

INVESTIGATIONS ON THE CONNECTION OF POLARIZATION AND FOREIGN QUENCHING IN FLUORESCENT SOLUTIONS

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At variance with earlier results, the true degree of polarization of fluorescence (the value corrected for secondary luminescence) has been found to depend linearly on the relative quantum yield for fluorescein dissolved in water and quenched with KI. The quenching of fluorescein and tryptaflavine with KI was found to be of pure dynamic character. A formula for the connection of the degree of polarization of fluorescence and the concentration of quencher is given and experimentally proved.

Introduction

1. A study of the degree of polarization of fluorescence of solutions quenched by foreign substances may give valuable informations about the character of the quenching process. In case of dynamic quenching the increase of concentration of quencher causes a diminution of the mean life time of fluorescence, consequently a decrease of the rotational depolarization and thus an increase of the degree of polarization, while in case of static quenching the degree of polarization does not depend on the concentration of quencher.

According to F. PERRIN [1] the degree of polarization of *unquenched fluorescence*, p_0 , may be given by the equation

$$\frac{1}{p_0} - \frac{1}{3} = \left(\frac{1}{p_\infty} - \frac{1}{3} \right) \left(1 + \frac{kT}{V\eta_v} \tau_0 \right), \quad (1)$$

where p_0 , k , T , τ_0 , V and η_v denote the limiting polarization ($p_0 \rightarrow p_\infty$ if $\eta_v \rightarrow \infty$ or $\tau_0 \rightarrow 0$), the Boltzmann-constant, the absolute temperature of the solution, the mean life time of the excited state, the molar volume of the solute molecule (including the solvent layer) and the viscosity of the solution, respectively. For the degree of polarization of quenched fluorescence of solutions, p , the following equations hold:

$$\frac{1}{p} - \frac{1}{3} = \left(\frac{1}{p_0} - \frac{1}{3} \right) \left(1 + \frac{kT}{V\eta_v} \tau \right), \quad (2)$$

or, provided T , V and η_v remain unchanged during the quenching process,

$$\frac{1}{p} - \frac{1}{p_\infty} = \left(\frac{1}{p_0} - \frac{1}{p_\infty} \right) \frac{\tau}{\tau_0}, \quad (3)$$

where τ denotes the mean life time of the excited state for quenched fluorescence. When $\tau/\tau_0 = \eta/\eta_0$ (η/η_0 is the relative yield of fluorescence),

$$\frac{1}{p} - \frac{1}{p_\infty} = \left(\frac{1}{p_0} - \frac{1}{p_\infty} \right) \frac{\eta}{\eta_0}, \quad (4)$$

thus $1/p$ is a linear function of η/η_0 .

2. Experiments showed that the linearity of the function $1/p = f(\eta/\eta_0)$ is fulfilled for fluorescein [2], fluorescein and rhodamin B [3] in glycerol-water mixture, fluorescein [1], rhodamin B [4] in water quenched by KI, but it is not valid for rhodamin B and riboflavine [5] in water quenched by hydrochinone, 3-amino-pyrene-8, 10-trisulfonate [6] in water quenched by different organic compounds and for fluorescein [7–8] in water quenched by KI. The divergence from linearity may be explained by assuming a mixed type of quenching, in which case $\tau/\tau_0 \neq \eta/\eta_0$, thus eq. (4) should not hold. In [2], [3], [8] and [9] different relations to be substituted into eq. (3) are given instead of $\tau/\tau_0 = \eta/\eta_0$, in [7] a new equation was derived to explain the non-linearity observed.

A comparison of experimental data obtained by different authors for fluorescein in water quenched by KI exhibits comparatively large deviations which should justify the assumption of contradictory quenching mechanisms (Fig. 1). In [7] the law of decay was supposed to be non-exponential, in [9] an initial static quenching was considered, in [1] an exponential law of decay and the role of BROWNIAN rotation was taken into account.

The aim of the present work was to repeat with greater accuracy the investigations about the system mentioned and to carry out further experiments allowing to draw some conclusions concerning the relation between the degree of polarization of fluorescence and concentration of quencher in the systems investigated.

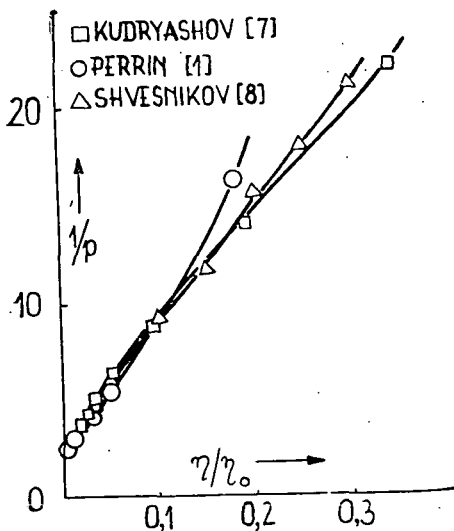


Fig. 1

Experimental

1. The degree of polarization of fluorescence has been measured by a photoelectric apparatus and a method described in [10] at a temperature of 30.0 ± 0.1 °C. The excitation of the fluorescence of fluorescein and tryptaflavine was carried out on a wavelength of 436 m μ and 460 m μ , respectively.

The temperature of the samples was maintained by a Höppler ultrathermostat and measured by means of a thermistor (2TH65) attached to the wall of the sample holder. The viscosities of the samples were measured by a Höppler viscosimeter at a temperature of 30.0 ± 0.1 °C and were found to be practically independent of the concentration of quencher, showing that the viscosity plays no part in changing the degree of polarization of fluorescence with the concentration of quencher.

The degree of polarization of fluorescence obtained by measurement (p') was corrected for secondary luminescence using the equation

$$p = \frac{p'}{1 - \kappa(1 - 0,6p')}$$

given in [11], where p denotes the true degree of polarization and κ ($\equiv S/P$, the ratio of the intensities of secondary and primary luminescences) is a quantity given in [12] as a function of the exciting wavelength, the wavelength of observation, and the yield of fluorescence. The spectra for calculating κ were determined by means of a photoelectric spectrophotometer (Optica Milano CF-4).

2. The systems investigated were fluorescein dissolved in NaOH of pH=12,5 in a concentration of $2,5 \cdot 10^{-4}$ mole/l and quenched with KI, tryptaflavine dissolved in ethanol containing $3 \cdot 10^{-3}$ mole/l acetic acid in a concentration of $1 \cdot 10^{-3}$ mole/l and quenched with hydrochinone.

The absorption spectra of all solutions were found to be independent of the concentration of quencher in accordance with earlier statements [13] for the case of fluorescein.

Discussion

1. As the absorption and emission spectra of the solutions do not change, the degree of polarization of fluorescence however, increases with the increase of the concentration of quencher, the quenching may be either of dynamic or mixed type. In order to decide which mechanism is to be made responsible for the quenching, the slope of the function $1/p = f(\eta/\eta_0)$ is to be examined. In [14] η/η_0 is given as a function of the concentration of quencher for fluorescein solutions, in [15] this value is given for tryptaflavine solutions. Making use of these data $1/p$ is represented as a function of η/η_0 in Fig. 2. The function $1/p = f(\eta/\eta_0)$ is linear in both cases, thus the quenching is of dynamic type in the studied range of concentration of dyestuff and Eq. (4) is valid for both systems. This means, further that, though conclusions based on the nonlinearity in case of fluorescein (see [1], [7] and [9]) may, in general, be acceptable, they are not reliable for the case in question.

2. Several papers dealing with the connection of luminescence of solutions and the dielectric relaxation of solvent [17] were recently published. As the dipole moment of an excited luminescent particle differs from that of an unexcited one, a rearrangement of the solvent molecules around a solute particle should take place when the latter goes over to the excited state. During this rearrangement, however, not only the solvent but also the solute particles should rotate. The rotation of the solute particles results in a rotational depolarization of fluorescence. The dielectric relaxation time of the solvent may be given by the DEBYE equation

$$\tau' = \frac{4\pi r'^3 \eta_v}{kT} = \frac{3V' \eta_v}{kT}, \quad (5)$$

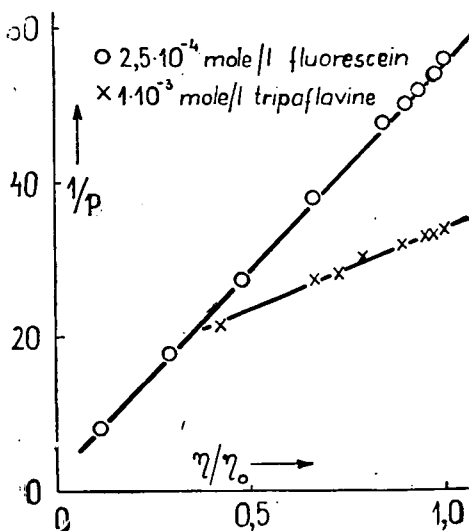


Fig. 2

which on substituting into Eq. (2) leads to the relation

$$\frac{1}{p} - \frac{1}{3} = \left(\frac{1}{p_\infty} - \frac{1}{3} \right) \left(1 + 3 \frac{V'}{V} \frac{\tau}{\tau'} \right), \quad (6)$$

where $V = 4\pi r^3/3$ and $V' = 4\pi r'^3/3$ are the volumes of the solute and solvent particles (considered as spheres). If $\tau/\tau_0 = \eta/\eta_0$ then

$$\frac{1}{p} - \frac{1}{3} = \left(\frac{1}{p_\infty} - \frac{1}{3} \right) \left(1 + 3 \frac{V'}{V} \frac{\tau_0}{\tau'} \frac{\eta}{\eta_0} \right). \quad (7)$$

In case of the function $1/p = f(\eta/\eta_0)$ being linear, the constant $V'\tau_0/V\tau'$ can be obtained by experiment. It is to be noted that the validity of Eq. (6) is restricted to cases in which Eq. (5) holds, *i. e.* — among others — V' must be very small compared to V^1 .

In [14] it has been shown that the concentration dependence of the relative yield (at least for the case of fluorescein dissolved in water and quenched with KI) can be given by the JABLOŃSKI-equation

$$\frac{\eta}{\eta_0} = \frac{1 - e^{-v}}{v} = \frac{1 - e^{-N'vc_q}}{N'vc_q}, \quad (8)$$

where $N' = 6,02 \cdot 10^{20}$ and v (the volume of an effective sphere) is an empirical constant. On putting (8) into Eq. (7) we obtain a connection between the degree of polarization of fluorescence (p) and the concentration of quencher (c_q):

$$\frac{1}{p} - \frac{1}{3} = \left(\frac{1}{p_\infty} - \frac{1}{3} \right) \left(1 + 3 \frac{V'}{V} \frac{\tau_0}{\tau'} \cdot \frac{1 - e^{-N'vc_q}}{N'vc_q} \right). \quad (9)$$

As Fig. 3 shows, the theoretical curves calculated by Eq. (9) and drawn by solid line it can be fitted to the experimental points fairly well. Curves (a) and (b) represent the values for fluorescein and tryptaflavine respectively. In case of fluorescein the following data were used for calculating $p_\infty/p = 2,748$; $V'\tau_0/V\tau' = 7,6$; $v = 2,94 \cdot 10^{-20} \text{ cm}^3$ (after [14]). According to fluorometric measurements $\tau_0 = 4,0 \cdot 10^{-9} \text{ s}$ [19]; the radius of the water molecule $r' = 1,36 \text{ \AA}$ (from [20], p. 88), thus $V' = 10,53 \cdot 10^{-24} \text{ cm}^3$; the volume of the fluorescein anion (including its hydrate envelope) $V = 58,9 \cdot 10^{-23} \text{ cm}^3$ (from a molar volume of 480 cm^3 given in [16]). These data give a dielectric relaxation time $\tau' = 0,99 \cdot 10^{-11} \text{ s}$ for water. According to [21] (p. 384) $\tau' = 0,96 \cdot 10^{-11} \text{ s}$ can be obtained by direct measurement of dielectric relaxation time. This value is in a very good agreement with that obtained by the measurement of degree of polarization of fluorescence².

In case of tryptaflavine $V'\tau_0/V\tau' = 1,18$ and $v = 0,76 \cdot 10^{-20} \text{ cm}^3$ [15]. In [16] the molar volume of tryptaflavine in ethanol with its solvate envelope is given to be

¹ In [18] similar considerations leading to an equation like Eq. (6) are given, though the conclusions drawn are somewhat different.

² The value of the molar volume of fluorescein is 247 cm^3 without hydration; using this value we obtain for $\tau' = 1,9 \cdot 10^{-11} \text{ s}$ which differs from $0,96 \cdot 10^{-11} \text{ s}$ to a high extent. It seems obvious, that in this phenomenon the solute particle must be taken into account together with its solvent envelope.

about 970 cm^3 . According to this value $V = 1,20 \cdot 10^{-21} \text{ cm}^3$, $V' = 7,19 \cdot 10^{-23} \text{ cm}^3$ (calculated from the molar weight and density of ethanol), $\tau_0 = 4,0 \cdot 10^{-9} \text{ s}$ [19]. For the dielectric relaxation time of ethanol we obtain a value of $\tau' = 20,3 \cdot 10^{-11} \text{ s}$.

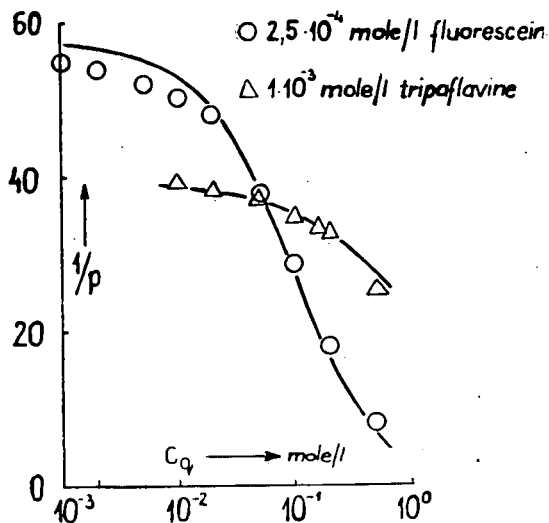


Fig. 3

In [21] (p. 348) $\tau' = 14,4 \cdot 10^{-11} \text{ s}$ is given from direct measurements. The agreement of these two values is not so good as that of the values for fluorescein, but considering the uncertain values of the volumes used for the calculation of τ' a better agreement may hardly be expected. In any case it would be very interesting to investigate further systems, especially of a type in which the DEBYE-equation surely does not hold. For us it seems very probable that even in these systems the dielectric relaxation time — as local characteristic of solvent — plays a deciding role in rotational depolarization, in which case, not the macroscopic, but the local viscosity is known to be important.

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ИССЛЕДОВАНИЯ О СВЯЗИ СТЕПЕНЬ ПОЛЯРИЗАЦИИ И ТУШЕНИЯ ПОСТОРОННЕГО ВЕЩЕСТВА ФЛУОРЕСЦЕНЦИРУЮЩИХ РАСТВОРОВ

Л. Салаи и Л. Соллоши

Потушенных KI-ом растворов флуоресцеина и трипропана тушение имеет динамический характер. Для степени поляризации флуоресценции растворов в зависимости от концентрации тушителя дано формула и контролирована экспериментально.

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