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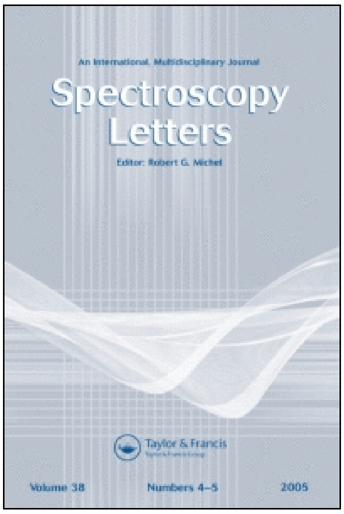

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ROTATIONAL DYNAMICS OF EXCITED PROBES: THE ANALYSIS OF EXPERIMENTAL DATA

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ROTATIONAL DYNAMICS OF EXCITED PROBES: THE ANALYSIS OF EXPERIMENTAL DATA

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ABSTRACT

The time course of molecular rotational motion can be observed directly using partially oriented samples produced by photoselection and fast laser techniques. The magnitude of the observed absorption dichroism or fluorescence anisotropy is related to the relative directions of the excitation and observation transition moment vector as well as the degree of orientation. The duration of the observed effect depends on the shape of the probe and the solvent viscosity. It has been the accepted procedure to assume isotropic rotational dynamics of the probe when calculating rotational time constants. This assumption is too severe. It has been shown that the assumption of isotropic rotational dynamics leads to incorrectly determined rotational time constants using simulated data. Systematic errors in these constants render them

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inappropriate for evaluating rotational dynamics theories such as Debye-Stokes-Einstein theory or extensions thereof.

Key Words: Fluorescence anisotropy; Time-resolved linear dichroism; Hydrodynamics; Debye-Stokes-Einstein diffusion theory

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INTRODUCTION

Theoretical descriptions of molecular motion in solution are of fundamental importance with relevance in areas ranging from diffusion and material transport to reactivity, solubility and self-assembly. Descriptions of solvent-solute interactions and material transport have been based on the study of the rotational dynamics of molecules in solution. Many early studies of molecular rotational motion were based on the Perrin relationship, which relates steady state fluorescence polarization measurements to the rotational dynamics of the probe through knowledge of the fluorescent lifetime[1]. More recently, the introduction of ultra-fast lasers to spectroscopy has allowed polarization-specific spectroscopic information to be obtained directly using time-resolved linear dichroism spectroscopy or fluorescence anisotropy measurements[2–4]. Information gleaned from these experiments can be used to study the structures of excited states, the topochemistry of excited state reactions and the dynamics of molecular rotation in solution[4–6].

It is now well established that the description of the dynamics of anisotropic rotation of an asymmetric probe requires a series of five exponential functions[7]. The optical density observed when the probe beam is polarized parallel to the excitation beam, as a function of time, $\mathrm{OD}_{=}(\mathrm{t})$, is given by Eq. (1).

$$\begin{aligned} OD_{=}(t) = P(t)\Bigg\{\frac{1}{9} &+ \left(\frac{4}{15}\right) q_L q_M \gamma_L \gamma_M \exp[-3(D_S + D)t] \\ &+ \left(\frac{4}{15}\right) q_L q_S \gamma_L \gamma_S \exp[-3(D_M + D)t] \\ &+ \left(\frac{4}{15}\right) q_M q_S \gamma_M \gamma_S \exp[-3(D_L + D)t] \\ &+ \left(\frac{1}{15}\right)(\beta + \alpha)\exp[-(6D + 2\Delta)t] \\ &+ \left(\frac{1}{15}\right)(\beta - \alpha)\exp[-(6D - 2\Delta)t]\Bigg\} \end{aligned} \tag{1}$$

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where

$$\begin{aligned}
\beta &= q_L^2\gamma_L^2 + q_M^2\gamma_M^2 + q_S^2\gamma_S^2 - \frac{1}{3}\\
\alpha &= (D_L/\Delta)(q_M^2\gamma_M^2 + q_S^2\gamma_S^2 - 2q_L^2\gamma_L^2 + q_L^2 + \gamma_L^2)\\
&\quad + (D_M/\Delta)(q_L^2\gamma_L^2 + q_S^2\gamma_S^2 - 2q_M^2\gamma_M^2 + q_M^2 + \gamma_M^2)\\
&\quad + (D_S/\Delta)(q_L^2\gamma_L^2 + q_M^2\gamma_M^2 - 2q_S^2\gamma_S^2 + q_S^2 + \gamma_S^2) - (2D/\Delta)\\
D &= \frac{1}{3}(D_L + D_M + D_S)\\
\Delta &= (D_L^2 + D_M^2 + D_S^2 - D_LD_M - D_LD_S - D_MD_S)
\end{aligned}$$

The subscripts L, M and S indicate the long, medium and short axes of the probe, which is assumed to rotate as a rigid ellipsoid. The symbols q_i and γ_i indicate the projections of the transition moment vectors for the excitation and absorption, respectively, onto the indicated axis. The function P(t) represents the time course of the excited state concentration. D is the average diffusion coefficient and Δ is a measure of the degree of rotational anisotropy.

In practice, the measured dichroic decay traces do not allow the complete set of decay constants to be obtained. The currently accepted procedure is to assume isotropic diffusion (ID) in which case Eq. (1) simplifies to the single exponential function given by Eq. (2)[8].

$$OD_{=}(t) = P(t)\left\{\frac{1}{9} + \left(\frac{4}{15}\right)\left(q_Lq_M\gamma_L\gamma_M + q_Lq_S\gamma_L\gamma_S + q_Mq_S\gamma_M\gamma_S + \frac{1}{2}\beta\right) \times \exp[-6D_{\mathrm{ID}}t]\right\} \tag{2}$$

Here D_{ID} is the diffusion coefficient assuming isotropic diffusion.

There have been numerous attempts to relate experimentally determined rotational time constants to solvent properties, specifically viscosity. Among the most prevalent is the Debye-Stokes-Einstein (DSE) theory for diffusional motion[1,8] depicted by Eq. (3).

$$\tau_{\mathrm{or}} = \frac{\eta V}{k_B T}(Cf) + \tau_0 \tag{3}$$

Here η is the viscosity of the solvent, V is the hydrodynamic volume of the probe molecule, k_B is the Boltzmann constant, and T is the absolute temperature. The free-rotor relaxation time, τ_0, given by $(2\pi/9)(I/k_BT)^{1/2}$, is generally on the order of a few picoseconds, much shorter than diffusional motion, and is frequently omitted. Variations in DSE theory, where the coefficients f and C take on values related to probe shape and diffusion boundary conditions, respectively, have also appeared[8–14].

If the parameters f and C are to take on physically meaningful values τ_{OR} must be correctly evaluated. Notice that the diffusion coefficient is related to the relaxation time by $\tau_{OR} = (6D_0)^{-1}$ where D_0 is the diffusion coefficient for a spherical probe[8]. Thus, the evaluation of dichroic data using Eq. (2) leads directly to a determination of τ_{OR} only if the assumption of isotropic diffusion is justified. A critical assessment of the theories of rotational motion is not the subject of this report. Instead the method of evaluating rotational time constants is examined. This report highlights the error associated with the measured values of D_{ID}, and by extension τ_{OR}, through the fitting of simulated dichroic data traces and presents a method for testing the assumption of isotropic diffusion.

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METHOD

Looking back to the general solution of anisotropic rotation (Eq. (1)) it is evident that the five exponential terms are not truly independent. In fact, all five exponential terms are related to the individual diffusion constants for rotation about the three principal hydrodynamic axes. In turn, it has been shown that each of the diffusion coefficients can be linked using the axial ratios of the probe[15]. Thus, the ratio of the diffusion coefficients for the principal axes of a general ellipsoid can be scaled relative to the diffusion coefficient of a sphere of the same volume using:

$$\frac{D_L}{D_0} = \frac{3lms(m^2Q + s^2R)}{2(m^2 + s^2)} \tag{4a}$$

$$\frac{D_M}{D_0} = \frac{3lms(l^2P + s^2R)}{2(l^2 + s^2)} \tag{4b}$$

$$\frac{D_S}{D_0} = \frac{3lms(l^2P + m^2Q)}{2(l^2 + m^2)} \tag{4c}$$

where l, m and s are the dimensions of the long, medium and short axes of the general ellipsoid, respectively, and P, Q and R are the elliptic integrals:

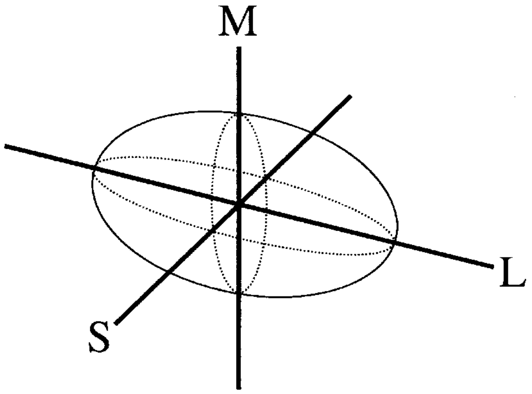
$$P = \int_0^\infty \frac{dx}{(l^2 + x)[(l^2 + x)(m^2 + x)(s^2 + x)]^{\frac{1}{2}}}$$

$$Q = \int_0^\infty \frac{dx}{(m^2+x)[(l^2+x)(m^2+x)(s^2+x)]^{\frac{1}{2}}}$$

$$R = \int_0^\infty \frac{dx}{(s^2+x)[(l^2+x)(m^2+x)(s^2+x)]^{\frac{1}{2}}}$$

In this context, the probe shape allows the scaling factors to be determined which are subsequently used to define the principal diffusion coefficients. Therefore, all of the exponential functions are linked to a single adjustable parameter. This parameter is identified as the diffusion coefficient of a spherical probe, D_0. What is then required is to determine how well the assumption of isotropic diffusion reproduces D_0 and how changes in q_i and γ_i influence the values determined[16].

In an effort to make our simulated data generally applicable we have chosen to assume a probe shape similar to a general ellipsoid and have adopted the axes labels as depicted in the following:



This probe shape has relevance for relatively high symmetry probes such as planar polyaromatic hydrocarbons. Specifically, the axial ratios used were $r_1 = l/m = 3$ and $r_2 = m/s = 2$, which are comparable to those of anthracene-based probes. The diffusion coefficients for the principal axis were obtained using the tabulated results of Small and Isenburg[15]. They are $D_L = 1.106D_0$; $D_M = 0.248D_0$; and $D_S = 0.273D_0$.

Further simplification of Eq. (1) results due to the requirement that the TMV directions for highly symmetric probes must be directed along the symmetry axes. If we assume the probe is indeed anthracene and that the hydrodynamic axes correspond to the symmetry axes, the projection of the TMV along the short axis will be zero for π-π* excited states. Thus

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$q_S = \gamma_S = 0$. The projections along the other two axis must be either 0 or 1 given by $q_L = \cos\theta$, $q_M = \sin\theta$ and $\gamma_L = \cos\phi$ and $\gamma_M = \sin\phi$, where θ and ϕ are either 0° or 90°. With these restrictions Eq. (1) simplifies to a biexponential function given by:

$$OD_{=}(t) = P(t)\left\{\frac{1}{9} + \left(\frac{1}{15}\right)(\beta + \alpha)\exp[-(6D + 2\Delta)t] + \left(\frac{1}{15}\right)(\beta - \alpha)\exp[-(6D - 2\Delta)t]\right\} \tag{5}$$

Notice that the exponents are independent of the projections q_i and γ_i but the relative contribution of each exponential the observed decay will change due to the dependence of α on q_i and γ_i. For high symmetry probes there are only three independent solutions to Eq. (5) corresponding to the sets of angles $(\theta,\phi) = (0,0)$, (0,90) or (90,0), and (90,90).

Dichroic traces were simulated using Eq. (5) and the assumed diffusion coefficients for the principle axes as described above using a standard spreadsheet program[17]. We have chosen to simulate the individual parallel and perpendicular dichroic traces as opposed to the dichroic ratio, orientation factor or anisotropy[18]. This choice has no impact on the conclusions drawn. The simulated traces corresponding to the optical density observed when the electric vector of the absorption beam is parallel to the excitation beam for the angles (θ,ϕ) of (0,0), (0,90) = (90,0) and (90,90) are show in Fig. 1 (closed circle: simulated data points). The perpendicular traces are also included[18]. All traces shown assume $P(t) = 1$ (i.e., long lived excited state) and $D_0 = 0.02$ au (au: arbitrary units)[19]. Each set of simulated traces was then fit to a single exponential decay using a non-linear least squares fitting routine to determine D_{ID}. The fits obtained are also included in Fig. 1 (solid lines). The D_{ID} values predicted where $D_{ID(0,0)} = 0.0052$ au, $D_{ID(0,90)} = 0.0053$ au and $D_{ID(90,90)} = 0.0113$ au. for panels a, b and c, respectively.

RESULTS AND DISCUSSION

Examination of Fig. 1 reveals that a single exponential function allows excellent, but not perfect, fits to the simulated data to be achieved. It is not surprising that most experimentally obtained dichroism decay traces, and similarly fluorescence anisotropy decay traces, are described by single exponential decays. The addition of even minimal random noise would

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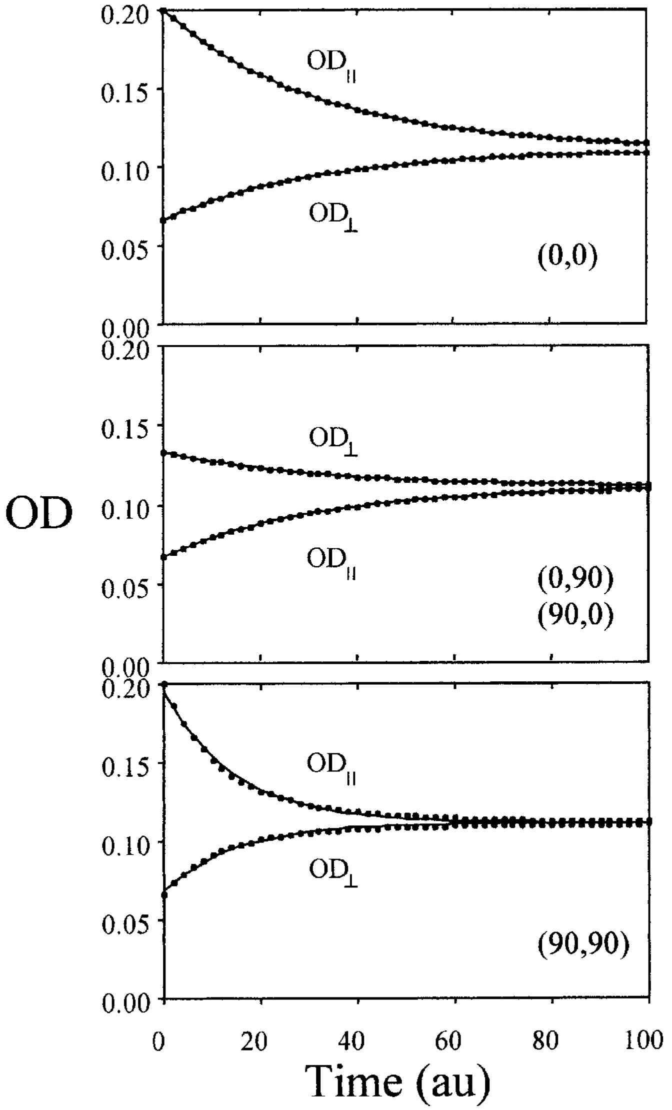

Figure 1. Simulated dichroic data traces (filled circles) obtained using Eq. (5) and $D_0 = 0.02$ au for different angles between the excitation TMV and the probe long axis (θ) and the absorption TMV and the probe long axis (ϕ) for both parallel and perpendicular polarizations of the absorption relative to the excitation. Top panel: $(\theta,\phi) = (0,0)$. The fit of a single exponential function gives $D_{ID} = 0.0052$ au. Middle panel: $(\theta,\phi) = (0,90) = (90,0)$. The fit of a single exponential function gives $D_{ID} = 0.0053$ au. Bottom panel: $(\theta,\phi) = (90,90)$. The fit of a single exponential function gives $D_{ID} = 0.0113$ au.

completely mask the differences between the simulated data and the fitted lines using single exponential functions. As a direct consequence of this finding, the ability to adequately fit experimental data with a single exponential function is not sufficient justification to assume isotropic diffusion.

The only difference between the sets of traces shown in Fig. 1 is the projection of the excitation and absorption (or emission) TMV onto the long axis of the probe. In effect, each simulated data set depicts a different orientation of the excited probe within the sample. The extent to which the long, medium and short axes must rotate to achieve randomization of sample will be different for each orientation. Thus, the overall decay will depend on D_L, D_M and D_S to differing extents. If the assumption of isotropic diffusion was justified, not only would the traces decay according to a single exponential function, but also the rate constants obtained would be identical for each orientation of the probe. Examination of the traces in Fig. 1 clearly shows that the rate of randomization is in fact different for each set of traces. This is confirmed by the results of the fitting where different D_{ID} values are obtained from fitting the three sets of traces. Clearly the assumption of isotropic diffusion is not justified, even though the fit of the simulated data to a single exponential function is acceptable.

Comparison of Eqs. (1) and (2) reveals that D_{ID} is related to D_L, D_M , and D_S through a complicated series of exponential functions. Clearly D_{ID} is not the average diffusion coefficient. Nor is it easily related to the spherical diffusion coefficient, D_0. Since the D_{ID} values obtained are significantly affected by the characteristics of the probe and details of the experimental observation conditions they cannot be good measures of the spherical diffusion coefficient D_0 and should not be used in the critical evaluation of diffusion theories.

The main difficulties with the measured values of D_{ID} arise from the fact that the assumption of isotropic diffusion is too severe. Isotropic diffusion requires the probe to be spherically symmetric or that solvent interactions conspire to allow long axes to rotate faster than short axis thus compensating for the differences in length. These requirements are certainly not appropriate for most organic dye molecules with, perhaps, one exception[14].

What is needed is a better method of determining the diffusion coefficients that does not require the assumption of isotropic diffusion. At first it might seem reasonable to fit the experimental data directly using Eq. (5). Unfortunately, reasonable fits to single exponential decays are observed in many cases and as described above are reasonable expected due relationship between the multiple exponential functions predicted by Eq. (1). The addition of a second or third exponential would simply increase the number of degrees of freedom in the fitting function, which in turn becomes hypersensitive to small fluctuations in the noise. Loose fits and poorly

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determined constants would result[20]. A different approach is needed which does not require increasing the number of degrees of freedom.

A possible solution is to link the diffusion coefficients for the individual axis to a spherical diffusion coefficient using the axial ratios of the probe. This is exactly what was done for the simulation of the data for this report. The experimental data would then be fit to a series of linked exponential decays with the same number of degrees of freedom as is used to fit a single exponential function. The advantage of the proposed method is that the shape of the probe dictates the scaling factors to be used and a physically meaningful value of the diffusion coefficient should result. The application of this linked exponential method for fitting the dichroic data obtained for symmetrical and unsymmetrical probes is currently under investigation.

CONCLUSION

Simulated dichroism data was used to examine the currently accepted procedure for evaluating rotational time constants. The assumption of isotropic diffusion was shown to be too severe and its use leads to the incorrect assessment of rotational time constants. Errors associated with the determination of these constants render them inappropriate for the critical evaluation of the Debye-Stokes-Einstein theory of rotational dynamics or extensions thereof.

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17. Microsoft Excel 98, Microsoft Corporation 1998.
18. The relationships that allow these order parameters to be evaluated are described in detail elsewhere (see for example Ref 5–8). The optical density observed when the probe beam is polarized perpendicular to the excitation beam, as a function of time, is given by: $OD_{\perp}(t) = \{1/6(P(t) - 1/2(OD_{=}(t))\}$. See reference 7.
19. Using the $D_0 = 0.02$ au the diffusion coefficients for the principal axes can be calculated: $D_L = 0.02212$ au; $D_M = 0.00496$ au; and $D_S = 0.00546$ au.
20. Technically, only one additional degree of freedom is required to fit a single exponential decay of dichroism (or anisotropy) data. P(t) is eliminated using the dichroic ratio or may be evaluated separately using magic angle data. The projections of the TMV onto the specific rotation axes can be determined a priori from theory or using symmetry considerations. However, in practice the evaluation of dichroic data generally requires two additional degrees of freedom because the observed initial dichroism (or anisotropy) is rarely as large as theory predicts. The linked exponential model also requires one additional degree of freedom.

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