Theory of Rotational Motion of Prolate Luminescent Molecules in Solution [*]

R. Alicki[**], M. Alicka, and A. Kawski

Luminescence Research Group, Institute of Physics, University of Gdańsk, Poland

On the ground of a generalized equation for rotational diffusion which takes the inertial effect into account, an expression for the steady-state fluorescence depolarization for prolate molecules with the emission transition moment parallel to the long axis, has been derived. The present theoretical results differ from those of previous studies and are in good agreement with the experimental results.

1. Introduction

The effect of the Brownian rotational motion upon the fluorescence anisotropy (FA)[1], r, of molecules has been so far an object of numerous experimental and theoretical investigations [1, 2]. For asymmetric molecules, the generalized Perrin theories predict extremely complex relations between parameters which are characteristic of the luminescent molecule itself (limiting fluorescence anisotropy, r_0, molecular volume of a molecule together with its solvent shell, V, mean fluorescence lifetime, τ) and the environment (solvent viscosity and temperature, η and T, respectively) in which the molecule is located [3—9]. It was found, however, that the theories of fluorescence depolarization based on the approximation of rotational diffusion do not satisfactorily describe the experimental results observed, in particular for prolate ellipsoid-of-revolution-shaped molecules for which the emission transition moment is parallel to the long molecular axis [10—12]. In such case, the complex relation mentioned above is simplified yielding the Perrin equa-

tion for a spherical rotator ($r_0/r = 1 + \tau/\theta$, where $\theta = V\eta/kT$) [13], which does not reflect properly $1/r$ versus T/η in the whole viscosity range.

In order to explain this discrepancy (i.e. the nonlinear dependence of $1/r$ upon T/η for a prolate molecule the emission transition moment of which lies along its longer axis), the libration motions were assumed to play the predominant role in thermal motions of prolate molecules [10]. Final expression obtained with such assumption describes properly the behaviour of $1/r$ as a function of T/η, nevertheless, the limitations assumed which were set upon the rotational motions of molecules in solutions raises certain doubts. The assumption as to the limited rotational motion is surely proper when the luminescent molecules are in anisotropic environment (e.g. in biological membranes, liquid crystals, etc.) [14, 15].

We shall show hereafter that the mentioned discrepancies between the experimental results and the existing theoretical studies result from the fact that the inertial effects have not been taken into account when considering rotational diffusion of molecules in liquid solutions. These effects turn out to influence substantially the decay of the fluorescence anisotropy for short lifetimes of a molecule after the excitation.

2. Theory

Let us consider in detail the rotational motion of ellipsoid of revolution (Fig. 1), axes 1, 2 und 3 of which are the main ones of a friction tensor (ξ_{kl}), being simultaneously those of the inertia tensor (I_{kl}). We assume moreover that $\xi_1 = \xi_2 = \xi$ and $I_1 = I_2 = I$, and that the direction of the emission

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[1] The FA is determined by

$$r = \frac{3}{2}\frac{J_\parallel}{J} - \frac{1}{2},$$

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.
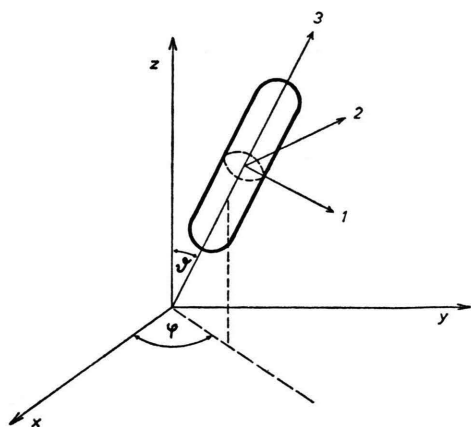
Fig. 1. Geometry of the system.

transition moment is parallel to the long axis 3 of the molecule. Electric vector E of the exciting light is directed along the z-axis.

We concern ourselves with the time changes in the direction of axis 3 determined by angles ϑ and φ. The anisotropy of the excited molecules at a moment t is described by the probability distribution $p_t(\vartheta, \varphi)$. The change of $p_t(\vartheta, \varphi)$ in time is given by the following generalized equation for rotational diffusion (Appendix):

$$\frac{\partial p_t(\vartheta, \varphi)}{\partial t}* = \int_0^t G(s)\, \Delta_\Omega\, p_{t-s}(\vartheta, \varphi)\, \mathrm{d}s, \tag{1}$$

where Δ_Ω is the angular part of the Laplacian operator, and

$$G(s) = \langle \omega_1(0)\, \omega_1(s) \rangle = \langle \omega_2(0)\, \omega_2(s) \rangle \tag{2}$$

is the autocorrelation function of the angular velocity with respect to axis 1 or 2. $\langle \cdots \rangle$ denotes the averaging process over the assembly of molecules.

Equation (1) describes the effect of "memory" related to the molecular inertia which is taken into account, i.e., the change of $p_t(\vartheta, \varphi)$ in time depends upon p_t also for $t' < t$. In order to calculate the autocorrelation function $G(s) = \langle \omega(0)\, \omega(s) \rangle$ we shall apply the Langevin equation

$$I \frac{\mathrm{d}\omega(t)}{\mathrm{d}t} = -\xi\, \omega(t) + M(t), \tag{3}$$

where: I the effective moment of inertia with respect to axes perpendicular to the long axis 3 of a molecule, ξ the rotational friction coefficient,

$M(t)$ the respective component of a stochastic force moment, $\omega = \omega_1$ or ω_2. The mean value of $M(t)$ over the assembly of molecules is assumed to satisfy the condition $\langle M(t) \rangle = 0$. The solution to (3) is

$$\omega(t) = \exp\{-(\xi/I)\,t\} \tag{4}$$
$$\cdot \left[\omega(0) + \int_0^t \exp\{-(\xi/I)\,t'\}\, M(t')\, \mathrm{d}t' \right].$$

Hence, we obtain

$$\langle \omega(0)\, \omega(t) \rangle = \langle \omega^2(0) \rangle \exp\{-(\xi/I)\,t\}. \tag{5}$$

From the rule of the equipartition of energy

$$\tfrac{1}{2} I \langle \omega^2(0) \rangle = \tfrac{1}{2} k T. \tag{6}$$

On the basis of (2), (5) and (6) we obtain the following expression for the autocorrelation function

$$G(s) = \frac{k T}{I} \exp\{-(\xi/I)\,s\}, \tag{7}$$

where the decay time of this function is $t_0 = I/\xi$.

When, e.g., $I \to 0$, then

$$G(s) \to D\, \delta(s), \tag{8}$$

where $D = kT/\xi$, and $\delta(s)$ is the delta-function. Hence, we can conclude that for $I \to 0$, the generalized diffusion equation (1) becomes the ordinary diffusion equation

$$\frac{\partial p_t(\vartheta, \varphi)}{\partial t} = D\, \Delta_\Omega\, p_t(\vartheta, \varphi),$$

where D is the rotational diffusion coefficient independent of time.

Instantaneous values of the fluorescence anisotropy (FA) of a solution on an impulse excitation at $t = 0$ is described by the following formula

$$r(t) = \int \mathrm{d}\Omega\, p_t(\vartheta, \varphi)\, [\tfrac{3}{2}\cos^2 \vartheta - \tfrac{1}{2}], \tag{9}$$

where $\mathrm{d}\Omega = \sin \vartheta\, \mathrm{d}\vartheta\, \mathrm{d}\varphi$.

On differentiating equation (9) and employing equation (1) we obtain

$$\frac{\mathrm{d}r(t)}{\mathrm{d}l} = \int_0^t \mathrm{d}s\, G(s) \int \mathrm{d}\Omega\, (\Delta_\Omega\, p_{t-s}(\vartheta, \varphi))$$
$$\cdot [\tfrac{3}{2}\cos^2 \vartheta - \tfrac{1}{2}]. \tag{10}$$

To find an equation describing function $r(t)$ we shall take advantage of the following facts:

a) the Laplacian operator Δ_Ω is self-adjoint

$$\int \mathrm{d}\Omega\, (\Delta_\Omega\, p)\, f = \int \mathrm{d}\Omega\, p\, (\Delta_\Omega\, f),$$

b) spherical functions $Y_{lm}(\vartheta, \varphi)$ are the eigenfunctions of the Laplacian operator Δ_Ω,

$$\Delta_\Omega Y_{lm} = -l(l+1)Y_{lm}, \quad l = 0, 1, 2, \ldots,$$
$$m = -l, \ldots, +l.$$

c) $\frac{3}{2}\cos^2\vartheta - \frac{1}{2} = CY_{20}, \quad C - \text{constant}.$

Applying properties a), b), and c) to (10) we obtain

$$\frac{dr(t)}{dt} = -6\int_0^t G(s)\,r(t-s)\,ds. \tag{11}$$

Function $r(t)$ possesses the following qualitative properties which meet (11):

1) $\left.\dfrac{dr(t)}{dt}\right|_{t=0} = 0,$ \hfill (12)

2) If the decay time, $t_0 = I/\xi$, of the autocorrelation function $G(s)$ is markedly shorter than that of function $r(t)$, and $t > t_0$, then the following approximated equation may be substituted for (11):

$$\frac{dr(t)}{dt} = -6\,D\,r(t), \tag{13}$$

where

$$D = \int_0^\infty G(s)\,ds.$$

(13) implies that for the properties mentioned above, function $r(t)$ is proportional to e^{-6Dt}. Function $r(t)$ is shown schematically in Figure 2.

For continuous excitation of an isotropic fluorescent solution, the mean FA can be calculated from the expression

$$\langle r \rangle = \frac{1}{\tau} \int_0^\infty \exp\{-t/\tau\}\, r(t)\,dt, \tag{14}$$

where τ is the mean lifetime of an excited molecule.

The knowledge of the solution of (11) is not indispensable to calculate $\langle r \rangle$ according to formula
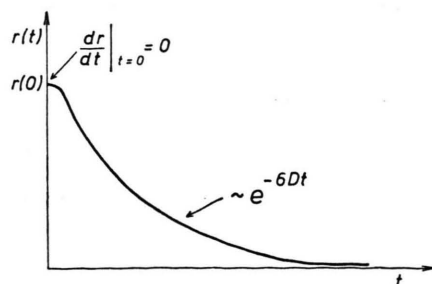
(14). It is sufficient to take advantage of the fact that $\langle r \rangle$ is a value of the Laplace transform of function $r(t)$ at a point $\lambda = 1/\tau$. Applying the Laplace transform to both sides of (11) in which function $G(s)$ is given by (7), after simple transformations we obtain

$$r = r_0 \frac{1 + A\,\tau/\theta}{1 + (1+A)\,\tau/\theta}, \tag{15}$$

where

$$A = I/6\,kT\,\tau^2, \tag{16}$$

$$\theta = \xi/6\,kT = V\eta/kT. \tag{17}$$

As is known from experiments [10—12, 16], $A^2 \ll 1$, so that (15) is identical with that obtained in paper [11], which properly describes the experimental values of $\langle r \rangle$ as function of τ/θ.

Figure 3 shows a family of curves calculated from (15) for different parameters A. When $A = 0$, or when the solvent viscosity, η, increases, i.e., $T/\eta \to 0$, the well-known Perrin equation is obtained which yields the linear dependence of $1/r$ upon T/η. Table 1 summarizes the values of r_0 and V_{eff}, which were determined basing on the linear dependence of $1/r$ upon T/η for high viscosities, of several compounds examined in our previous papers [10—12, 16, 17]. Parameters A were obtained for the above-mentioned compounds by comparing the experimental
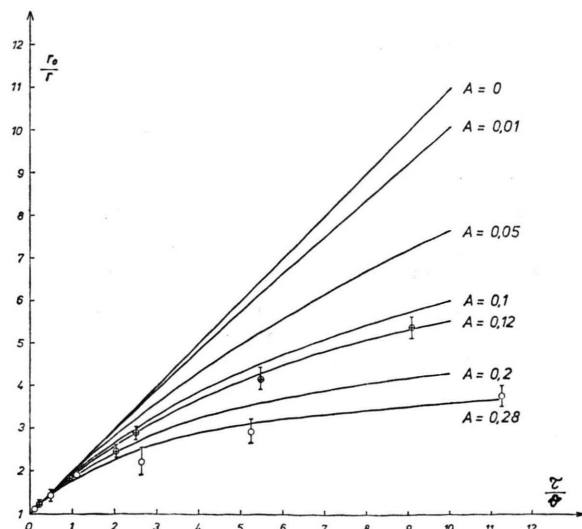
Fig. 3. Theoretical curves of FA as a function of τ/ϑ [Eq. (15)] for different values A. Experimental values: $\oplus$ for diphenylenestilbene and $\bigcirc$ for 4-amino-4′-nitrostilbene from [10, 11].

Fig. 2. Qualitative properties of function $r(t)$ given by (11).

Table 1.

Compound	r_0	$V_{\text{eff}} \times 10^{30}$ (m³)	τ (ns)	A	$I_{\text{eff}} \times 10^{41}$ (kg m²)
p-terphenyl	0.3450	206	1.15	0.007	22.6
diphenylenestilbene (DPS)	0.3030	823	1.1	0.12	390
2,2'-p-phenylenebis(5-phenylooxazole); (POPOP)	0.3125	963	1.35	0.14	625
p-bis[2-(5-α-naphthyloxazolyl)]-benzene; (α-NOPON)	0.3067	1001	1.64	0.11	725
4-amino-4'-nitrostilbene (ANS)	0.3125	1533	2.3	0.28	3635

results ($1/r$ versus T/η) for r_0 and V_{eff} determined previously, with theoretical values obtained according to formula (15) within the whole viscosity range examined. The knowledge of A and τ enables the determination of the moment of inertia, I, of the molecules under study. The value of I obtained with trans-stilbene is 4.3×10^{-41} kg · m² which is consistent with a value of 3×10^{-41} kg · m², as estimated by Sarshevskii et al. [18].

It is worthy of mention that the effect of molecular inertia upon the FA $\langle r \rangle$ was recently considered by Sarshevskii and coworkers [18], who made use of the results contained in a paper by Steele [19]. The modified diffusion equation given by Steele [19] was derived previously in a monograph [20] basing on less precise theoretical considerations and therefore it describes worse the process of rotational diffusion of molecules.

Appendix

Derivation of a motion rule for a system with n degrees of freedom (e.g., a Brownian particle), interacting with a thermal bath with temperature T.

1. Let us consider an open system described by generalized coordinates $x(x_1, \ldots, x_n)$, and momenta $p = (p_1, \ldots, p_n)$, which interacts with the environment characterized by coordinates $\xi(\xi_1, \ldots, \xi_N)$ and momenta $\pi = (\pi_1, \ldots, \pi_N)$ (dynamical variables), with N very high. The state of the whole system is described by the probability distribution $f_t(x, p; \xi, \pi)$ over a phase space, meeting the Liouville equation

$$\partial f_t/\partial t = \{H^\lambda, f_t\}, \qquad (\text{I})$$

where $\{\ \}$ denotes the Poisson bracket with respect to all canonical variables, and H^λ is the total Hamiltonian

$$H^\lambda = \tfrac{1}{2} \sum_k (p_k^2/M_k) + H_{\text{R}}(\pi, \xi) + \lambda V(\xi, x), \qquad (\text{II})$$

where the first term corresponds to the kinetic energy of an open system, the second one describes the total energy of a reservoir, and the last one — the interaction between the open system and the reservoir.

It is assumed that the system is invariable with respect to a translation in space (x, ξ).

2. The first stage consists in the elimination of the variables of reservoir (ξ, π) assuming a thermal reservoir (thermal bath) with temperature T. This can be achieved applying the so-called projection technique [21]. We obtain an equation for

$$\varphi_t(x, p) = \int d\xi \, d\pi \cdot f_t(x, p; \xi, \pi):$$

$$\frac{\partial \varphi_t(x, p)}{\partial t} = -\sum_k \frac{p_k}{M_k} \frac{\partial}{\partial x_k} \varphi_t(x, p) + \int_0^t \mathbb{L}_s \varphi_{t-s}(x, p) \, ds, \qquad (\text{III})$$

where $\mathbb{L}_s$ is a certain operator acting upon φ_{t-s} and depending on time s.

3. Assuming a weak interaction with the reservoir (a standard assumption) we can substitute the Markov equation for (III)

$$\frac{\partial \varphi_t}{\partial t} = -\sum_k \frac{p_k}{M_k} \frac{\partial}{\partial x_k} \varphi_t + \mathbb{L} \varphi_t, \qquad (\text{IV})$$

where

$$\mathbb{L} = \int_0^\infty \mathbb{L}_s \, ds.$$

The form of operator $\mathbb{L}$ is not essential for further considerations, nevertheless, the so-called Fokker-Planck equation [22] can be in general substituted to a good approximation for (IV).

4. Momenta $p = (p_1, \ldots, p_n)$ are eliminated assuming that the momentum distribution at the initial moment is given by

$$\varrho_{\text{T}}(p) = \sum{}' \exp\left(-\frac{\tfrac{1}{2} \sum (p_k^2/M_k)}{kT}\right).$$

On employing the projection method once more, we obtain a generalized diffusion equation for a translational motion with the probability distribution $p_t(x) = \int \mathrm{d}p\, \varphi_t(x, p)$:

$$\frac{\partial p_t(x)}{\partial t} = \sum \int_0^t G_k(s)\, \frac{\partial^2}{\partial x_k^2}\, p_{t-s}(x)\, \mathrm{d}s, \qquad (V)$$

where[2]

$$G_k(s) = \langle v_k(0)\, v_k(s)\rangle \quad \text{and} \quad v_k = \frac{p_k}{M_k}.$$

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[2] When $G_k(s)$ decays to zero very rapidly (e.g., when $M_k \to 0$), the Markov approximation can be also employed, yielding a common diffusion equation

$$\frac{\partial p_t}{\partial t} = \sum D_k\, \frac{\partial^2}{\partial x_k^2}\, p_t,$$

where

$$D_k = \int_0^\infty G_k(s)\, \mathrm{d}s.$$

For rotational Brownian motions of molecules we assumed that axis 3 (see the theory) is the axis of symmetry of the friction tensor and the moment of inertia, and we are interested only in the position of this axis in space. Other degrees of freedom are included in the thermal reservoir. For this case, (V) can be easily applied to a particular case of rotational diffusion (with the assumptions under discussion) yielding

$$\frac{\partial p_t(\vartheta, \varphi)}{\partial t} = \int_0^t G(s)\, \Delta_\Omega\, p_{t-s}(\vartheta, \varphi)\, \mathrm{d}s, \qquad (VI)$$

where

$$\Delta_\Omega = \frac{1}{\sin \vartheta}\, \frac{\partial}{\partial \vartheta}\left(\sin \vartheta\, \frac{\partial}{\partial \vartheta}\right) + \frac{1}{\sin^2 \vartheta}\, \frac{\partial^2}{\partial \varphi^2},$$

$$G(s) = \langle \omega_1(0)\, \omega_1(s)\rangle = \langle \omega_2(0)\, \omega_2(s)\rangle,$$

and ω_1 and ω_2 are the components of angular velocity with respect to axes 1 and 2, which are perpendicular to axis 3.

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