Thermal Motion of Prolate Luminescent Molecules in Solvents with Different Viscosities

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Dedicated to Professor Albert Schmillen on the occasion of his 65th birthday

The emission anisotropies r of the fluorescence of the prolate molecules 4-dimethylamino-4′-nitrostilbene (DNS) and 4-amino-4′-nitrostilbene (ANS) in solvents of different viscosities η were investigated. The non-linear dependence of $1/r$ on T/η was confirmed. Agreement between the experimental data and theory was obtained by the assumption that the prolate luminescent molecules perform irregular rotational motions within a limited solid angle. The theory developed for prolate molecules with the emission transition moment parallel to the long axis of the molecules describes well the experimental results. Rotational relaxation times in different solvents and effective volumes of the studied molecules were obtained.

1. Introduction

According to the theory of rotational depolarization of fluorescence [1, 2] the partial polarization of the fluorescence light emitted by molecules in solution depends on the ratio of their lifetime τ in the excited fluorescent state to the rotational relaxation time Θ. The non-linear dependence of $1/r$ on T/η of several luminescent systems [1, 3—7] has been attributed to the asphericity of the luminescent molecule, to the invalidity of the law of Brownian rotation at low viscosity and to solvation effects.

($r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$ is the emission anisotropy (EA), T the absolute temperature, and η the viscosity of the solvent.)

Up to now the rotational depolarization of fluorescence has been studied for many dyes of spherical or ellipsoidal shape in mixed solvents at different concentrations and in one solvent at different temperatures.

The present study concerns the rotational depolarization of the fluorescence of the prolate molecules DNS and ANS, with the emission transition dipole moment parallel to the long axis, in solvents with different viscosities.

In the case of steady excitation, the EA of a rotational ellipsoid is given by the sum of three terms [5, 8, 9]:

$$\frac{r}{r_0} = \frac{A_1(\vartheta)}{1+\tau/\Theta_1} + \frac{A_2(\vartheta)}{1+\tau/\Theta_2} + \frac{A_3(\vartheta)}{1+\tau/\Theta_3}\,, \qquad (1)$$

where r_0 is the limiting EA, and the constants

$$\begin{aligned} A_1(\vartheta) &= (\tfrac{3}{2}\cos^2\vartheta - \tfrac{1}{2})^2\,,\\ A_2(\vartheta) &= 3\cos^2\vartheta\sin^2\vartheta\,,\\ A_3(\vartheta) &= \tfrac{3}{4}\sin^4\vartheta\,, \end{aligned} \qquad (2)$$

are simple trigonometric functions of the angle ϑ between the emission transition dipole moment and the long axis of the molecule. $\Theta_{i(1,2,3)}$ are the three rotational relaxation times depending on the ratio γ' of the longitudinal axis to the equatorial axis of the ellipsoid. γ' is greater than unity for a prolate rotor (rod shaped molecule).

When the transition dipole moment is parallel to the long axis ($\vartheta = 0$) one obtains from Eqs. (2)

$$A_1(\vartheta) = 1 \quad \text{and} \quad A_2(\vartheta) = A_3(\vartheta) = 0\,, \qquad (3)$$

which gives the Perrin equation for a spherical rotor.

The aim of the present work is to verify whether the prolate molecules DNS and ANS, which fulfill the condition (3), are really subjected to the Perrin equation.

2. Experimental

The compounds examined were: 4-dimethylamino-4′-nitrostilbene (DNS) and 4-amino-4′-nitrostilbene (ANS). The solvents used were spectroscopically pure. To find out whether concentration effects interfere with the measurements, solutions of two different solute concentrations, 5×10^{-5} and 10^{-6} M, were prepared.

The absorption and emission spectra of the investigated substances were measured as previously described [10]. The temperature of the samples was

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20 °C and kept constant within ±0.2 °C with an ultrathermostat. The mean EA of the solution was measured on a Bauer-Rozwadowski compensation polarimeter [11, 12] built in our Laboratory by J. Kukielski. A Xenon lamp XBO 450 W was used as excitation source and the exciting wavelength was selected according to the 0—0 transition of the investigated substances by a SPM-2 Monochromator.

The EA values were found to be reproducible within $\Delta r = \pm 0.001$ and constant within the fluorescence band, for all investigated fluorescent compounds (see also Ref. [13, 14]).

The solutions were excited by an appropriate filtered Hg-arc lamp, and their mean decay times τ were measured on a phase fluorometer [15] with an accuracy of 0.05 ns.

3. Experimental Results

The results obtained for EA and τ of DNS and ANS in different solvents of different viscosities are given in Table 1. The mean decay times in the investigated solvents of different viscosities are constant for a given luminescent compound. It is also evident that the observed variation of $1/r$ on τ/Θ (Perrin plot) is not linear (see also Figs. 4 and 5). The influence of the dielectric constant of the solvent on the EA is very small and in our approach can be neglected [16].

4. Theory

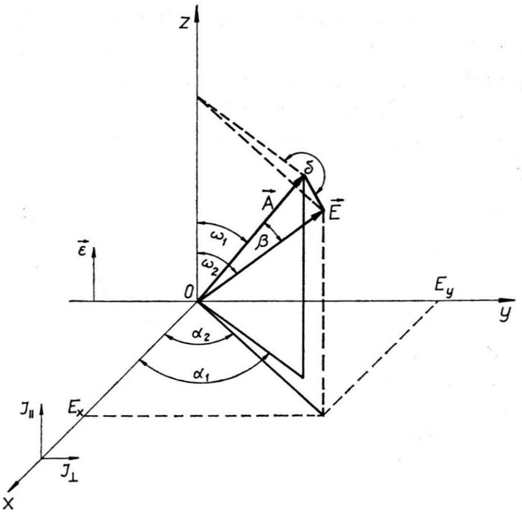

Fig. 1. Geometry of the system. Excitation light $\boldsymbol{\epsilon}$ is polarized in the direction z. Fluorescence is observed along x. The vectors $\boldsymbol{A}$ and $\boldsymbol{E}$ represent the direction of absorption and emission transition moments. δ is the angle between the planes formed by the OZ, $\boldsymbol{A}$ and $\boldsymbol{E}$, $\boldsymbol{A}$ directions; ω_1 the angle between the vector $\boldsymbol{A}$ and the z-axis, ω_2 the angle between the vector $\boldsymbol{E}$ and the z-axis, and α_1, α_2 the angles between the x-axis and the vertical projections of the $\boldsymbol{A}$ and $\boldsymbol{E}$ vectors.

Let us consider free rotational motion of a prolate molecule with its directions $\boldsymbol{A}$, $\boldsymbol{E}$ of the absorption and emission oscillators fixed in the molecule (Figure 1). The electric vector $\boldsymbol{\epsilon}$ of the exciting light may be oriented along the z-axis. $I_\parallel$ and $I_\perp$ designate the components of the fluorescence intensity parallel and perpendicular to $\boldsymbol{\epsilon}$, respectively, as observed along the x-axis.

The angles ω_1, ω_2, β and δ (Fig. 1) obey the relation

$$\cos\omega_2 = \cos\omega_1 \cos\beta + \sin\omega_1 \sin\beta \cos\delta\,. \quad (4)$$

Table 1. Measured emission anisotropies r and mean decay times τ of DNS and ANS in solvents of different viscosities.

No.	Solvent	Viscosity at 20 °C	DNS			ANS		
			r	τ ns	Θ from Eqs. (24)—(27) ns	r	τ ns	Θ from Eqs. (24)—(27) ns
1	benzene	0.652	0.088	2.78	0.21	0.082	2.25	0.20
2	chlorobenzene	0.900	0.106	2.36	0.3	—	—	—
3	dioxan	1.450	—	—	—	0.107	2.31	0.44
4	n-butyl alcohol	2.948	0.153	2.68	0.98	0.143	2.36	0.89
5	n-heptyl alcohol	6.961	0.177	2.55	2.32	0.167	2.26	2.11
6	n-undecyl alcohol	15	0.234	2.49	5.13	0.228	2.35	4.67
7	ethylene glycol	19	0.253	2.65	6.5	—	—	—
8	cyclohexanol	68	0.299	2.67	22.74	0.281	2.28	20.72

In an isotropic solution all orientations of the vectors $\boldsymbol{E}$ and $\boldsymbol{A}$, determined by the angles ω_1, ω_2, α_1, α_2 and δ are equally probable.

As the total fluorescence intensity $I = I_{\parallel} + 2 I_{\perp}$ is proportional to $\boldsymbol{\epsilon}^2$, according to Fig. 1 the $I_{\parallel}$ component is given by

$$I_{\parallel} = I \langle \cos^2 \omega_2 \rangle . \tag{5}$$

In the case of a rigid solution without intermolecular transfer processes the expression

$$\langle \cos^2 \omega_2 \rangle = I_{\parallel}/I$$

is independent of time. However if molecular rotation occurs, this fraction is a function of time. Therefore a kinetic equation describing this change in time, caused by molecular rotation, should be found.

At first we shall find the initial value of $\langle \cos^2 \omega_2 \rangle$ $(t=0)$ taking into account the photoselection by linearly polarized exciting light, in which case we have

$$I_{\parallel}/I = \langle \cos^2 \omega_2 \rangle = \tag{6}$$

$$\frac{\int\limits_{\alpha_1=0}^{2\pi} \int\limits_{\alpha_2=0}^{2\pi} \int\limits_{\delta=0}^{2\pi} \int\limits_{\omega_1=0}^{\pi/2} (\cos^2 \omega_2) \cos^2 \omega_1 \sin \omega_1 \, \mathrm{d}\omega_1 \, \mathrm{d}\alpha_1 \, \mathrm{d}\alpha_2 \, \mathrm{d}\delta}{\int\limits_{\alpha_1=0}^{2\pi} \int\limits_{\alpha_2=0}^{2\pi} \int\limits_{\delta=0}^{2\pi} \int\limits_{\omega_1=0}^{\pi/2} \cos^2 \omega_1 \sin \omega_1 \, \mathrm{d}\omega_1 \, \mathrm{d}\alpha_1 \, \mathrm{d}\alpha_2 \, \mathrm{d}\delta} = \int\limits_{\omega_1=0}^{\pi/2} (\cos^2 \omega_2) \cos^2 \omega_1 \sin \omega_1 \, \mathrm{d}\omega_1 = \tfrac{2}{5} \cos^2 \beta + \tfrac{1}{5} .$$

In the integration of (6) the relation (4) was used. Analogically we find

$$I_{\perp}/I = \langle \sin^2 \omega_2 \rangle = \tfrac{2}{5} - \tfrac{1}{5} \cos^2 \beta , \tag{7}$$

and from (6) and (7) we obtain very simply the known Perrin equation for the fundamental EA

$$r_0 \equiv r(0) = \frac{I_{\parallel}(0) - I_{\perp}(0)}{I(0)} = 0.6 \cos^2 \beta - 0.2 . \tag{8}$$

When the transition moments $\boldsymbol{A}$ and $\boldsymbol{E}$ are parallel $(\beta = 0)$ one obtains from (6) and (7)

$$\frac{I_{\parallel}(0)}{I(0)} = \frac{3}{5} \quad \text{and} \quad \frac{I_{\perp}(0)}{I(0)} = \frac{1}{5} . \tag{9}$$

The variation of the polarization with time for the rotational motion of fluorescing molecules shall be described by a relaxation mechanism, i.e. the three components of the relative polarization

$$(I_{\parallel}(t)/I)_z = W_1(t); \quad (I_{\perp}(t)/I)_x = W_2(t); \quad (I_{\perp}(t)/I)_y = W_3(t) , \tag{10}$$

shall obey the differential equations

$$\mathrm{d}W_1/\mathrm{d}t = -2(D_1 + D_2) W_1 + 2 D_1 W_2 + 2 D_2 W_3 , \tag{11}$$

$$\mathrm{d}W_2/\mathrm{d}t = 2 D_1 W_1 - 2(D_1 + D_3) W_2 + 2 D_3 W_3 , \tag{12}$$

$$\mathrm{d}W_3/\mathrm{d}t = 2 D_2 W_1 + 2 D_3 W_2 - 2(D_2 + D_3) W_3 , \tag{13}$$

valid for times $t > t_0$, t_0 being the end of excitation. If the exciting light is polarized in the z direction, according to the rotational symmetry about the z axis we should have $D_1 = D_2 = D$.

The emission anisotropy is determined solely by $W_1(t)$:

$$r(t) = \tfrac{3}{2} W_1(t) - \tfrac{1}{2} . \tag{14}$$

Thus we need only the solution of (11) and as by definition

$$W_1(t) + W_2(t) + W_3(t) = 1 ,$$

we get

$$\begin{aligned} \mathrm{d}W_1/\mathrm{d}t &= -4 D W_1 + 2D(1 - W_1) \\ &= -6 D W_1 + 2D \end{aligned} \tag{15}$$

with

$$\mathrm{d}W_1(\infty)/\mathrm{d}t = 0$$

and

$$\mathrm{d}W_1(t)/\mathrm{d}t = -6D[W_1(t) - W_1(\infty)] . \tag{16}$$

Substituting $W_1(t)$ by $r(t)$ by means of (14), (16) transforms into

$$\mathrm{d}r/\mathrm{d}t = -6D[r(t) - r(\infty)] \tag{17}$$

with the general solution

$$r(t) = c \cdot e^{-6Dt} + r(\infty) . \tag{18}$$

For freely rotating molecules $r(\infty) = 0$, and $r(0)$ is given by (8). If $\boldsymbol{A}$ and $\boldsymbol{E}$ are parallel $(\beta = 0)$ we get $r(0) = \tfrac{2}{5}$ and

$$r(t) = r(0) \, e^{-6Dt} . \tag{19}$$

$r(0)$ is identical with the EA r_0 at continuous excitation. If the rotation of fluorescing molecules is limited to a solid angle, we have $r(\infty) \neq 0$ and $r(0) = c + r(\infty)$ or

$$r(t) = [r(0) - r(\infty)]\, e^{-6D't} + r(\infty);$$
$$r(t)/r(0) = (1 - A)\, e^{-6D't} + A\,, \tag{20}$$

where D' may be different from D (see also [17]).

The molecular rotation after the end of excitation is equivalent to a change of direction of the emitting oscillator against the direction the absorbing oscillator. Thus we can describe $r(t)$ by the time dependent mean value of $\langle \cos^2 \beta \rangle_0{}^t$, averaged over the interval $(0, t)$, using (6) and (14):

$$r(t) = \tfrac{3}{2}\left(\tfrac{2}{5}\langle \cos^2 \beta \rangle_0{}^t + \tfrac{1}{5}\right) - \tfrac{1}{2} \tag{21}$$

and

$$r(\infty) = \tfrac{3}{5}\langle \cos^2 \beta \rangle_0{}^\infty - \tfrac{1}{5}\,. \tag{22}$$

The interval $(0, \infty)$ is very large compared to the relaxation time $1/6D$. Moreover, in the case of limited rotation we suppose $(\mathrm{d}r/\mathrm{d}t)_{t=0}$ to be equal to the value at free rotation, which means that

$$-\,6D'[r(0) - r(\infty)] = -\,6D\, r(0) \tag{23}$$

or

$$\frac{1}{\Theta} = 6D' = \frac{6D\, r(0)}{r(0) - r(\infty)} = \frac{6D}{1 - A} \tag{24}$$

with

$$A = \frac{r(\infty)}{r(0)} = \frac{3}{2}\langle \cos^2 \beta \rangle_0{}^\infty - \frac{1}{2}\,. \tag{25}$$

In the case of stationary excitation the EA is given by $r \equiv \langle r(t) \rangle_t$ (averaged over the decay process) and may be expressed in the form

$$\frac{r}{r_0} = \frac{1}{\tau}\int_0^\infty \frac{r(t)}{r_0}\, e^{-t/\tau}\, \mathrm{d}t \tag{26}$$
$$= A + \frac{1 - A}{1 + 6D'\tau} = A + \frac{1 - A}{1 + 6D\,\tau/(1 - A)}\,,$$

where $r_0 \equiv r(0)$.

The coefficient D for free rotation is given by [18]

$$6D = kT/V\eta\,, \tag{27}$$

where V (in cm^3) is an effective volume of a spherical molecule of the same volume as that of the prolate molecule.

Figures 2 and 3 show the system of curves computed from Eqs. (20) and (26) for different parameters A in the case when an isotropic solution is excited with a very short light pulse and continuous light, respectively. In a special case, when $\langle \cos^2 \beta \rangle = \frac{1}{3}$, that means $A = 0$ (and when A is small, for example $A = 0.001$ in Fig. 3), the EA decay in time is given by (19), and when the illumination is continuous, according to (26) we obtain the Perrin formula.

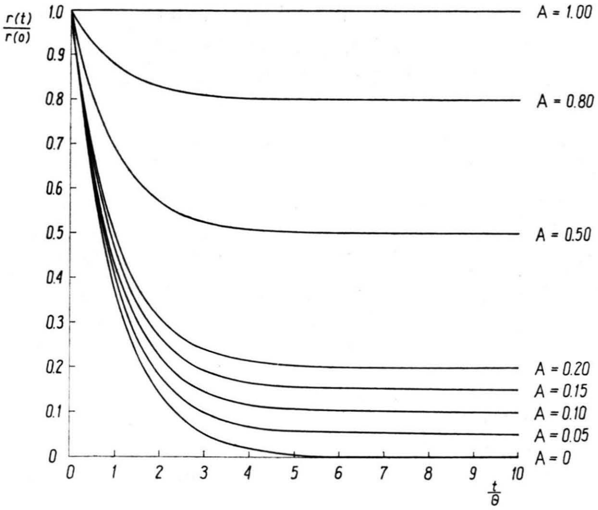

Fig. 2. Theoretical curves of fluorescence anisotropy decay (Eq. (20)) for different values A.

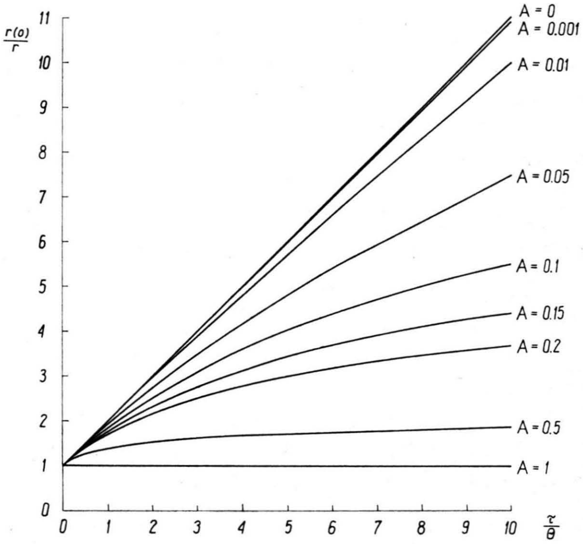

Fig. 3. Theoretical curves of fluorescence *EA* as a function of τ/Θ (Eq. (26)) for different values A.

So the deviation from the linear dependence of $1/r$ on $\tau\, 6D' = \tau/\Theta$ (see Fig. 3) may be explained by the fact that the rod-shaped luminescent molecules do not freely rotate but perform irregular libration, within a definite solid angle. Equations

(20) and (26) can be used in all cases of limited rotational motion, i.e. in heterogeneous complex media and biological membranes [19—22].

5. Comparison of Theory and Experiment

Comparison of experimental results for DNS and ANS with theoretical curves computed from Eq. (26) is shown in Figs. 4 and 5. Independently, the limiting EA, r_0, has been evaluated from the intercepts of the linear dependence of $1/r$ for $T/\eta \to 0$ (the beginnings of the curves exhibit linear dependence). On comparison of the theory with the experimental results, the parameter A (and hence $r(\infty)$) and the mean effective volume V of the investigated luminescent molecule were evaluated from the r_0/r dependence on $\tau/\Theta = c\,T/\eta$, where $c = k\tau/(1-A)\,V = \text{const}$ (according to Eqs. (24) and (27)). Table 2 compares the determined quantities.

DNS and ANS are highly polar compounds ($\mu_g = 7.2$ D, $\mu_e = 23.1$ D for DNS and $\mu_g = 6.5$ D, $\mu_e = 20.7$ D for ANS [23]), and therefore the volumes of these molecules together with their solvation shells are large. Specific interactions of alcohols do not influence significantly the measured EA. Within the big viscosity range (solvents No. 5, 6, 7, 8 from Table 1) the Perrin dependence of $1/r$ on T/η is well satisfied.

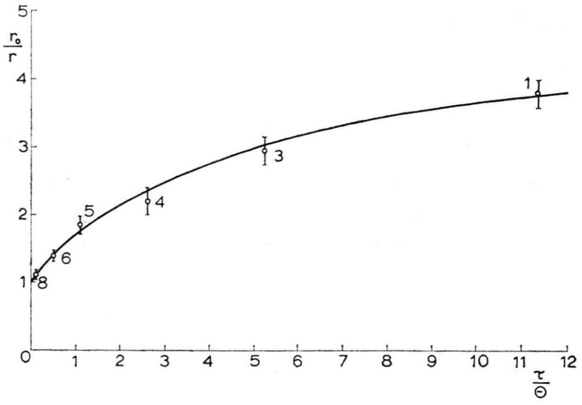

Fig. 5. The same dependence as in Fig. 4 for ANS and $A = 0.2$.

These EA studies demonstrate that DNS and ANS undergo more than one kind of rotational motion in solvents with different viscosities. When the viscosities of the solvents are low, the highly polar compounds DNS and ANS arrange the environment of the solute [16] and the rotational motions of the transition moments of DNS and ANS are not isotropic.

It is well known that the emission transition moment and the electric dipole moments in the ground ($\boldsymbol{\mu}_g$) and excited ($\boldsymbol{\mu}_e$) states of DNS and ANS are along the long axis of these molecules [23,

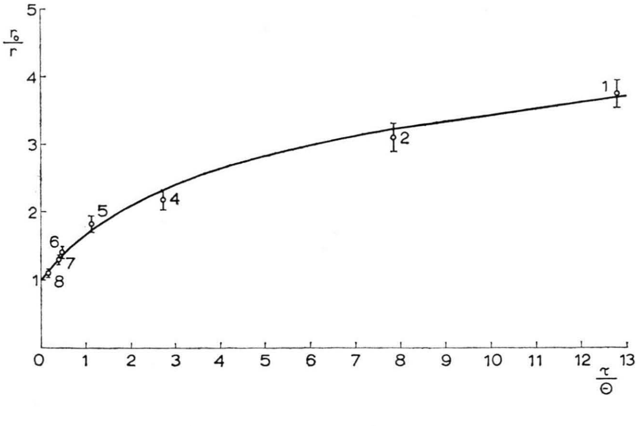

Fig. 4. Reciprocal EA versus $\tau/\Theta = c\,T/\eta$ (where $c = k\tau/(1-A)\,V$) for DNS. — The points are the experimental values and the drawn line is the theoretical curve (Eq. (26)) for $A = 0.22$.

Table 2.

Compound	r_0	A Eq. (26)	$r(\infty)$ Eq. (25)	$\langle\beta\rangle$ Eq. (25)	V $[10^{-24}\ \text{cm}^3]$	R [Å]
DNS	0.3278	0.22	0.0721	47.7°	1724	7.44
ANS	0.3125	0.20	0.0625	48.6°	1533	7.15

24]. The above compounds meet the assumptions of our theory and the character of the experimental curves is well described by Eq. (26), as it is seen in Figs. 4 and 5.

The rotational relaxation times $\Theta = 1/6D'$ determined from Eqs. (24)—(27) in the viscosity region from 0.652 to 68 cP, varies between 0.2 and 20 ns.

More information on the nature of the environments of DNS and ANS in single solvents can be obtained by nanosecond EA studies. Such investigations are in progress and will be published soon.

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