also used for absorption measurements) with entry and exit slit widths of 0.05 mm giving a spectral band width of 0.5 nm at 480 nm and 1.4 nm at 670 nm. As in absorption measurements, further reduction of the slit widths did not result in further spectral resolution. Light passing

through the exit slit fell directly onto the photocathode of the EMI 9558B photomultiplier which was run at a negative potential of 1200 volts from a stabilized power source (WN 2.5, "Polon", Warsaw, Poland). The output was fed directly to the Zeiss galvanometer registering a maximum of about 0.05 μA (the 1000 scale divisions represented 0.5 μA).

The detection system (monochromator set at the given slit width plus photomultiplier) was calibrated with a tungsten strip projection lamp (Philips 6V, 16-17A Type 6002c) fitted with a plane quartz window, evacuated and flushed with about half an atmosphere of argon four times at a diffusion pump and finally filled with argon to about 100 Torr, which gave a colour temperature of 2854°K when run at 17.6 A⁸. The quantum response of the detector system was compared with the relative quantum flux of the lamp obtained by multiplying the relative energy per unit wavelength interval at a given wavelength [50] by the wavelength:

$$E = nh\nu \therefore n = \frac{E}{h\nu} \propto E\lambda$$

(see Lippert and co-workers [51]). The change in flux transmitted through the quartz window due to change in refractive index with wavelength was about 0.6% between wavelengths of 380 and 780 nm and was disregarded. The calibration light was unpolarized and no selective detection by the photomultiplier itself ("eigen-polarization" [52]) was observed, but the monochromator introduced a wavelength-dependent polarization in the detection system as a whole. For this reason it was important to ensure that the calibration source was unpolarized (the original lamp gave quite an appreciable polarization) and that all relative measurements carried out were also of unpolarized emission, *e.g.* fluorescence spectra, relative fluorescence yields. Five determinations of the calibration curve for wavelengths between 380 and 780 nm at intervals of 5 nm gave a standard error of less than 0.7% of the value for all wavelengths included.

The excitation source was an Osram 650W, 115-120V coiled coil tungsten-iodine filament lamp mounted in a water-cooled jacket and operated at 60-90V from a stabilised A.C. source. The light was passed through a Russian UM-2 prism monochromator with slit widths set at 1 mm and focussed onto the sample at an angle of about 40° to the observation direction which was perpendicular to the surface of the cell, after passing through a Zeiss polaroid oriented so as to produce emission unpolarized in the direction of observation (see Appendix A). Excitation wavelengths of 465 and 510 nm (for observation of emission between 480 and 580 nm and between 560 and 670 nm respectively) were employed, the spectral band width being 8 and 12 nm respectively.

Relative quantum yields were also determined in the apparatus described above using the thin layer cell and, for the higher concentrations, 1 mm path length glass or silica cuvettes which showed effectively complete absorption of the exciting light. While a correction for the small change of polarization across the fluorescence emission band was not applied

⁸ Colour temperature calibration was carried out at the "Centralny Urząd Jakości i Miar", Warsaw with a quoted accuracy of $\pm 30^\circ$.

in determination of the true fluorescence spectrum, the much larger changes in polarization for relative yield measurements for different concentrations did necessitate such a correction (see Appendices A and B).

(d) Fluorescence polarization

Determinations of the EA of fluorescence were made in an apparatus previously described by Bauer and Rozwadowski [53, 54], modified for front-surface illumination of thin layers. Excitation light passing from a power-stabilized xenon arc source (XBO-150, "Osram", The Netherlands) through a double quartz prism monochromator (Hilger-Müller "Uvisir") set at a slit width of 0.5 mm to give a spectral band width of 4.2 to 6.3 nm between wavelengths of 440 and 520 nm, was focussed onto the sample in the thin layer apparatus or, for the higher concentrations, 1 mm path length glass or silica cuvettes at an angle of about 40° after passing through a Zeiss polaroid. To eliminate scattered excitation light from the observation path as much as possible, sharp cut Schott-Jena filters OC11 or OC14 with 90, 10 and approximately 0% transmission at wavelengths of 560, 525 and 515 nm and 600, 575 and 560 nm respectively were placed in the observation path.

Unpolarized emission for zero setting of the apparatus was produced by appropriate orientation of the excitation polaroid (see Appendix A). The EA , with polaroid vertically oriented, was read off as a compensation angle to which depolarizing plates were set to regain the zero position (zero modulation of the emission passing through the Billings cell indicated that the emission incident on the cell was unpolarized). The EA itself was determined from this compensation angle using tables of the Kawski-Lisica relationship [55, 56]. Two determinations of the right and left compensation angles consistently gave an accuracy in the EA of better than 0.0005 over the whole range of EA measured (included in 0.013–0.34). Long term reproducibility, however, was not as good as this and provided the main source of error in these determinations over the whole range of concentration. This source of error was observed to be greater than any change in polarization resulting from changes in the viscosity of the solvent in the range of temperature employed (22 to 27°).

(e) Fluorescence lifetime

Measurements of fluorescence lifetime were carried out on a phase and modulation fluorometer based on an earlier model of Bauer and Rozwadowski [57]. The delay in fluorescence compared with scatter by a dilute suspension of Ludox colloidal silica (E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware, USA) [58] was given by the angular displacement, φ , of the zero in one cycle recorded by running through a delay line with stops at intervals of 2.076° (corresponding to 10 cm of delay line), the linear frequency, f , of the modulated exciting light being 11.40 MHz. The lifetime, τ , was calculated from the relationship given by Bailey and Rollefson [59]:

$$\tau = \frac{\tan \varphi}{2\pi f} = 13.97 \tan \varphi \text{ [ns]}.$$

Excitation was by a cooled, stabilised mercury arc *via* a Zeiss filter isolating the Hg line at 436 nm. Fluorescence or scatter originating from the thin layer, placed at about 50° to

the excitation light path to prevent observation of excitation scattered from the cell faces, was observed at right angles to the excitation path. Scatter and fluorescence were separated by filters in the observation path (Zeiss Hg436 for scatter and OC11 or OC14 for fluorescence). No account was taken of the small differences in lifetime present for the polarized components of the fluorescence observed compared with the total fluorescence (see Jabłoński [60] and Spencer and Weber [61]).

4. Results

Absorption spectra determined in the range of wavelengths 400 to 560 nm (5 nm intervals) for concentrations 10^{-4} to 5×10^{-3} M and normalized to unity at the maximum (Fig. 1) were identical within experimental error. Above this concentration the spectra appeared to broaden and the molar extinction coefficient, $\epsilon_m(\lambda)$, to fall, although the

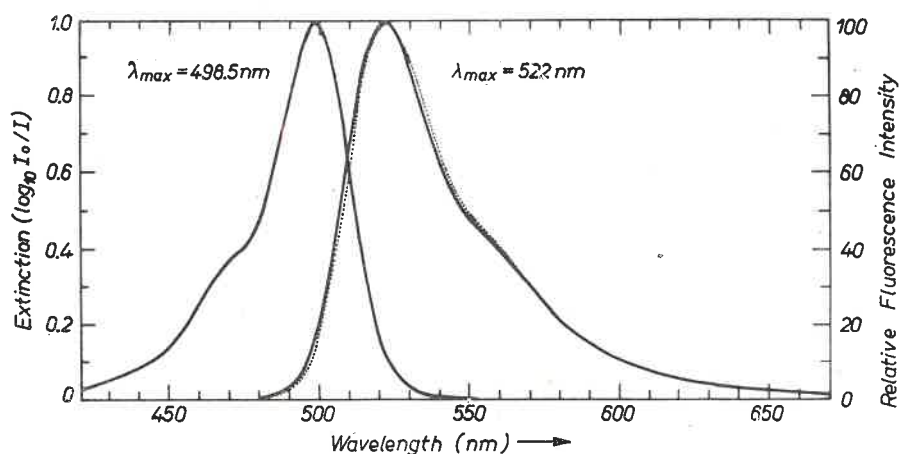


Fig. 1. Absorption and emission spectra of fluorescein in alkaline-aqueous glycerolic solution. Legend: fluorescence ————— 5×10^{-5} M, - - - - - 10^{-3} M

wavelength of maximum absorption remained constant. The extinction coefficient at the visible maximum, 498.5 nm, was determined as the average over the concentration range 10^{-4} to 2×10^{-3} M. Each value was taken from the slope of extinction $\left(\log_{10} \left(\frac{I_0}{I}\right)\right)$ vs layer thickness to an extinction of about 0.7, beyond which the Lambert law appeared not to hold. The value obtained was $\epsilon_m(\lambda_{\max}) = (0.729 \pm 0.010) \times 10^5 \text{ cm}^2/\text{mmole}$. This compares quite well for the glycerolic medium of high refractive index with the higher figures given by Seybold, Gouterman and Callis [62] of 0.923×10^5 in alkaline 95% ethanol and 0.879×10^5 in alkaline aqueous solutions. The broadening of the spectrum above a concentration of 5×10^{-3} M was, to at least a large extent, due to the failure of the Lambert law cited above. This was demonstrated by the double-peaked difference spectrum between the same solution in two thin layer cells of different path length (the comparison cell was merely two glass plates pressed closely together and enclosing the solution), with the minimum located

at the maximum of the true absorption spectrum. For the higher concentrations a layer thin enough to obtain the required low extinctions for validity of this law could not be obtained. Failure of Beer's law was already observed, however, at 5×10^{-3} M and this is probably due to dimer formation [10]. Investigation of this with the present apparatus was precluded due to the concurrent failure of the Lambert law.

Fluorescence spectra were determined using thin layers such that the extinction was less than 0.1 at the absorption maximum in order to eliminate the effect of reabsorption on the blue edge of the spectrum. The difference between spectra thus determined and those for even lower extinctions were the same within experimental error. The spectrum taken at 5 nm intervals between 480 and 580 nm ($\lambda_{\text{ex}} = 465$ nm) for a concentration of 10^{-3} M was slightly red-shifted compared with that taken for a 5×10^{-5} M solution (compare general result of Weber and Shinitzky [63]). The spectrum between 560 and 670 nm ($\lambda_{\text{ex}} = 510$ nm), being unaffected by reabsorption, was determined for 10^{-3} and 10^{-2} M solutions with extinction at the maximum of about 1 and found to be identical for the two concentrations. The complete spectra are given in Figure 1 as relative quanta per unit wavelength interval ($F(\lambda)$) normalized to 100 at the maximum, 522 nm, the two ranges being normalized in their overlap region, 560 to 580 nm.

Relative fluorescence quantum yields, observed at 570 nm, were determined in the concentration range 2×10^{-4} to 2×10^{-2} M using the thin layer cell set to give an extinction of about 0.7 at the maximum. Excitation was at 510 nm (extinction about 0.4) and a correction

TABLE I

Change in quantum yield and relative lifetime with concentration for fluorescein in alkaline-aqueous glycerolic solution

c_M	Φ	$0.95 \left(\frac{\tau}{\tau_0} \right)$	
		(i)	(ii)
1×10^{-4}	—	0.92 ± 0.01	—
2×10^{-4}	—	0.98 ± 0.04	—
5×10^{-4}	—	0.94 ± 0.02	—
1×10^{-3}	—	0.95 ± 0.03	—
2×10^{-3}	0.946 ± 0.004	0.95 ± 0.07	—
5×10^{-3}	0.953 ± 0.004	0.95 ± 0.01	0.95 ± 0.03
1×10^{-2}	0.875 ± 0.005	0.90 ± 0.02	0.87 ± 0.04
2×10^{-2}	0.636 ± 0.008	0.65 ± 0.02	0.67 ± 0.03
5×10^{-2}	0.190 ± 0.005	—	0.18 ± 0.02
0.1	0.0435 ± 0.002	—	0.04 ± 0.015

Quantum yields determined as relative yields with totally absorbing solutions and $\Phi_0 = 0.95 \pm 0.05$ [11].

Lifetimes given as $0.95 \left(\frac{\tau}{\tau_0} \right)$ for comparison with quantum yields:

(i) determined with solutions of extinction at the absorption maximum of about 0.7

(ii) determined with totally absorbing solutions.

applied for the percentage of incident light absorbed by samples of slightly different extinctions. For concentrations of 5×10^{-3} M and above, determinations were also made in 1 mm cells with effectively complete absorption of the exciting light. A comparison standard (10^{-4} M solution in a 1 mm cell) read between each measurement varied by less than 0.5% throughout the determinations. The results calculated on the basis $\Phi_0 = 0.95$ (see Szalay and co-workers [12] and compare Seybold and co-workers [62]) and corrected for the effects of polarization (Appendices A and B) are presented in Table I and Figure 2. The onset of con-

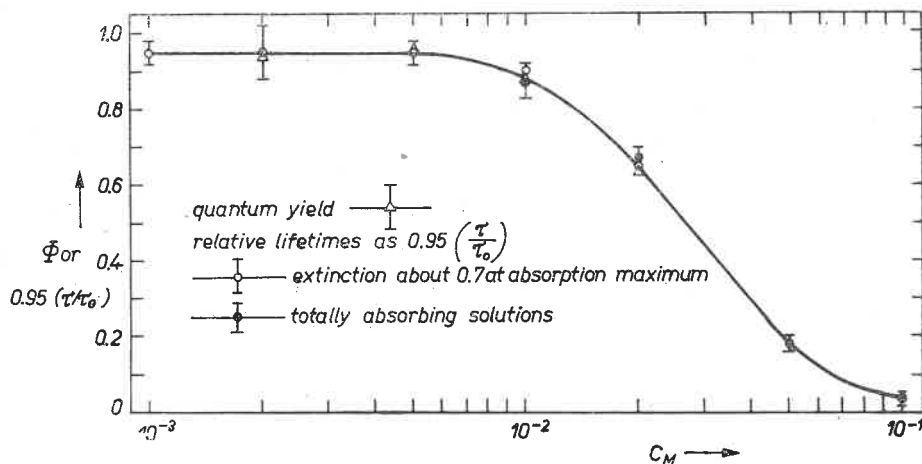


Fig. 2. Concentration quenching of fluorescein in alkaline-aqueous glycerolic solution

centration quenching coincided well with the appearance of a non-negligible fraction of dimers (non-or weakly fluorescent) inferred from the failure of Beer's law evident by a concentration of 5×10^{-3} M.

Fluorescence lifetimes determined in the range 10^{-4} to 2×10^{-2} M (thin layer cell) and 5×10^{-3} to 0.1 M (1 mm cuvettes) are also given in Table I and Figure 2 for comparison with the quantum yield results. It is seen that, within the quite large experimental error, the changes in yield and lifetime are proportional. In the lower concentration range, with an extinction of about 0.7 at the absorption maximum, the technical lifetime measured was (3.69 ± 0.05) ns.

The EA of fluorescence was determined as a function of concentration for excitation wavelengths 465 and 510 nm, the latter representing approximately the '0-0' transition⁹. The effect of secondary fluorescence was allowed for by a simple correctional procedure (see Appendix C): after correction of the experimental values for depolarization due to

⁹ The '0-0' transition is most accurately determined from a "mirrorsymmetry" plot of absorption and fluorescence as $\frac{E(\bar{\nu})}{\bar{\nu}}$ and $\frac{F(\bar{\nu})}{\bar{\nu}^3}$ vs $\bar{\nu}$ according to the relationship of Förster [36]. The data presented here in Figure 1 are $E(\lambda)$ and $F(\lambda)$ vs λ ($E(\bar{\nu}) = E(\lambda)$ and $F(\bar{\nu}) = \lambda^2 F(\lambda)$ [51]). The appropriate plot showed that the mirrorsymmetry relationship is not well followed in this case, (see also [62]) but in so far as an '0-0' transition wavelength could be assigned: $\lambda_{0-0} = 511$ nm.

the finite solid angles of excitation and observation (Appendix B), the data collected in the concentration range 2×10^{-4} to 2×10^{-2} M (extinction at absorption maximum about 0.7) was normalized to data obtained in the range 10^{-5} to 2×10^{-3} M (extinction maximum about 0.07), the factor being constant in the range of overlapping data to $\pm 0.7\%$; the range of concentration 2×10^{-3} M to 0.1 M (1 mm cuvettes, totally absorbing) was similarly normalized to the already normalized range above with about the same accuracy, giving an overall error of less than $\pm 1.5\%$ (but see *Discussion*). Limiting EA values (r_0) were determined by extrapolation of the results to zero concentration in a plot of $\left(\frac{1}{\bar{r}}\right)$ vs c_M . These plots were linear over only a part of the range of concentration examined. However, limiting EA 's of reasonable accuracy could be determined and are given in Table II. The ratios, $\left(\frac{\bar{r}}{r_0}\right)$, of the EA at a given concentration to the limiting value are given as the average for the two

TABLE II

Limiting EA values extrapolated from plots of the reciprocal of the EA vs concentration

Excitation wavelength (nm)	Filter	r_0
510 (0.0')	OC11	0.344
	OC14	0.3405
465	OC11	0.3415
	OC14	0.338

The limiting EA values are quoted to the nearest 0.0005.

filter systems (OC11 and OC14) for both $\lambda_{\text{ex}} = 465$ nm and 510 nm in Table III and, as a function of $\log_{10} c_M$ in Figures 3 and 4. The predictions of the Jabłoński¹⁰ and Förster-Ore¹¹ models (Fig. 3) and the present model (Fig. 4) are also shown for comparison. In addition, Table III gives the ratio of EA 's determined for $\lambda_{\text{ex}} = 465$ nm and 510 nm, shown also in Figure 4. The inverse ratio $\left(\frac{r_0}{\bar{r}}\right)$ is given as a function of concentration to include the range of linearity up to 2×10^{-3} M in Table IV and Figure 5.

¹⁰ Fitted to the point $\left(\frac{\bar{r}}{r_0}\right) = 0.5$ where $\frac{\nu}{2} - 1 = \exp\left(\frac{-\nu}{2}\right)$ for a physical solutions of Eq. (3) in absence of concentration quenching.

¹¹ Fitted to $\nu = 1$ ($c = c_0$ in absence of concentration quenching) at $\left(\frac{\bar{r}}{r_0}\right) = 0.46$.

TABLE III

Course of Concentration Depolarization of Fluorescein in alkaline-aqueous glycerolic solution

c_M	(i)	(ii)	(iii)	(iv)	(v)	$\left(\frac{\tau_{465}}{\tau_{510}}\right)$
1×10^{-5}	0.990 ± 0.003	0.998	—	0.997	0.983 ± 0.004	0.986 ± 0.001
2×10^{-5}	0.982 ± 0.004	0.995	—	0.994	0.972 ± 0.004	0.983 ± 0.003
5×10^{-5}	0.960 ± 0.010	0.988	—	0.985 ± 0.001	0.947 ± 0.006	0.980 ± 0.002
1×10^{-4}	0.948 ± 0.003	0.977	—	0.971 ± 0.001	0.936 ± 0.006	0.980 ± 0.003
2×10^{-4}	0.943 ± 0.008	0.955	—	0.943 ± 0.003	0.928 ± 0.007	0.974 ± 0.004
5×10^{-4}	0.876 ± 0.012	0.893	—	0.871 ± 0.006	0.867 ± 0.017	0.980 ± 0.007
1×10^{-3}	0.789 ± 0.008	0.802	—	0.776 ± 0.008	0.765 ± 0.006	0.961 ± 0.003
2×10^{-3}	0.659 ± 0.008	0.658	—	0.640 ± 0.012	0.622 ± 0.008	0.929 ± 0.004
5×10^{-3}	0.406 ± 0.008	0.412	0.410	0.408 ± 0.014	0.357 ± 0.004	0.865 ± 0.008
1×10^{-2}	0.246 ± 0.006	0.251	0.226	0.228 ± 0.014	0.197 ± 0.003	0.785 ± 0.004
2×10^{-2}	0.137 ± 0.012	0.158	0.115	0.112 ± 0.010	0.100 ± 0.007	0.697 ± 0.010
5×10^{-2}	0.109 ± 0.006	0.145	0.069	0.068 ± 0.007	0.064 ± 0.006	0.575 ± 0.022
0.1	0.136 ± 0.013	0.224	0.075	0.073 ± 0.009	0.072 ± 0.006	0.522 ± 0.012

(i) experimental data, $\lambda_{\text{ex}} = 510$ nm (approximate '0-0' transition)

(ii) active sphere model predictions according to Eq. (3)

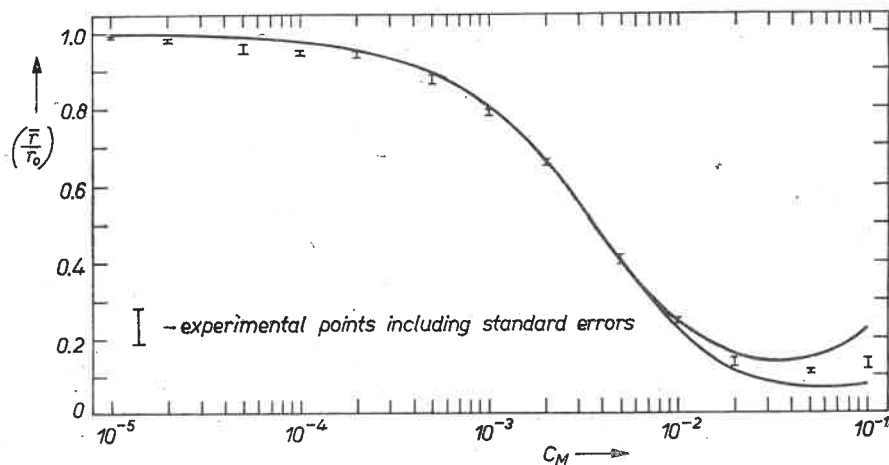
(iii) Förster-Ore model predictions with $R_0 = 45.6$ Å; values from Kawski and Kamiński [14](iv) stepwise transfer model predictions with $R'_0 = 48.8$ Å according to Eqs (17) and (19)(v) experimental data, $\lambda_{\text{ex}} = 465$ nm.

Fig. 3. Concentration depolarization of fluorescein in alkaline-aqueous glycerolic solution. Legend: upper curve: active sphere model, Eq. (3), lower curve: Förster-Ore model, Eq. (4), values taken from Kawski and Kamiński [14]

Calculations of $R_0(R'_0)$ were made using the average of overlap integrals determined from the presented absorption and fluorescence spectra as:

$$\mathcal{J}(\bar{\nu}) = \frac{\epsilon_m(\lambda_{\max}) \sum_{\lambda} \epsilon'(\lambda) F(\lambda) \lambda^4 \Delta\lambda}{\sum_{\lambda} F(\lambda) \Delta\lambda}$$

with $\Delta\lambda = 5$ nm, $\epsilon'(\lambda)$ the absorption spectrum normalized to a maximum of unity and summation carried out between 480 and 740 nm (fluorescence spectrum extrapolated from

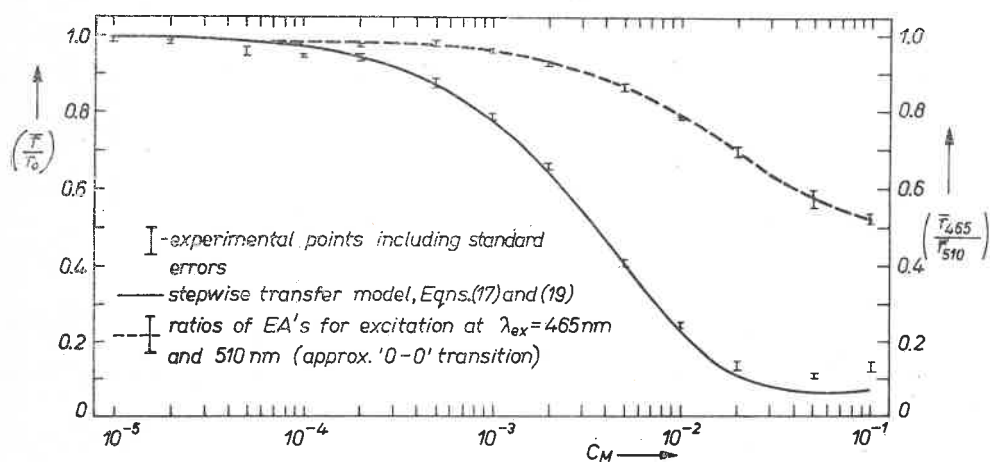


Fig. 4. Concentration depolarization of fluorescein in alkaline-aqueous glycerolic solution

TABLE IV

Course of Concentration Depolarization of Fluorescence in alkaline-aqueous glycerolic solution

c_M	$\left(\frac{r_0}{r}\right)$	
	$\lambda_{\text{ex}} = 510$ nm	$\lambda_{\text{ex}} = 465$ nm
1×10^{-5}	1.011 ± 0.004	1.015 ± 0.002
2×10^{-5}	1.018 ± 0.004	1.029 ± 0.006
5×10^{-5}	1.042 ± 0.010	1.056 ± 0.008
1×10^{-4}	1.055 ± 0.006	1.069 ± 0.009
2×10^{-4}	1.061 ± 0.018	1.079 ± 0.002
5×10^{-4}	1.144 ± 0.026	1.155 ± 0.030
1×10^{-3}	1.268 ± 0.013	1.308 ± 0.014
2×10^{-3}	1.520 ± 0.011	1.611 ± 0.047
5×10^{-3}	2.463 ± 0.056	2.806 ± 0.055

670 nm to zero at 740 nm). The value obtained was $\mathcal{J}(\bar{\nu}) = (62.2 \pm 1.6) \times 10^{-15}$ cm⁶/mmole. Φ_0 was taken as 0.95 ± 0.05 [11], the measured refractive index of the solvent was 1.465 and the R_0 value was then (45.6 ± 0.8) Å, taking the orientation factor, η^2 , as the average:

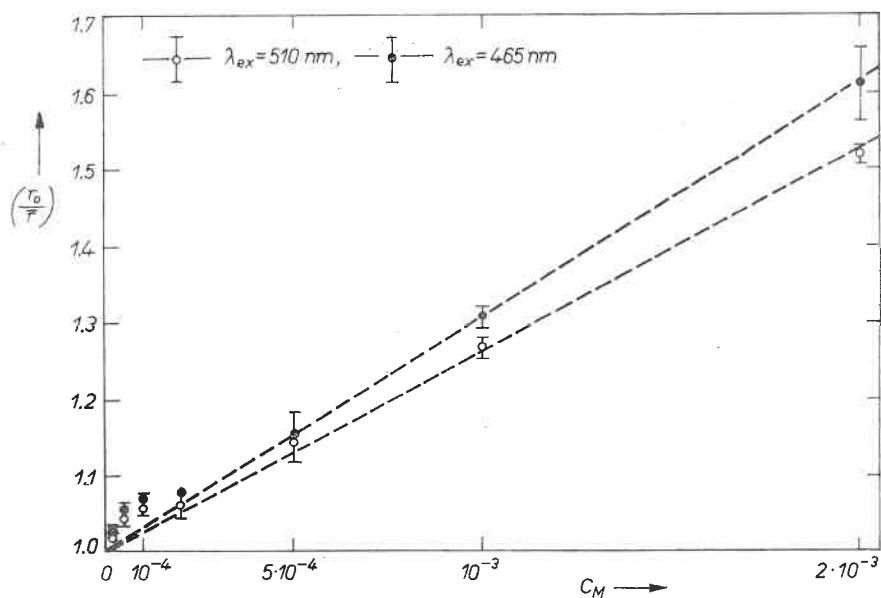


Fig. 5. Concentration depolarization of fluorescein in alkaline-aqueous glycerolic solution — reciprocal plot to indicate range of linearity and deviation at low concentrations

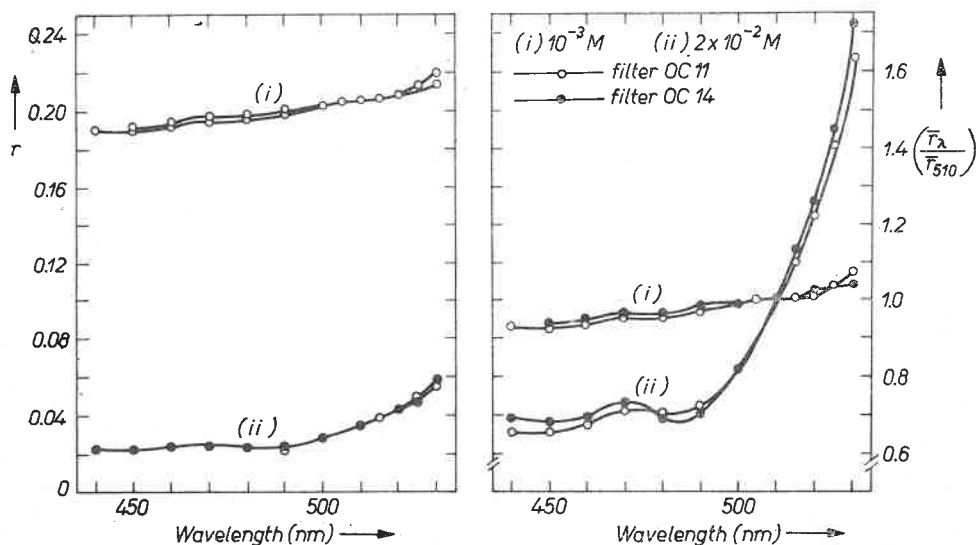


Fig. 6. Excitation polarization spectra for fluorescein in alkaline-aqueous glycerolic solution

$\bar{\kappa}^2 = \frac{2}{3}$. This R_0 value compares extremely well with that given by c_0 determined from the fluorescence depolarization results as $R_0 = (45.6 \pm 0.7) \text{ \AA}$. Data-fitting of the active sphere of Förster-Ore models to the linear portion of $\left(\frac{r_0}{r}\right)$ vs c_M gave a mean value of $R_0 = 47.5 \text{ \AA}$. This apparently small difference, however, represents almost a 30% higher (one-way) transfer rate than that given by the above value of R_0 .

Fitting the stepwise transfer model to the half-polarization point, $\left(\frac{\bar{r}}{r_0}\right) = 0.46$, gave a mean value $\alpha = 0.5936$ used in calculating the remainder of the course of concentration depolarization for this model (Table III, Fig. 4) with R_0' calculated from the spectral data and $\bar{\kappa} = 0.690$.

Excitation polarization spectra (not corrected for the influence of secondary fluorescence) for solutions of concentration 10^{-3} and 2×10^{-2} M of the same optical thickness (extinction at the visible maximum of about 0.7) are presented in Figure 6 as \bar{r} and $\left(\frac{\bar{r}}{r_{0-0'}}$) vs λ_{ex} to show clearly the "red-edge" effect of Weber [63, 64].

5. Discussion

The most striking result obtained in this work is the correspondence of R_0 values obtained independently from spectral data and from depolarization data ('0-0' excitation), respectively $(45.6 \pm 0.8) \text{ \AA}$ and $(45.6 \pm 0.7) \text{ \AA}$. This apparently justifies the relationship given by Förster [36]:

$$c_0 = \frac{3}{4\pi N R_0^3}$$

in the approximation for the course of concentration depolarization derived by Ore [1], even though the relationship is only considered as approximate and the expression derived for depolarization, Eq. (4), involves a strictly incorrect use of the average value of the orientation factor κ^2 (see Knox [9]). It is noted that the relationship between R_0 and R_f (the radius of the active sphere) derived by comparison of the Förster-Ore and Jabłoński expressions in the middle range of depolarization is thus also justified. However, there are obvious deviations in the experimental results for both low and high concentrations from the predictions of all the theoretical models presented. It is also noted that the R_0 values determined here are considerably lower than those previously derived (see particularly [12, 14]).

In the high concentration range, the stepwise transfer model presented here and the Förster-Ore model predict similar greater depolarizations than those observed, while the active sphere model results in a considerably smaller depolarization than observed. The differences between the Förster-Ore and Jabłoński models in this region are accentuated on taking into account the effect of concentration quenching. The corrected experimental data are, however, presented with some reserve for this region. Deviations from the Beer-Lambert law in concentrated solutions probably causes changes in reabsorption of fluorescence in totally absorbing solutions of different concentration, whereas the correction applied assumes a constant degree of reabsorption. Thus the correction factor calculated for the 2×10^{-2} M solutions was lower by more than 15% than that used (the average for 2×10^{-3} to 10^{-2} M solutions). This seems to imply that the corrected experimental EA 's presented are too high in the three solutions of highest concentration and therefore that the Förster-Ore or stepwise transfer models are more appropriate in this region than the active sphere model. Further technical refinement should enable a more definitive test to be made here.

In the concentration range 10^{-5} to 10^{-4} M, all model predictions agree well among themselves, but between 10^{-4} M and c_0 , the stepwise transfer model predicts rather larger depolarizations than the other two models. None, however, corresponds well with the experimental data for all $c_M \leq 10^{-3}$ M. The latter two models indicate that there should be considerably less depolarization than observed, while the stepwise transfer formulation varies between over- and underprediction. The deviations are particularly large at the lowest concentrations where all models predict, to a fair approximation, a linear change in the reciprocal of the EA with concentration. As readily seen from Table IV and Figure 5, such a relationship exists only over a quite small concentration range, and even then does not correspond with any of the models. The effect was quite reproducible and is also seen to be qualitatively similar in the data of Szalay and co-workers [11]¹². A possible interpretation of this effect is suggested as that, at very low concentrations, solvent structural changes in the vicinity of the large charged dye molecules are such as to facilitate the formation of large aggregates of "structured" solvent containing on average higher concentrations of the solute than expected from a random (Poisson) distribution, a kind of "super"-solvation shell or cage. The quite high molar aqueous content of the solvent may or may not be specifically involved in such a process. As the solute concentration rises there would come a point at which such "structured" solvent formed essentially the whole of the solvent system in which the solute would now be normally distributed. The contention from the results presented is that this occurs at about 1.5×10^{-3} to 2×10^{-3} M where the average inter-dye molecular spacing is about 100 Å. Further testing of this hypothesis would require at least investigation of polar fluorescent dyestuffs and their non-polar analogues in a range of polar and non-polar solvent systems to vary the interaction of solute and solvent medium as much as possible.

In the light of the interpretation above it is probable that the stepwise transfer model tends to overestimate depolarization in the lower and middle concentration ranges compared with the other two models. The main conclusion to be drawn from these results is that care is necessary in interpreting depolarization data in both high and low concentration regions, although the present theories are quite satisfactory in the range of depolarization:

$$0.3 < \frac{\bar{r}}{r_0} < 0.7$$

using R_0 determined from spectral data or $R_J \simeq 1.43 R_0$ in the active sphere approximation. There seems little justification in using complicated extensions of the simple active sphere model when the experimental accuracy and the approximations inherent in the model are taken into account.

The "red-edge" effect of Weber has recently been attributed to selection of molecules absorbing (and fluorescing) at longer wavelengths as a result of stronger interaction with the solvent [65]. The results presented here indicate that before-relaxation transfer may also contribute to the effect. The earlier results of Bauer [66] show even more clearly than here the correspondence of detail in the excitation polarization spectrum with vibrational detail

¹² Further confirmation of the anomalous course of concentration depolarization observed in the above work is to be found in the publications of Losev and Zenkevich [68] and of Bojarski and Dudkiewicz [69].

in absorption and are also capable of at least partial explanation by invoking this heterogeneity of solute-solvent interaction. Such heterogeneity would also cast some doubt on the validity of assigning a unique '0-0' transition wavelength and, more importantly, a unique R_0 value. In any case, in the presence of before-relaxation transfer, the unique rate constant for transfer in the case of very weak interaction does not apply and therefore the various models for concentration depolarization are also invalid (the active sphere approximation also assumes a single interaction mode) and the deviation (displayed in Table III and Figure 4) becomes very large at high concentrations.

APPENDIX A

(i) Zero setting in measurement of EA .

For excitation at an angle to the surface of the fluorescent solution, different fractions of differently polarized excitation will be transmitted through the interfaces. Regarding the excitation incident in the xy -plane and polarized at an arbitrary angle, ψ , to the vertical (z -) axis as resolved into vertical (V) and horizontal (H) components transmitted into solution, then:

$$\frac{I_V}{I_H} = \frac{A \cos^2 \psi}{\sin^2 \psi}$$

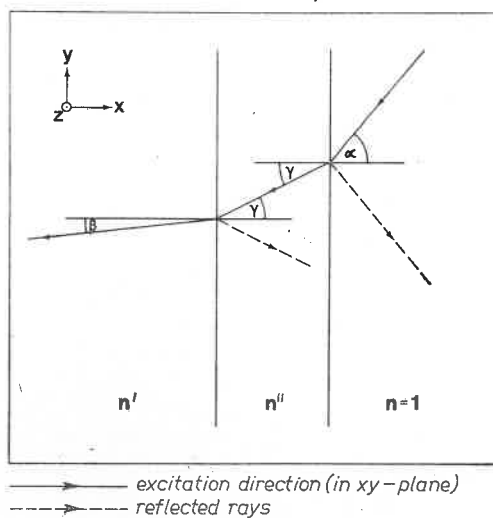


Fig. A1. Geometry of excitation at an angle to the surface of a thin layer of fluorescent solution. Observation is along the x -axis

where A is the ratio of the transmissions of vertically and horizontally polarized excitation through the interfaces. For excitation light rays incident on the air-cell interface at an angle α to the normal to the face, on the cell-solution interface at γ and leaving the cell-solution interface at β (Fig. A1), these angles being related by Snell's law to the refractive indices:

$$\sin \gamma = \frac{\sin \alpha}{n''}, \quad \sin \beta = \frac{\sin \alpha}{n'}$$

it follows from Fresnel's equations (see *e. g.* Longhurst pp. 467-469 [67]) that:

$$A = \cos^2(\alpha - \gamma) \cos^2(\gamma - \beta). \quad (\text{A1})$$

For fluorescence with emission anisotropy r , the relative intensities polarized in the z , y and x directions (the last not seen in observation along the x -axis), I_{\parallel} , I_{\perp} and $I_{\perp'}$ respectively are:

$$\begin{aligned} I_{\parallel} &= (1+2r) A \cos^2 \psi + (1-r) \sin^2 \psi \\ I_{\perp} &= (1-r) A \cos^2 \psi + (1+2r) \sin^2 \psi \cos^2 \beta + (1-r) \sin^2 \psi \sin^2 \beta \\ I_{\perp'} &= (1-r) A \cos^2 \psi + (1+2r) \sin^2 \psi \sin^2 \beta + (1-r) \sin^2 \psi \cos^2 \beta. \end{aligned}$$

Observation of zero polarization along the x -axis implies that:

$$I_{\parallel} - I_{\perp} = 0$$

so that the requirement on ψ is given by:

$$\tan^2 \psi = A \sec^2 \beta. \quad (\text{A2})$$

In the determinations carried out here, $n = 1$, $n' = 1.475$ and $n'' = 1.40$ to 1.55 (covering the range for glass or silica cells), while $\alpha = 41.2^\circ$ in the polarimeter. Thus: $\psi = (47.0 \pm 0.3)^\circ$ and the range is less than the error involved in setting the polaroid.

(ii) Relative yield correction

In measurement of the relative quantum yield for different concentrations, unpolarized emission was also used in order to eliminate the effect of selective detection of differently polarized components of the emission. A correction for the EA observed, \bar{r}_{ex} (for the given excitation geometry, see Appendix B) is then necessary to the relative intensities observed to obtain the relative total intensities:

$$\text{total intensity} = I_{\parallel} + I_{\perp} + I_{\perp'} = 3(A \cos^2 \psi + \sin^2 \psi)$$

$$\text{observed intensity} = I_{\parallel} + I_{\perp} = 2(A \cos^2 \psi + \sin^2 \psi) + \bar{r}_{\text{ex}}[A \cos^2 \psi + \sin^2 \psi(1 - 3 \sin^2 \beta)].$$

Thus:

$$\frac{\Phi}{\Phi_{\text{obs}}} = \frac{3}{2 + \bar{r}_{\text{ex}} \left[1 - \frac{3 \sin^2 \beta \sin^2 \psi}{A \cos^2 \psi + \sin^2 \psi} \right]}. \quad (\text{A3})$$

The corresponding form using the degree of polarization, \bar{p}_{ex} , is:

$$\frac{\Phi}{\Phi_{\text{obs}}} = \frac{3 - \bar{p}_{\text{ex}}}{2 - \bar{p}_{\text{ex}} \left[\frac{2 \sin^2 \beta \sin^2 \psi}{A \cos^2 \psi + \sin^2 \psi} \right]}. \quad (\text{A3a})$$

In determination of the relative yields for solutions of different polarization, the expression in terms of EA is much the simpler, *e. g.* in the apparatus used here, $\alpha = 45.5^\circ$ giving $\psi = (47.3 \pm 0.3)^\circ$ and:

$$\frac{\Phi_1}{\Phi_2} = \frac{\Phi_{1\text{obs}}}{\Phi_{2\text{obs}}} \left[\frac{0.951 + 0.632 \bar{r}_{\text{ex}2}}{0.951 + 0.632 \bar{r}_{\text{ex}1}} \right] \quad (\text{A4})$$

which changes the relative observed yield by about 8% over the range of concentration quenching.

APPENDIX B

Correction of EA for non-ideal observation and excitation geometry

The EA (or degree of polarization) excited by plane polarized light is defined for the ideal case in terms of intensity components of emission polarized parallel and perpendicular to the polarization vector of the excitation and observed in the plane at right angles to this. In practice, out-of-plane emission is invariably observed and excitation is often focussed down onto the sample giving a range of excitation directions. Both these effects depolarize the observed emission, giving a technical EA lower than that defined, the extent being determined by the particular geometry of excitation and observation.

Emission of $EA \bar{r}_{\text{ex}}$ (under ideal excitation conditions $\bar{r}_{\text{ex}} = \bar{r}$) observed at an elevation θ (out of the xy -plane) has relative intensity components:

$$I_{\parallel}(\theta) = (1 + 2\bar{r}_{\text{ex}}) \cos^2 \theta + (1 - \bar{r}_{\text{ex}}) \sin^2 \theta$$

$$I_{\perp}(\theta) = (1 - \bar{r}_{\text{ex}})$$

giving an observed EA over the range of θ considered:

$$\bar{r}_{\text{obs}} = \frac{\sum_{\theta} I_{\parallel} - \sum_{\theta} I_{\perp}}{\sum_{\theta} I_{\parallel} + 2 \sum_{\theta} I_{\perp}} = \frac{\bar{r}_{\text{ex}} \cdot \overline{\cos^2 \theta}}{1 - \bar{r}_{\text{ex}} \cdot \overline{\sin^2 \theta}} \quad (\text{B1})$$

so that:

$$\bar{r} = \frac{\bar{r}_{\text{obs}}}{\cos^2 \theta + \bar{r}_{\text{obs}} \cdot \overline{\sin^2 \theta}}. \quad (\text{B2})$$

The equivalents in terms of degree of polarization turn out to be of exactly the same form:

$$\bar{p}_{\text{obs}} = \frac{\bar{p}_{\text{ex}} \cdot \overline{\cos^2 \theta}}{1 - \bar{p}_{\text{ex}} \cdot \overline{\sin^2 \theta}} \quad (\text{B1a})$$

$$\bar{p}_{\text{ex}} = \frac{\bar{p}_{\text{obs}}}{\cos^2 \theta + \bar{p}_{\text{obs}} \cdot \overline{\sin^2 \theta}}. \quad (\text{B2a})$$

Approximating the observed emission to a conically divergent beam of homogeneous intensity and aperture 2ψ arising from a (pseudo-) point source, it can be seen from the geometry (Fig. B1) that:

$$\overline{f(\theta, \Phi)} = \frac{\int_{\theta=-\psi}^{\psi} f(\theta, \psi) \Phi \cos \theta d\theta}{\int_{\theta=-\psi}^{\psi} \Phi \cos \theta d\theta} \quad (\text{B3})$$

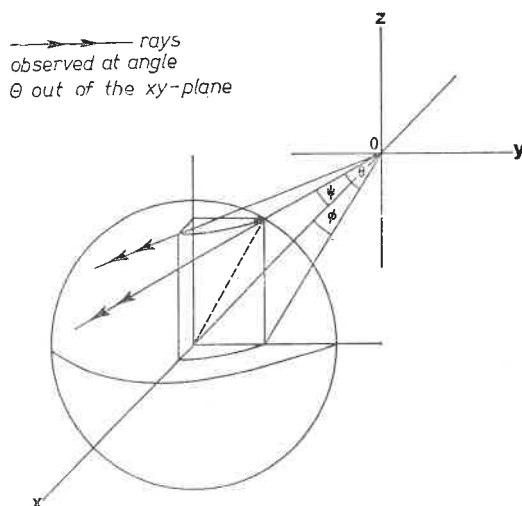


Fig. B1. Geometry of out of plane observation of fluorescence. Observation directions are centred along the x -axis

where the longitude, Φ , is given by $\sin \Phi \cos \theta = \sqrt{\sin^2 \psi - \sin^2 \theta}$ ($-\psi \leq \Phi \leq \psi$). For small angles, $\Phi \simeq \sin \Phi$ (good to 2% even at 20°) so that:

$$\overline{\cos^2 \theta} = \frac{\int_{\theta=0}^{\psi} \cos^2 \theta \sqrt{\sin^2 \psi - \sin^2 \theta} d\theta}{\int_{\theta=0}^{\psi} \sqrt{\sin^2 \psi - \sin^2 \theta} d\theta} \quad (\text{B4})$$

$$\text{while } \overline{\sin^2 \theta} = 1 - \overline{\cos^2 \theta}. \quad (\text{B5})$$

In the polarimeter used in this work, $\psi = 6^\circ 50'$ or approximately 0.12 radian (corrected from the aperture of the instrument in air for the refractive index of the aqueous glycerolic medium) so that:

$$\overline{r}_{\text{ex}} = \frac{\overline{r}_{\text{obs}}}{0.9965 + 0.0035 \overline{r}_{\text{obs}}} \quad (\text{B6})$$

with the average functions of θ calculated from Eqs (B4) and (B5) by summation using Simpson's rule with intervals of 0.01 radian.

Excitation with a beam of non-negligible convergence also causes a technical depolarization, as pointed out by Weber [26]. Rewriting his Eqs (35) for a ray of latitude θ and longitude Φ taken here from the x -axis (see Fig. B2):

$$I_{\parallel}(\theta, \Phi) = (1+2\bar{r}) \cos^2 \theta + (1-\bar{r}) \sin^2 \theta$$

$$I_{\perp}(\theta, \Phi) = (1+2\bar{r}) \sin^2 \theta \sin^2 \Phi + (1-\bar{r}) (\cos^2 \theta \sin^2 \Phi + \cos^2 \Phi)$$

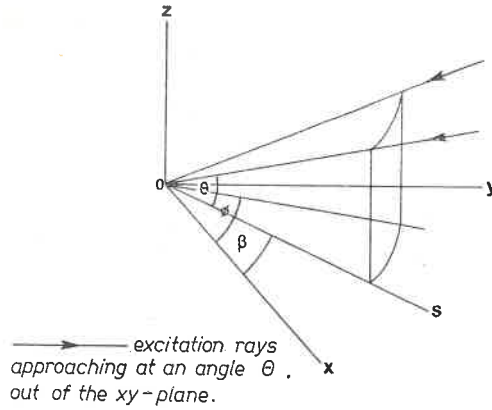


Fig. B2. Geometry of converging excitation at an angle to the surface of a thin layer of fluorescent solution. Excitation is centred along SO , observation in the x -direction

where \bar{r} is the "true" EA ($\theta \rightarrow 0, \Phi \rightarrow \frac{\pi}{2}$). The AE resulting from the sum of these components over θ and Φ is then:

$$\bar{r}_{ex} = \frac{\bar{r}[1-\sin^2 \theta (1+\sin^2 \Phi)]}{1+\bar{r}[\sin^2 \theta (2 \sin^2 \Phi - 1)]} \tag{B7}$$

Thus:

$$\bar{r}_{ex} = \frac{\bar{r}_{ox}}{[1-\sin^2 \theta (1+\sin^2 \Phi)] - \bar{r}_{ex} [\sin^2 \theta (2 \sin^2 \Phi - 1)]} \tag{B8}$$

or in terms of degree of polarization:

$$\bar{p}_{ex} = \frac{\bar{p} [1-\sin^2 \theta (1+\sin^2 \Phi)]}{1-\bar{p} [\sin^2 \theta \cdot \cos^2 \Phi]} \tag{B7a}$$

and

$$\bar{p} = \frac{\bar{p}_{ex}}{[1-\sin^2 \theta (1+\sin^2 \Phi)] + \bar{p}_{ex} [\sin^2 \theta \cdot \cos^2 \Phi]} \tag{B8a}$$

Approximating to cylindrical symmetry about the z -axis rather than spherical symmetry about 0, it can be seen from Figure B2 that:

$$\overline{f(\theta, \Phi)} = \frac{\int_{\theta} \int_{\psi} f(\theta, \Phi) \frac{d\theta}{\cos^2 \theta} d\Phi}{\int_{\theta} \int_{\psi} \frac{d\theta}{\cos^2 \theta} d\Phi} \quad (\text{B9})$$

With the exciting beam centred on OS , then:

$$\overline{\sin^2 \theta} = \frac{\int_{\theta=-\psi}^{\psi} \tan^2 \theta d\theta}{\int_{\theta=-\psi}^{\psi} \sec^2 \theta d\theta} = 1 - \frac{\psi}{\tan \psi} \quad (\text{B10})$$

and

$$\overline{\sin^2 \Phi} = \frac{\int_{\Phi=\beta-\delta}^{\beta+\delta} \sin^2 \Phi d\Phi}{\int_{\Phi=\beta-\delta}^{\beta+\delta} d\Phi} = \frac{1}{2} - \cos 2\beta \left(\frac{\sin 2\delta}{4\delta} \right) \quad (\text{B11})$$

for apertures 2ψ and 2δ in the θ and Φ senses respectively.

In the polarimeter, $\psi = \delta = 1^\circ 20'$ (corrected for refractive index of the medium), with $\alpha = 26^\circ 30'$, and $\bar{r} = \bar{r}_{\text{ex}}$ within 0.01%. In yield measurements the excitation geometry was different and the polarization correction to be applied to the relative yields dependent on \bar{r}_{ex} determined from Eq. (B7) with \bar{r} the value measured in the polarimeter and not corrected for the effect of secondary fluorescence. Here $\psi = 12^\circ 20'$, $\delta = 3^\circ 25'$ and $\beta = 28^\circ 55'$ so that:

$$\bar{r}_{\text{ex}} = \frac{0.982 \bar{r}}{1 - 0.008 \bar{r}} \quad (\text{B12})$$

Again this is a small correction here, changing the relative yield correction according to Eq. (A4) by less than 0.5%.

APPENDIX C

Normalization of AE's measured under different conditions of reabsorption of the emission

As in the case of energy transfer, the "trivial" reabsorption of emission in the body of the fluorescent solution and subsequent emission depolarizes the total emission on average. The EA observed under reabsorption regime a for a solution of given concentration may be defined as:

$$\bar{r}_a = \frac{I_{||a} - I_{\perp a}}{I_{||a} + 2I_{\perp a}} \quad (\text{C1})$$

Following Pheofilov and Sveshnikov [16], the polarized components of the emission from a solution of the same concentration but under reabsorption regime b in which a greater degree of reabsorption takes place are considered to be depolarized, the resulting components being $k_{\parallel}I$ and $k_{\perp}I$ (two components of this second value), the subscripts being related to the original polarization vector. Thus:

$$\bar{r}_b = \frac{[k_{\parallel}I_{\parallel a} + 2k_{\perp}I_{\perp a}] - [k_{\perp}I_{\parallel a} + (k_{\parallel} + k_{\perp})I_{\perp a}]}{[k_{\parallel}I_{\parallel a} + 2k_{\perp}I_{\perp a}] + 2[k_{\perp}I_{\parallel a} + (k_{\parallel} + k_{\perp})I_{\perp a}]} \quad (C2)$$

where the second component $k_{\perp}I_{\perp}$ enters for the "unseen" component $I_{\perp a}$ of regime a .

Writing $\alpha = \frac{k_{\parallel}}{k_{\perp}}$ as the coefficient of depolarization between these two reabsorption conditions gives:

$$\bar{r}_a = \frac{(1-\alpha) [I_{\parallel a} - I_{\perp a}]}{(1+2\alpha) [I_{\parallel a} + 2I_{\perp a}]} = \beta \bar{r}_a \quad (C3)$$

The argument now simplifies compared with that involving degrees of polarization given previously [16]. Since β is a constant depending only on the reabsorption conditions involved, Eq. (C3) holds for all concentrations provided there are no absorption or fluorescence spectral changes which would affect the reabsorption properties (see *e. g. Discussion* for the validity of this result in totally absorbing solutions). Under such conditions there is thus an exact linear relationship between the EA measured at one optical thickness (in particular under conditions of negligible reabsorption) and another, allowing direct comparison over a wide range of concentrations.

The same result is obtained more directly from Perrin's law of isotropic depolarization, Eq. (7), on substituting θ_m by θ_a and θ_b as the appropriate equivalent depolarizing angles for regimes a and b and eliminating r_0 between the two expressions obtained.

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