# Influence of Quenching upon the Rotational Depolarization of Fluorescence*

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Z. Naturforsch. **40 a,** 313 – 316 (1985); received September 29, 1984

The theory of the rotational depolarization of fluorescence taking into account the inertial effect was verified experimentally on the example of 2,2′-p-phenylenebis(5-phenyloxazole) (POPOP), 2,2′-p-phenylenebis(4-methyl-5-phenyloxazole) 2MPOPOP), p-bis[2-(5-α-naphthyloxazolyl)]-benzene (α-NOPON), diphenylenestilbene (DPS) and 1,6-diphenylhexatriene (DPH) in cyclohexane by the method of quenching with nitrobenzene. The volumes obtained for the centers and moments of inertia are lower than those obtained by the use of solvents of different viscosities, but they still refer to the solvated molecules.

## 1. Introduction

The influence of quenching upon the rotational depolarization of the fluorescence of solutions was an object of numerous investigations [1, 2]. In the case of dynamic quenching, the decrease of the fluorescence lifetime $\tau$ with growing quencher concentration results in an enhancement of the fluorescence anisotropy (FA) $r$**. For the correlation between $\tau$ and $r$ Levshin and Perrin [3, 4] have given the classical equation

$$\frac{r_0}{r} = -1 = \frac{kT}{V\eta} \cdot \tau \tag{1}$$

($\eta$ the viscosity of the solvent, $V$ the effective volume of the luminescence center).

In the case of static quenching, however, $\tau$ and $r$ are independent of the quencher concentration.

Equation (1) can be transformed to a linear relation between $r_0/r$ and $\varphi/\varphi_0$, setting $\tau/\tau_0 = \varphi/\varphi_0$ ($\varphi/\varphi_0$ is the relative quantum yield). Experimentally observed deviations from the linearity were ascribed to mixed types of quenching or nonexponential decays of the fluorescence [5 – 8].

For short lifetimes, the inertial effect influences essentially the decay of the fluorescence anisotropy [9 – 11].

The aim of the present paper is the experimental verification of our theory [10, 11] for the fluorescence quenching of selected elongated molecules in a solvent. We used nitrobenzene as a suitable quencher with a diffusion controlled dynamical quenching mechanism [12].

## 2. General Bases

A more generalized consideration of rotational diffusion [10, 11, 13] gives the relation

$$\frac{\tau^2}{r_0/r - 1} = \frac{I}{6kT}\left(1 + \frac{\tau}{\tau_R}\right) \tag{2}$$

with the rotational relaxation time $\tau_R = I/6V\eta$. $I$ is the effective moment of inertia, $r_0 = \frac{1}{5}(3\cos^2\beta - 1)$ the fundamental emission anisotropy; $\beta$ denotes the angle between the dipole transition moments of absorption and emission. In the case under investigation $\beta = 0$, i.e. the excitation takes place in the region of the 0 – 0 transition.

If $\tau/\tau_R \gg 1$, relation (2) becomes (1).

Already Pringsheim [14] has found that in the Levshin-Perrin equation [3, 4] the $\tau$ values cannot be assumed identical in solvents with different viscosities, as confirmed in our previous papers [15 – 17] for a series of luminescent substances. Therefore, to obtain a more reliable information on $V$ and $I$, one should use (2) instead of (1). Thus, simultaneous measurement of $r$ and $\tau$ of the molecule investigated in a certain solvent is indispensable.

Moments of inertia $I$ so far measured using formula (2) for several luminescent compounds in solvents of different viscosities and polarities are given

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* Carried out under Research Project MR.I.5.

* * The FA is determined by $r = \frac{(J_\parallel - J_\perp)}{J}$, where $J = J_\parallel + 2J_\perp$ is the total fluorescence intensity; $J_\parallel$ and $J_\perp$ designate the components of the fluorescence intensity parallel and perpendicular to the direction of the electric vector of the exciting light.

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in Table 1 together with the values of $I$ calculated by geometrical considerations. Obviously, the geometrically calculated $I$ values differ considerably from those obtained experimentally. The differences are due to the solvent-solute interactions. Evidently the luminescent molecule is "coated" with solvent molecules forming the so-called "solvation shell". In order to avoid, even in part, this solvation effect one should investigate the behaviour of the luminescent molecules in a weakly interacting solvent.

To achieve this, cyclohexane has been selected as solvent and the mean lifetime $\tau$ of the luminescent molecules has been changed by adding nitrobenzene in various concentrations as quenching substance.

## 3. Experimental

### a) Substances

The fluorescent substances: 2,2′-p-phenylenebis(5-phenyloxazole) (POPOP), 2,2′-p-phenylenebis(4-methyl-5-phenyloxazole) (2MPOPOP), p-bis[2-(5-α-naphthyloxazolyl)]-benzene (α-NOPON), disphenylenestilbene (DPS) and 1,6-diphenyl-hexa-1,3,5-triene (DPH) were investigated in cyclohexane (spectrograde). Nitrobenzene was used as quencher.

### b) Fluorescence anisotropy measurements

FA measurements were carried out by means of the measuring apparatus shown in Figure 1. The exciting light, after passing a monochromator $M$ and a modulating system consisting of crossed Glan prims $G_1$, $G_2$ and a quartz modulator $Q$ (the modulation factor is close to 100%, the modulation frequency $f = 105.26$ kHz) falls on a cuvette. The polarization direction is perpendicular to the plane of the figure. The modulated fluorescence light, after passing the interference filter $F$, is splitted in a Wollastone prism into two components $J_{\parallel}$ and $J_{\perp}$, which enter the photomultipliers PM1 and PM2, respectively. The dividing system DR measures the rate of both independent of the changes in the intensity of the exciting light. The system is calibrated using the unpolarized excimer fluorescence of pyrene in a solution of low viscosity (Benzene) having $J_{\perp}/J_{\parallel} = 1$. Substituting the sample under investigation for the pyrene solution one finds the degree of depolarization $\varrho = J_{\perp}/J_{\parallel}$, which is of our interest. The above method of calibrating the system enables the elimination of the effect of the instrumental polarization of the system. By the employment of this method, the emission anisotropy can be measured in the range $0.003 < r < 0.997$.

Table 1. Mean moments of inertia $I$ calculated for free molecules and those measured in solvents of different viscosities.

| Compound | $I^{\parallel a}$ | $I^{\perp b}$ | $I_{\text{mean}}$ | Measured [13, 19] $I$ |
|---|---|---|---|---|
| | in $10^{-44}$ kg · m$^2$ | | | in $10^{-40}$ kg · m$^2$ |
| POPOP | 18.7 | 20.7 | 19.7 | 62.5 |
| 2MPOPOP | 20.7 | 22.7 | 21.7 | 83.5 |
| α-NOPON | 32.3 | 33.9 | 33.1 | 67.7 |
| DPS | 18.5 | 19.2 | 18.8 | 36.4 |
| DPH | 8.9 | 9.3 | 9.1 | – |

[a] $I^{\parallel}$ – Moment of inertia relative to the axis in the molecular plane and perpendicular to the long axis of the molecule.

[b] $I^{\perp}$ – Moment of inertia relative to the axis perpendicular to the molecular plane and to the long axis of the molecule.

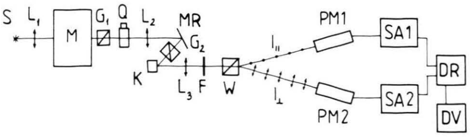

Fig. 1. Fluorescence anisotropy measuring apparatus $S$ the excitation light source, XBO 250; $L_1$, $L_2$, $L_3$ lenses; $M$ monochromator SPM2; $G_1$, $G_2$ Glan prisms; $Q$ quartz modulator; $K$ couvette with the sample; $F$ filter; $W$ Wollastone prism; PM1, PM2 photomultipliers; SA1, SA2 selective amplifiers (selective + lock-in); DR dividing recorder; DV digital voltmeter.

### c) Lifetime measurements

The fluorescence decay times were measured by the pulse method. The fluorescence was excited by a nitrogen laser pulses $h_1$ with a half-width of about 300 ps and a repetition frequency of 200 Hz. The excitation and fluorescence pulses were measured by means of a photomultiplier – sampling oscilloscope – multichannel analyzer system. The measured "excitation" $h$ and "fluorescence" $g$ pulses with this system perform the following dependences:

$$h = h_1 * e\,, \quad g = h_1 * e * f = h * f\,,$$

where $h_1$ is the real excitation puls from the laser, $e$ the answer of the measuring system upon the $\delta$-puls, $h$ the measured excitation puls, $g$ the measured fluorescence puls, $f$ the real (explored) fluorescence decay, and $*$ the symbol of the convolution integral.

The pulses $h(t)$ have the following parameters: a rise time $t \approx 900$ ps and a half-width of about 2 ns. The pulses $h(t)$ and $g(t)$ were measured 250 times for their averaging in the multichannel analyzer, and alternately to avoid effects of thermal fluctuations in the measuring system and changes in the parameters of the excitation pulses during the measurements.

The computations of the fluorescence decay times were carried out by a new method [18]. In accordance with this method for a single exponential fluorescence decay $f(t) = 1/\tau \exp(-t/\tau)$ the following relation holds:

$$\tau = \int_0^\infty \int_0^t [h(x) - g(x)] \,\mathrm{d}x \,\mathrm{d}t \,,$$

where

$$g(t) = \int_0^t h(x) f(t-x) \,\mathrm{d}x$$

and

$$\int_0^\infty h(t) \,\mathrm{d}t = \int_0^\infty g(t) \,\mathrm{d}t = 1 \,.$$

## 4. Results and Discussion

Four of the luminescent compounds, except DPH, have a short mean lifetime $\tau$ and a marked fluorescence anisotropy in cyclohexene having the viscosity $\eta = 1.01$ cP at 20 °C (Table 2), whereas DPH evidences a strong rotational depolarization in this solvent. By adding nitrobenzene in different concentrations (from $0.25 \cdot 10^{-2}$ to $1.5 \cdot 10^{-1}$ M), a strong fluorescence quenching has been observed. The decay time $\tau(c)$ decreases with increasing concentration $C$ of nitrobenzene according to the Stern-Volmer relation $\tau_0/\tau = 1 + KC$, where $K$ is the quenching constant. At high nitrobenzene concentrations a deviation from that relation has been observed, similarly as by Albat and Schmillen (12). We have therefore confined the concentration range of nitrobenzene to that obeying the Stern-Volmer relation.

The simultaneous measurements of $\tau$ and $r$ enables the application of the relation $\dfrac{\tau^2}{r_0/r - 1}$ according to (2). Figure 2 shows the experimental results

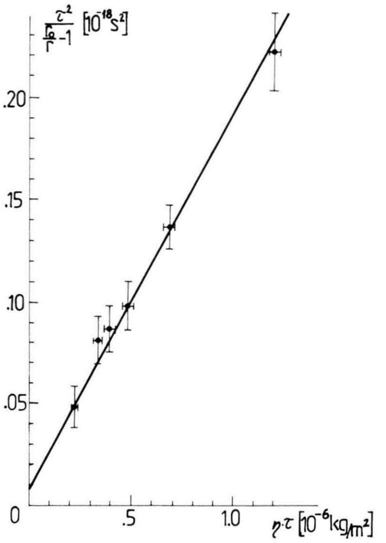

Fig. 2. Dependence of $\dfrac{\tau^2}{r_0/r - 1}$ on $\eta\,\tau$ for POPOP in cyclohexane quenched by nitrobenzene.

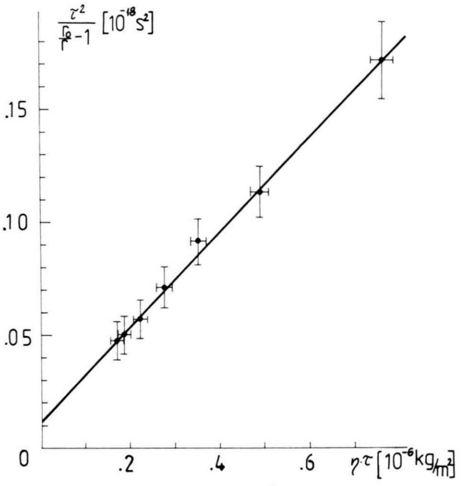

Fig. 3. Dependence of $\dfrac{\tau^2}{r_0/r - 1}$ on $\eta\,\tau$ for POPOP in benzene quenched by nitrobenzene.

Table 2. Mean lifetimes, $\tau$, and the fluorescence anisotropies, $r$, of the luminescent compounds in cyclohexane at 20 °C (without quencher).

| Compound | $\tau$ [ns] | $r$ | $r_0$ |
|---|---|---|---|
| POPOP | 1.09 | 0.049 | 0.3125 |
| 2MPOPOP | 1.25 | 0.034 | 0.3086 |
| α-NOPON | 1.25 | 0.036 | 0.3067 |
| DPS | 0.86 | 0.065 | 0.3030 |
| DPH | 8.8 | 0.003 | – |

Table 3.

| Compound | $T$ [K] | $C_{\text{comp}}$ [$10^{-5}$ M] | $C_{\text{nitrob.}}$ [$10^{-2}$ M] | $V/kT$ [$10^{-6}$ ms$^2$/kg] | $I/6kT$ [$10^{-18}$ s$^2$] | $V$ [$10^{-30}$ m$^3$] | $I$ [$10^{-40}$ kg · m$^2$] | $\tau_r$ [ps] |
|---|---|---|---|---|---|---|---|---|
| POPOP | 293 | 1 | $0 \div 15$ | $0.193 \pm 0.006$ | $0.0074 \pm 0.0045$ | 782 | 1.8 | 38 |
| 2MPOPOP | 303 | 5 | $0 \div 12.5$ | $0.147 \pm 0.006$ | $0.009 \pm 0.0045$ | 615 | 2.26 | 60 |
| α-NOPON | 303 | 1 | $0 \div 12.5$ | $0.150 \pm 0.005$ | $0.018 \pm 0.004$ | 627 | 4.52 | 119 |
| DPS | 293 | 1 | $0 \div 10$ | $0.222 \pm 0.004$ | $0.010 \pm 0.002$ | 898 | 2.42 | 45 |
| DPH | 293 | 40 | $0 \div 15$ | $0.079 \pm 0.002$ | $0.013 \pm 0.002$ | 319 | 3.15 | 163 |

obtained for one of the five substances investigated. In all five experiments a linear dependence according (2) has been observed. $V/kT$, $I/6kT$ and the values of $V$ and $I$ calculated from (2) are summarized in Table 3. As can be seen, the effective volumes $V$, as well as the effective moments of inertia $I$ differ considerably from those obtained previously. The moments of inertia found in the present paper are lower (slightly more than by one order of magnitude) but still higher than those calculated (cf. Tables 1 and 3) based on the geometry of the molecules.

In order to additionally convince of the effect of solvent upon $V$ and $I$ of POPOP, these magnitudes were determined in benzene (a solvent of high polarizability) using the method described above. The results obtained are shown in Figure 3. The dependence of $\tau^2/(r_0/r) - 1$ on $\eta\tau$ is well described by (2). The values $I = 2.72 \times 10^{-40}$ kg · m$^2$ and $V = 853 \times 10^{-30}$ m$^3$ are indeed slightly higher than those obtained with POPOP in cyclohexane. The volume of the POPOP molecule together with its shell varies between $V = 782 \times 10^{-30}$ m$^3$ in cyclohexane to $V = 963 \times 10^{-30}$ m$^3$ obtained from measurements in different solvents.

The results evidence unequivocally the change in the composition of the solvent shell when changing the solvent, and show that the molecular moment of inertia $I$ in liquids is markedly higher than that of a free molecule. The reasonable value of $I$ in liquids is evidenced by the rotational relaxation time $\tau_r$ given in Table 3.

The values of the limiting fluorescence anisotropy $r_0$ of the luminescent molecules investigated are lower than $r_f = 0.4$, which could have been expected from the theoretical considerations [20]. This, according to Jabłoński [21], indicates the occurrence of torsional vibrations of the luminescent molecules in solutions inside the solvation shell, immediately following excitation.

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