ROTATIONAL DEPOLARIZATION OF FLUORESCENCE OF DYE SOLUTIONS

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The degree of fluorescence polarization has been measured for dilute solutions of fluorescein, eosin and trypaflavine in glycerol-water, glycerol-ethanol, and glycerol-water-ethanol mixtures as a function of solvent composition. The non-linear variation of $1/p$ with reciprocal viscosity observed for eosin and trypaflavine is explained in terms of a dependence of molar volume on solvent composition assuming spherical symmetry for eosin and ellipsoidal symmetry with an axis ratio of 0,53 for trypaflavine.

Introduction

Rotational depolarization of fluorescence is observed as a viscosity (η) and temperature (T) dependence of the degree of polarization (p) in dilute viscous solutions [1—3]. The decrease of p with increasing T/η is ascribed to an increase in the thermal rotation of fluorescing particles [1,4—11]. For excitation by linearly polarised light, the degree of polarization of fluorescence emitted by spherically symmetrical species is given by

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{RT\tau}{V\eta}\right)$$

(1)

where p_0, V and τ denote the fundamental polarization, the molar volume of the solute together with the solvate shell and the mean lifetime of the excited state, respectively.

The non-linear dependence of $1/p$ on $1/\eta$ observed for several systems [12—16] has been attributed either to the asphericity of the luminescent particles, or the invalidity of the laws of Brownian rotation at low viscosity or solvation effects. If torsional vibrations of the molecules are taken into account the appropriate from of Eq. (1) becomes [17][1]

$$\frac{1}{P} - \frac{1}{3} = \left[\frac{1}{P_0} - \frac{1}{3}\left(1 + \frac{2a}{\eta}\right)\right]\left(1 + \frac{RT\tau}{V\eta}\right),$$

(2)

where $a \gtrsim 0$ is a constant to be fitted to the experimental curves.

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[1] In [17] Eq. (2) is given in terms of emission anisotropy $r = 2p/(3-p)$ in form of $1/r = (1 + RT\tau/V\eta)(r_0 - a/\eta)$.

Though several theories have been developed for the rotational depolarization of fluorescence of ellipsoid-molecules [10, 18], an expression containing a minimum number of parameters and claimed to be of general validity is given only in [16]. When the transition moments of absorption and emission are parallel and the moments lie in the symmetry axis of the luminescent particle

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_0} - \frac{1}{3}\right)(1 + 6D_x\tau), \tag{3}$$

where $D_x \equiv RT\Phi_x(\gamma)/8V\eta$, and $\Phi_x(\gamma)$, a shape factor, is a given function of $\gamma \equiv b/a$ (b and a denote the axes of the ellipsoid). If the transition moments are perpendicular to the symmetry axis of the luminescent particle

$$\frac{1}{P} - \frac{1}{3} = \frac{20}{\dfrac{3}{1+6D_x\tau} + \left[\dfrac{20}{\dfrac{1}{P_0} - \dfrac{1}{3}} - 3\right]\dfrac{1}{1+2(2D_z+D_x)\tau}}, \tag{4}$$

where $D_z \equiv RT\Phi_z(\gamma)/8V\eta$ and $\Phi_z(\gamma)$ is another shape factor. (For spherical particles $\Phi_x(\gamma) = \Phi_z(\gamma) = 4/3$ and Eq. (4) is identical with Eq. (1)).

A consideration of fluorescence reabsorption and secondary emission effects has led to the relationship [20]

$$P = \frac{P'}{1 - \varkappa(1 - 0,6p')} \tag{5}$$

between the true (p) and measured (p') degree of polarization, where $k_L \equiv S/P$, the ratio of the intensities of secondary (S) and primary (P) luminescence, is given as $\varkappa = \int_0^\infty \gamma_F(\lambda) f(\lambda) M d\lambda$; $\gamma_F(\lambda)$ and $f(\lambda)$ are the absolute quantum yield and the normalized emission quantum spectrum, and M a function defined in [21]. M depends on the absorption coefficients of the solution. Eq. (1) may be rewritten by means of Eq. (5) to give

$$\frac{1}{P'} = \frac{1}{P_0'}\left(1 + \frac{RT\tau}{V\eta}\right) + \left[\frac{0,6\varkappa - \dfrac{1}{3}}{1 - \varkappa}\right]\left[\frac{RT\tau}{V\eta}\right], \tag{6}$$

which for $\varkappa = 0$ (in the absence of secondary luminescence) is identical with Eq. (1). If, however, $\varkappa \neq 0$ and is viscosity dependent (owing to the change in overlap of absorption and emission spectra with the viscosity), the functions $1/p = f(1/\eta)$ and $1/p' = f'(1/\eta)$ may differ considerably [19].

The aim of the present paper is to examine the theories outlined above in their application to experimental results obtained for typical fluorescent solutions.

Experimental

The rotational depolarization of fluorescence of fluorescein, trypaflavine and eosin in glycerol-water, glycerol-ethanol and glycerol-ethanol-water mixtures has been studied at a dye concentration of 2.10^{-4} mole/l and a temperature of 30.0 ± 0.1 centigrade maintained by a Höppler-ultrathermostat. The purity of the dyestuffs was checked by absorption measurements and by the independence of the peak wavelength emission on exciting wavelength in the region of 275—475 nm, 370—480 nm and 470—515 nm for solutions of fluorescein, trypaflavine and eosin, respectively. The absorption and emission spectra were recorded on an Optica Milano CF4 spectrophotometer. The photoelectric apparatus used for measuring the degree of polarization and the method of measurements are described in [25]; in all cases practically the whole long wave region of the emission was observed by use of appropriate complementary filters, the excitation wavelengths being 436 nm (for fluorescein) and 500 nm. The layer thickness of the samples was 0.633 cm and viscosities were measured using a Höppler-viscosimeter. The solutions of fluorescein, trypaflavine and eosin contained 1 per cent NaCH, 2 per cent acetic acid and 5.10^{-3} mole/l NaCH, respectively. True degrees of polarization were obtained from Eq. (5).

Results and Discussion

As shown in Fig. 1, $1/p$ varies linearly with $1/\eta$ in accordance with Eq. (1) for the solutions of fluorescein examined; deviations from linearity reported by various authors are attributed to the neglected effects of secondary luminescence [19]. For this solute the application of Eq. (2) requires that $a = 0$, i.e. the depolarising effect of torsional vibrations is negligible, moreover it is not necessary to invoke
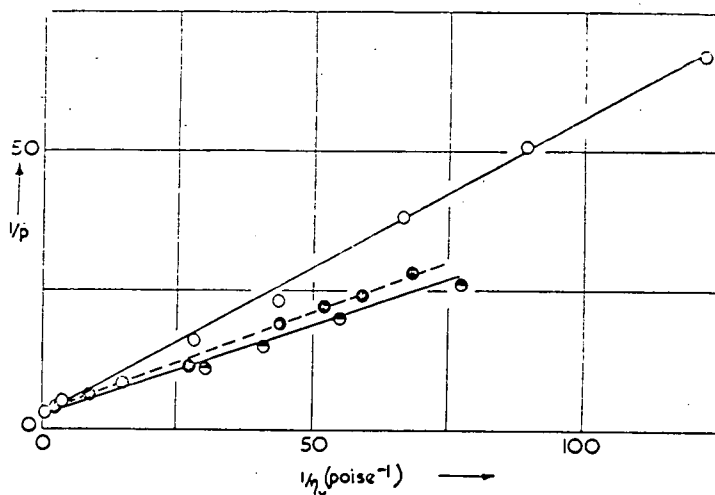

Fig. 1. Rotational depolarization of fluorescence of fluorescein in glycerol-water (○), glycerol-ethanol (●) and glycerol-ethanol-water (◔) mixtures. Solid line calculated from Eq. (3)

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solute hydration previously introduced to accoung for the non-linear variation of $1/p$ with $1/\eta$ [13].

The non-linear dependence of $1/p$ on, $1/\eta$ shown in Fig. 2, for eosin may be attributed either to a dependence of τ (due to O_2 quenching) or of V on solvent composition. If the variation of τ with viscosity is expressed by [24]

$$\frac{1}{\tau} = \frac{1}{\tau_0} + \frac{8RT[O_2]}{3000\eta} \tag{7}$$

where τ_0 is the lifetime of the unquenched excited molecule. The concentration of dissolved oxygen $[O_2]$ required to account for the magnitude of the observed effects is $\sim 10^{-1}$ M which exceeds by far the equilibrium concentration of $\sim 10^{-3}$ M reported for air-saturated ethanol in this temperature region; moreover it was found that the intensity of fluorescence of both eosin and trypaflavine in outgassed ethanolic solutions was reduced by less than 10 per cent when these solutions were saturated with air.

The molar volume V of the solute in solution is given by [23]

$$V = Cw + V_s, \tag{8}$$

where w and V_s denote the molar volume of solvent and solid solute, respectively, and the constant C has a value of 16,9 in the system examined. With a molecular weight of 648 and a density of 2.2 g/cm³ measured picnometrically for a chloro-
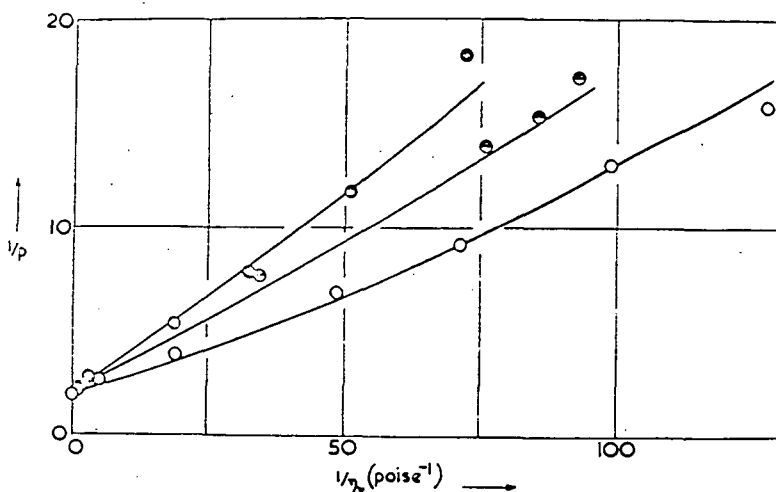

Fig. 2. Rotational depolarization of fluorescence of eosin
in glycerol-water (o), glycerol-ethanol (●) and glycerol-ethanol-water
(☺) mixtures. Solid line calculated from Eq. (3)

benzene suspension, $V_s = 295$ cm³ for eosin. Values of V computed from (8) are substituted in Eq. (1) to obtain the solid curves drawn in Fig. 2.

Values of τ of 4.0×10^{-9} s and 3.6×10^{-9} s have been reported for trypaflavine dissolved in water and glycerol, respectively [22], and quenching by dissolved air

reduces the fluorescene intensity of an ethanolic solution by less than 10 per cent. This solvent dependence of τ is insufficient to account quantitatively for the non-linear dependence of $1/p$ on $1/\eta$ shown for trypaflavine in Fig. 3. The solid curves drawn in this Figure are in accordance with Eq. (3) with $\Phi_x(\gamma) = 1,2$ and values of V obtained from equation (8) with $V_s = 185$ cm³ satisfactorily express the observed viscosity dependence of $1/p$.
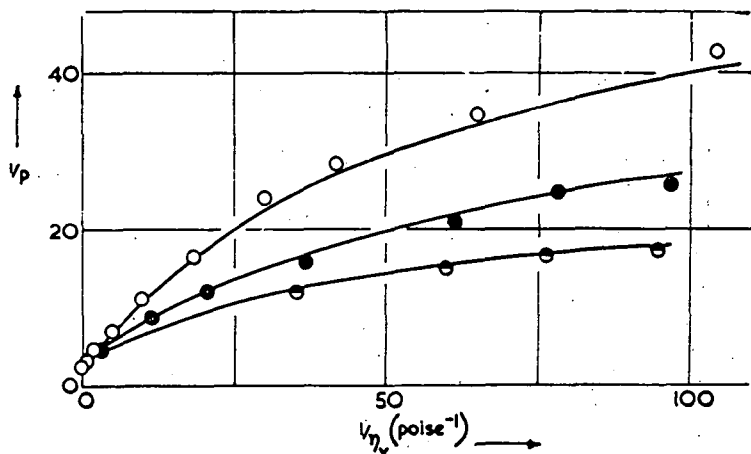

Fig. 3. Rotational depolarization of fluorescence of trypaflavine in glycerol-water (o), glycerol-ethanol (●) and glycerol-ethanol-water (◉) mixtures. Solid line calculated from Eq. (3)

Under the conditions of excitation and emission involved, the same electronic levels for the ions examined and Eq. (3) should be valid; this is confirmed by the data plotted in Figs. (4—6), where the solid lines are drawn according to Eq. (3)
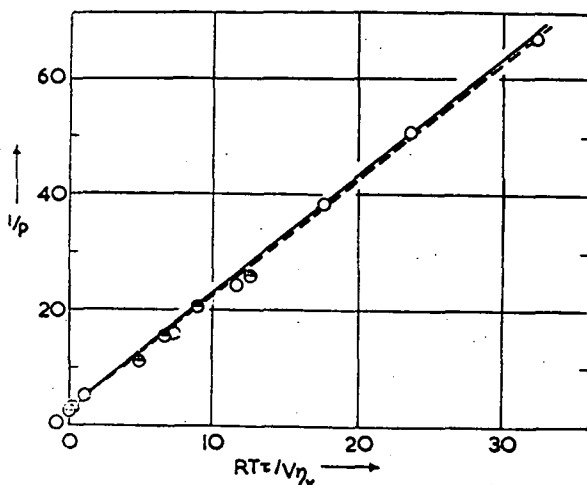

Fig. 4. The validity of Eq. (3) for fluorescein. Solid line calculated from Eq. (3), with $1/p_0 = 2,262$; $\Phi_x(\gamma) = 1,33$; $\tau = 5,05$ ns, $V = 479$ cm³ (for glycerol-water mixtures), $\tau = 4,48$ ns, $V = 780$ cm³ (for glycerol-ethanol-mixtures). Broken line is least square's plot of experimental data

with $\Phi_x(\gamma)=1,33$ for fluorescein and eosin and $\Phi_x(\gamma)=1,2$ for trypaflavine, and the broken lines represent least square plots of the experimental points. Since $\Phi_x(\gamma)=1,33$ corresponds to $\gamma=1$, fluorescein and eosin exhibit spherical symmetry with respect to rotational depolarization of fluorescence. In the case of trypaflavine on the other hand, the value of $\gamma=0,53$ (or 1,90) corresponding to $\Phi_x(\gamma)=1,20$ [16] is close to that expected for an ellipsoid of axes 9,5 Å and 5,2 Å
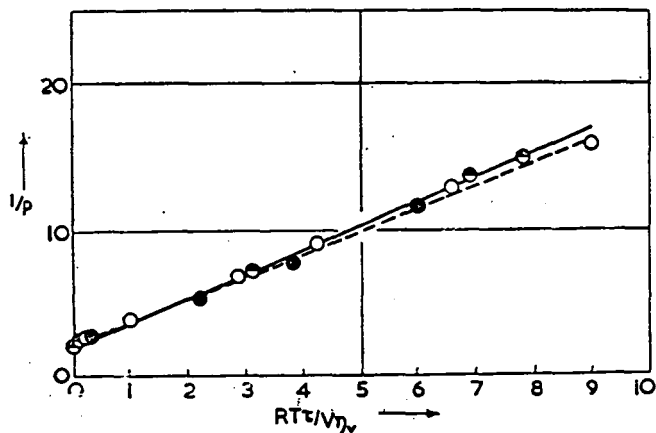

Fig. 5. The validity of Eq. (3) for cosine. Solid line calculated from Eq. (3), with $1/p_0=2,04$; $\Phi_x(\gamma)=1,33$; $\tau=4,7$ ns; V calculated from (8). Broken line is least square's plot of experimental data
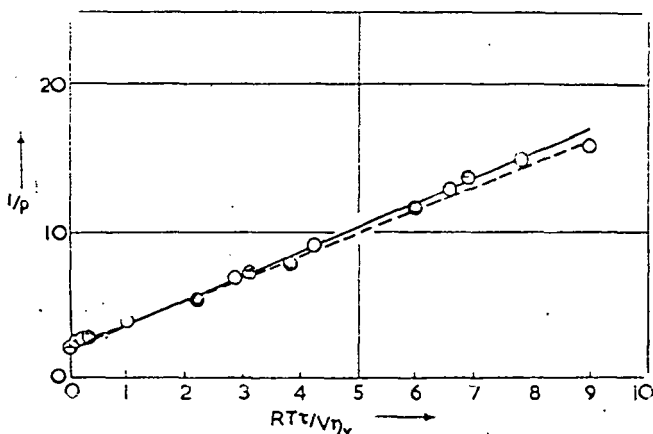

Fig. 6. The validity of Eq. (3) for trypaflavine. Solid line calculated, from Eq. (3) with $1/p_0=2,15$; $\Phi_x(\gamma)=0,53$; $\tau=3,91$ ns, V from (8). Broken line is least square's plot of experimental data

obtained from a model of this cation [26]; it is not possible, however, to correlate the transition moment with a particular molecular axis from such measurements since $\Phi_x(\gamma)$ has the same value for a long or a short axis polarized transition.

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РОТАЦИОННАЯ ДЕПОЛЯРИЗАЦИЯ ФЛУОРЕСЦЕНЦИИ ВЯЗКИХ РАСТВОРОВ

Л. Салаи и Л. Визе

Измерялось степень поляризации флуоресценции трипофлавина, эозина и флуоресценина в зависимости от состава растворителя в смесях глицерин-вода, глицерин-спирт и глицерин-вода-спирт. Отсутствие линейного изменения обратного значения степени поляризации от обратного значения вязкости объясняется тем, что молекулярный объём со сольватной оболочкой флуоресцирующих молекул зависит от состава растворителя. Форма частицы эозина сферическая, а трипофлавина эллипсоид с отношением осей 0,53.