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FLUORESCENCE DEPOLARIZATION FOR A PROBE IN A CYLINDRICAL PHASE

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A theory is presented relating polarized fluorescence intensities to orientational order parameters and correlation functions for a probe reorienting in a mesophase with local uniaxial symmetry and a random distribution of directors in a plane perpendicular to the axis of a macroscopically cylindrical structure. The theory should be applicable, for example, to experiments on lyotropic hexagonal phases as well as on nerve membranes. Explicit results are given for probes with transition moments parallel or perpendicular to the molecular axis.

1. Introduction

The theory of fluorescence depolarization for a probe in an ordered fluid such as a mono-domain nematic has been recently developed [1]. The theory has been generalized [2] to describe the fluorescence depolarization for a probe in a vesicle bilayer and applied to the interpretation of experiments performed on model and tumour cell membranes [3,4]. In both cases, we have written down equations for observables in terms of model-independent quantities, such as order parameters and correlation functions. This approach has the advantage of making contact with other techniques such as NMR and ESR where the same type of formalism is adopted. In turn, having a unifying formalism for the various techniques provides a first step in a combined-techniques attack on a problem as complex as the description of order and dynamics in liquid crystals and membranes. This seems particularly relevant since there is no universal technique which can determine with optimum precision second- and higher-rank order parameters, correlation functions or correlation times, etc. Thus, NMR is probably the best technique to measure order parameters of molecules or of submolecular groups, especially when coupled with isotopic substitution [5]. On the other hand, time-dependent fluorescence depolarization has the possibility, at least in principle, of yielding orientational correlation functions and, in suitable cases, fourth-rank order parameters not available from NMR. As an example, we have shown in ref. [1] that the time-dependent anisotropy of polarization of fluorescence can give, for a rigid rod-like probe with absorption and emission transition moments parallel to the long axis, the second-rank orientational correlation function $\phi_{00}(t)$, where

$$\phi_{mn}(t) = \langle D^2_{mn}(0)\, D^2_{mn}(t)^* \rangle , \tag{1}$$

and D^L_{mn} is the Wigner rotation matrix carrying the laboratory into the molecular frame. Here the angular brackets ⟨ ⟩ indicate an orientational ensemble average. We have also shown that for vesicles, i.e. macroscopically isotropic systems with a locally ordered structure, this very detailed dynamic information is somewhat reduced. Thus, instead of $\phi_{00}(t)$ or $\phi_{mn}(t)$ for other orientations of the transition moments only a relative orientation correlation function $\langle D^2_{nn}(t-0)\rangle$ can be obtained because of the overall isotropic symmetry of the system. Application of the combination properties of the Wigner rotation matrices [6] shows that

$$\langle D^2_{nn}(t-0)\rangle = \sum_q \phi_{qn}(t) , \tag{2}$$

i.e. only a sum of correlation functions can be obtained. It seems clear that having at hand a theory for systems of interest with different symmetry constraints can be useful in planning experimental work as well as being essential for the interpretation of actual experimental data.

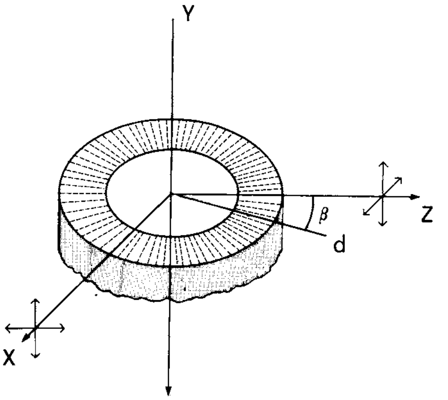

Fig. 1. A schematic section of a cylindrical phase or a nerve membrane with a radial two-dimensional isotropic distribution of directors. The geometry of the fluorescence depolarization experiment is also shown. The light used to excite the probe comes from the X direction with vertical or horizontal polarization and is observed along the Z direction through a vertical or horizontal polarizer. We call β the angle between a local director d and the Z axis.

In this paper, we generalize the theory of fluorescence depolarization to systems with locally uniaxial structure and macroscopic cylindrical symmetry. Cylindrical phases are quite common among lyotropic liquid crystalline phases. Indeed, theories for interpreting ESR and NMR data for molecules dissolved in these phase have already been put forward by, for example, Seelig [7]. Cylindrical distributions of bilayers are also found in nerve membranes [8]. Here fluorescence experiments have been performed, for example by Tasaki et al. [9], on giant squid axons. An interesting review of such work has been given by Conti [8].

We consider the schematic arrangement shown in fig. 1 for the fluorescence polarization experiment. The cylinder axis is along Y, with the local directors isotropically distributed in the XZ plane. Light enters from the X direction through a polarizer set along the vertical (Y) or horizontal (Z) direction. The fluorescence light emitted by the probe is observed along the Z direction (perpendicular geometry) as shown in fig. 1 or along the X axis itself (transparency arrangement). In any case, the emitted light is collected through vertical or horizontal polarizers. The intensities of interest are therefore

(a) I_{ZZ}, I_{ZY} (horizontal excitation, transparency arrangement),

(b) I_{YY}, I_{YZ} (vertical excitation, transparency arrangement),

(c) I_{YY}, I_{YX} (vertical excitation, perpendicular arrangement),

(d) I_{ZY}, I_{ZX} (horizontal excitation, perpendicular arrangement),

where I_{if} indicates the excitation polarizer along i and emission polarizer along f. Because of the symmetry of the system, we have with the geometry chosen the following five independent intensities:

$$I_{ZZ}(t) = I_{XX}(t)\,, \tag{3a}$$

$$I_{ZY}(t) = I_{XY}(t)\,, \tag{3b}$$

$$I_{ZX}(t) = I_{XZ}(t)\,, \tag{3c}$$

$$I_{YY}(t)\,, \tag{3d}$$

$$I_{YX}(t) = I_{YZ}(t)\,. \tag{3e}$$

We now proceed to evaluate these intensities in terms of molecular properties through a generalization of the formalism introduced in refs. [1,2].

2. Theory

We have shown [2] that the time-dependent fluorescence intensities $I_{if}(t)$ for incoming polarization direction $\boldsymbol{e}_i$ and analyzing direction $\boldsymbol{e}_f$ can be written as a sum of rank-labeled contributions $I_{if}^{LL'}(t)$:

$$I_{if}(t) = F(t) \sum_{L,L'} I_{if}^{LL'}(t)\,, \quad L, L' = 0, 2\,, \tag{4}$$

where $F(t)$ is the intrinsic fluorescence decay function. The contribution $I_{if}^{LL'}(t)$ is

$$I_{if}^{LL'}(t) = \sum E_i^{L,m*} E_f^{L'm'} \langle A_{\text{lab}}^{L,m}(0) \bar{A}_{\text{lab}}^{L',m}(t)^* \rangle\,, \tag{5}$$

with $E_i^{L,m}$ and $E_f^{L,m}$ irreducible components of the polarization tensor [2] in excitation and emission, while $\langle A_{\text{lab}}^{L,m}(0) \bar{A}_{\text{lab}}^{L'm'}(t)^* \rangle$ is a cross correlation function of the irreducible components of the absorption and emission tensors $\mathbf{A}$ and $\bar{\mathbf{A}}$. These are, in turn, constructed from the absorption and emission dipole moments μ, $\bar{\mu}$ as the direct product $\mathbf{A} = \boldsymbol{\mu} \otimes \boldsymbol{\mu}$, $\bar{\mathbf{A}} = \bar{\boldsymbol{\mu}} \otimes \bar{\boldsymbol{\mu}}$. Note that all the geometric information about the experiment is contained in the tensors $\mathbf{E}_i$ and $\mathbf{E}_f$. Explicit expressions for the relevant intensities can be obtained by substituting components of $\mathbf{E}_i$ and $\mathbf{E}_f$

for various geometries and working out the necessary correlation functions. We now consider the calculation of the absorption–emission cross correlation function. To do this, we rewrite our laboratory components $A_{\mathrm{lab}}^{L,m}$ in terms of those in the molecular frame by first rotating from laboratory to local director frame and then from this system to the molecule frame:

$$A_{\mathrm{lab}}^{L,m} = \sum D_{mn}^{L}(d-L)^{*} D_{nq}^{L}(M-d)^{*} A_{\mathrm{mol}}^{L,q} , \qquad (6)$$

where $D_{mn}^{L}(F'-F)$ indicates the Wigner rotation matrix from F to F'. Here the local director lies in the XZ plane (cf. fig. 1) and the rotation $D_{mn}^{L}(d-L) = = d_{mn}^{L}(\beta)$, where $d_{mn}^{L}(\beta)$ indicates a reduced or small Wigner rotation [6]. Substitution of $A_{\mathrm{lab}}^{l,m}$, $\bar{A}_{\mathrm{lab}}^{L',m}$ gives

$$I_{i,f}^{0,0} = 1/9 , \qquad (7)$$

$$I_{i,f}^{0,2} = \frac{1}{3} \sum (-)^{m} E_{f}^{2,m} \langle d_{-m0}^{2}(\beta)\rangle_{\mathrm{d}} \langle P_2\rangle \bar{A}_{\mathrm{mol}}^{2,0} , \qquad (8)$$

$$I_{i,f}^{2,0} = \frac{1}{3} \sum E_{i}^{2,m^{*}} \langle d_{m0}^{2}(\beta)\rangle_{\mathrm{d}} \langle P_2\rangle A_{\mathrm{mol}}^{2,0} , \qquad (9)$$

$$I_{i,f}^{2,2} = \sum E_{i}^{2,m^{*}} E_{f}^{2,m'} (-)^{m'-n} \langle d_{mn}^{2}(\beta) d_{-m'-n}^{2}(\beta)\rangle_{\mathrm{d}}$$
$$\times \phi_{nq}^{2}(t) A_{\mathrm{mol}}^{2,q} \bar{A}_{\mathrm{mol}}^{2,q^{*}} , \qquad (10)$$

where $\langle\ \rangle_{\mathrm{d}}$ indicates an average over the director distribution and we neglect the possibility of director fluctuations during the timescale of the experiment. It can be seen that only $I_{i,f}^{2,2}$ contains the dynamic information. In deriving eqs. (7)–(10), the relation

$$D_{mn}^{L^{*}} = (-)^{m-n} D_{-m-n}^{L}$$

has been used. We have made the assumption that the locally ordered structure has uniaxial symmetry, and that the fluorescent probe molecule also has cylindrical symmetry. This means that the molecule is assumed to be rod-like or disk-like. In this case, symmetry requires [10]

$$\langle D_{mn}^{2}(M-d)\rangle = \langle P_2\rangle \delta_{m0}\delta_{n0} , \qquad (11)$$

$$\langle D_{mn}^{2}(M_0-d) D_{m'n'}^{2}(M_t-d)^{*}\rangle =$$
$$= \langle D_{mn}^{2}(M_0-d) D_{mn}^{2}(M_t-d)^{*}\rangle \delta_{mm'}\delta_{nn'}$$
$$= \phi_{mn}(t)\delta_{mm'}\delta_{nn'} . \qquad (12)$$

The average over the director distribution in eq. (10) can be performed after coupling the two small Wigner matrices using the Clebsch–Gordan series [6] as

$$\langle d_{mn}^{2}(\beta) d_{-m'-n}^{2}(\beta)\rangle_{\mathrm{d}}$$
$$= \sum_{J} C(22J; m, -m') C(22J; n, -n) \langle d_{m-m',0}^{J}(\beta)\rangle_{\mathrm{d}} . \qquad (13)$$

Thus, for eqs. (8)–(10) we only need the two-dimensional isotropic averages of the small Wigner rotation matrices, i.e.

$$\langle d_{mn}^{L}\rangle_{\mathrm{d}} = \frac{1}{\pi}\int_{0}^{\pi} d_{mn}^{L}(\beta)\,\mathrm{d}\beta . \qquad (14)$$

These are obtained by direct integration of the standard Racah expression [6]. We find

$$\langle d_{mn}^{L}\rangle_{\mathrm{d}} = \sum_{k} (-)^{k} [(L+m)!(L-m)!(L+n)!(L-n)!]^{1/2}$$
$$\times \Gamma(L-k+\tfrac{1}{2}(m-n+1)\Gamma(k+\tfrac{1}{2}(n-m+1) ,$$
$$\times [(L+m-k)!(L-n-k)!k!(k+n-m)!L!]^{-1} , \qquad (15)$$

where $\Gamma(x)$ is the gamma function [12] and the sum over k is taken over all integers leaving the argument of the factorials non-negative.

We can now compute explicit formulas for the fluorescence intensities of a probe with given transition moments in terms of order parameters and correlation functions. This entails a large number of algebraic manipulations, which we have performed on a computer using a procedure written in the computer algebra system SCHOONSCHIP [11]. We obtain for a probe with absorption and emission moments parallel to the molecular axis, a case approximated by the popular probe 1,6-diphenylhexatriene (DPH),

$$I_{ZZ}(t)/F(t) = \tfrac{1}{9} + \tfrac{1}{9}\langle P_2\rangle + \tfrac{11}{72}\phi_{00}(t) + \tfrac{1}{6}\phi_{10}(t)$$
$$+ \tfrac{1}{8}\phi_{20}(t) , \qquad (16a)$$

$$I_{ZY}(t)/F(t) = \tfrac{1}{9} - \tfrac{1}{18}\langle P_2\rangle - \tfrac{1}{18}\phi_{00}(t) - \tfrac{1}{6}\phi_{20}(t) , \qquad (16b)$$

$$I_{ZX}(t)/F(t) = \tfrac{1}{9} + \tfrac{1}{9}\langle P_2\rangle - \tfrac{7}{72}\phi_{00}(t) - \tfrac{1}{6}\phi_{10}(t)$$
$$+ \tfrac{1}{24}\phi_{20}(t) , \qquad (16c)$$

$$I_{YY}(t)/F(t) = \tfrac{1}{9} - \tfrac{2}{9}\langle P_2\rangle + \tfrac{1}{9}\phi_{00}(t) + \tfrac{1}{3}\phi_{20}(t)\,, \quad (16\mathrm{d})$$

$$I_{YX}(t)/F(t) = \tfrac{1}{9} - \tfrac{1}{18}\langle P_2\rangle - \tfrac{1}{18}\phi_{00}(t) - \tfrac{1}{6}\phi_{20}(t)\,. \quad (16\mathrm{e})$$

As an example, we shall evaluate the fluorescence polarization anisotropy ratio

$$\begin{aligned} r_{YY,XX}(t) &= [I_{YY}(t) - I_{YX}(t)]/[I_{YY}(t) + 2I_{YX}(t)] \\ &= [\phi_{00}(t) + 3\phi_{20}(t) - \langle P_2\rangle]/(2 - 2\langle P_2\rangle)\,, \end{aligned} \quad (17)$$

obtainable for the geometry in fig. 1. We note first that eq. (16d) shows that, for complete local order, i.e. $\langle P_2\rangle = \langle P_4\rangle = 1$, there is no absorption for a DPH-like probe. The limiting values for short and long times are in general

$$r_{YY,YX}(0) = (\tfrac{2}{15} - \tfrac{11}{42}\langle P_2\rangle + \tfrac{9}{70}\langle P_4\rangle)/(\tfrac{1}{3} - \tfrac{1}{3}\langle P_2\rangle) \quad (18)$$

and

$$r_{YY,YX}(\infty) = -\tfrac{1}{2}\langle P_2\rangle\,. \quad (19)$$

Thus, we see that in contrast to the case of three-dimensional isotropic systems, such as vesicles, some information on fourth-rank order parameters is contained in the decay provided the probe has a short fluorescence time compared with the reorientation decay time. It is important to arrive at explicit expressions such as these because then a computer program to simulate or predict fluorescence depolarization can be written even on a microcomputer, when a model for the correlation functions $\phi_{mn}(t)$ is also available. As an illustration, we have calculated the predicted fluorescence polarization decay for a probe with both transition moments parallel to the long axis according to the strong-collision model [4,13] of reorientation. The results for $\langle P_2\rangle$ varying between 0.2 and 0.8 are shown in fig. 2. It is interesting to note that, at least for times short compared with the reorientation τ_0, the anisotropy ratio changes sign as the order increases. This hints that the experiment should provide a sensitive way of obtaining information on local ordering in a relatively viscous environment.

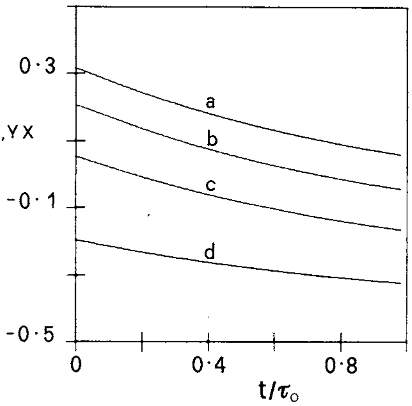

Fig. 2. The time-dependent polarization anisotropy ratio $r_{YY,YX}$ for a rod-like probe with absorption and emission moments parallel to the molecule axis. Here we assume a strong-collision model for the long-axis reorientation with a characteristic time τ_0 and order parameter $\langle P_2\rangle = 0.2$ (a), 0.4 (b), 0.6 (c) and 0.8 (d). The values of $\langle P_4\rangle$ required by the model are those expected for a Maier–Saupe distribution (see, for example, ref. [3]).

Exact intensity expressions can be obtained for any other orientation of transition moments. Here we report just another case, that of a probe with absorption and emission moments parallel to each other but perpendicular to the molecule axis (i.e. $\boldsymbol{u}\|\boldsymbol{u}\|y$), which is relevant, for example, to various condensed aromatic hydrocarbons. Working through eqs. (4)–(10), we find

$$\begin{aligned} I_{ZZ}(t)/F(t) &= \tfrac{1}{9} - \tfrac{1}{18}\langle P_2\rangle + \tfrac{11}{288}\phi_{00}(t) + \tfrac{11}{96}\phi_{02}(t) \\ &\quad + \tfrac{1}{24}\phi_{10}(t) + \tfrac{1}{8}\phi_{12}(t) + \tfrac{1}{32}\phi_{20}(t) + \tfrac{3}{32}\phi_{22}(t)\,, \end{aligned} \quad (20\mathrm{a})$$

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$$\begin{aligned} I_{XY}(t)/F(t) &= \tfrac{1}{9} + \tfrac{1}{36}\langle P_2\rangle - \tfrac{1}{72}\phi_{00}(t) - \tfrac{1}{24}\phi_{02}(t) \\ &\quad - \tfrac{1}{24}\phi_{20}(t) - \tfrac{1}{8}\phi_{22}(t)\,, \end{aligned} \quad (20\mathrm{c})$$

$$\begin{aligned} I_{YY}(t)/F(t) &= \tfrac{1}{9} + \tfrac{1}{9}\langle P_2\rangle + \tfrac{1}{36}\phi_{00}(t) + \tfrac{1}{12}\phi_{02}(t) \\ &\quad + \tfrac{1}{12}\phi_{20}(t) + \tfrac{1}{4}\phi_{22}(t)\,, \end{aligned} \quad (20\mathrm{d})$$

$$\begin{aligned} I_{YX}(t)/F(t) &= \tfrac{1}{9} + \tfrac{1}{36}\langle P_2\rangle - \tfrac{1}{72}\phi_{00}(t) - \tfrac{1}{24}\phi_{02}(t) \\ &\quad - \tfrac{1}{24}\phi_{20}(t) - \tfrac{1}{8}\phi_{22}(t)\,. \end{aligned} \quad (20\mathrm{e})$$

From these expressions, we find the polarization anisotropy ratio

$$\begin{aligned} r_{YY,YX}(t) &= 6\,\{\tfrac{1}{12}\langle P_2\rangle + \tfrac{1}{72}\phi_{00}(t) \\ &\quad + \tfrac{1}{24}[\phi_{02}(t) + \phi_{20}(t)] + \tfrac{1}{8}\phi_{22}(t)\} \\ &\quad \times (2 + \langle P_2\rangle)^{-1}\,, \end{aligned} \quad (21)$$

with limiting values

$$r_{YY,YX}(0) = \left(\tfrac{2}{15} + \tfrac{11}{84}\langle P_2\rangle + \tfrac{27}{560}\langle P_4\rangle\right) \times \left(\tfrac{1}{3} + \tfrac{1}{6}\langle P_2\rangle\right)^{-1} \tag{22}$$

for short times and

$$r_{YY,YX}(\infty) = \tfrac{1}{4}\langle P_2\rangle \tag{23}$$

for long times. Note that, for this configuration of transition moments, absorption can occur even in the limit of complete order. Explicit models of reorientation could be used, as we did previously, to obtain a plot of the predicted time evolution. This is, however, quite straightforward and for our purpose it is enough to point out that a good indication of the behaviour can be obtained even by interpolating between the two limiting values in eqs. (22), (23).

In conclusion, we have shown that the analysis of fluorescence polarization data for a dye in a cylindrical phase can provide information on the local order and dynamics of the molecule. Expressions for the fluorescence intensities have been obtained for any orientation of the absorption and emission moments in the molecular frame for a probe with effective cylindrical symmetry [1]. We believe these expressions will be particularly useful when planning experimental work in order to choose the most appropriate fluorescent probe and geometry.

Acknowledgement

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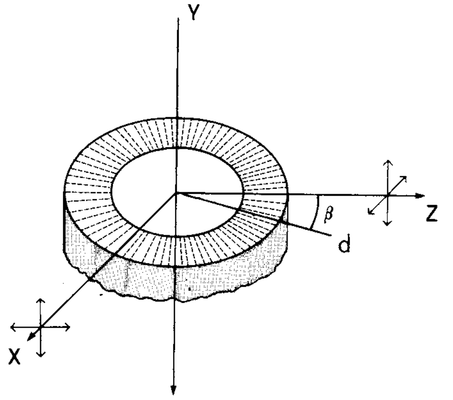

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We now proceed to evaluate these intensities in terms of molecular properties through a generalization of the formalism introduced in refs. [1,2].

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We have shown [2] that the time-dependent fluorescence intensities $I_{if}(t)$ for incoming polarization direction $\boldsymbol{e}_i$ and analyzing direction $\boldsymbol{e}_f$ can be written as a sum of rank-labeled contributions $I_{if}^{LL'}(t)$:

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with $E_i^{L,m}$ and $E_f^{L,m}$ irreducible components of the polarization tensor [2] in excitation and emission, while $\langle A_{\mathrm{lab}}^{L,m}(0)\, \bar{A}_{\mathrm{lab}}^{L'm'}(t)^{*} \rangle$ is a cross correlation function of the irreducible components of the absorption and emission tensors $\mathbf{A}$ and $\bar{\mathbf{A}}$. These are, in turn, constructed from the absorption and emission dipole moments μ, $\bar{\mu}$ as the direct product $\mathbf{A} = \boldsymbol{\mu} \otimes \boldsymbol{\mu}$, $\bar{\mathbf{A}} = \bar{\boldsymbol{\mu}} \otimes \bar{\boldsymbol{\mu}}$. Note that all the geometric information about the experiment is contained in the tensors $\mathbf{E}_i$ and $\mathbf{E}_f$. Explicit expressions for the relevant intensities can be obtained by substituting components of $\mathbf{E}_i$ and $\mathbf{E}_f$

$$I_{YY}(t)/F(t) = \tfrac{1}{9} - \tfrac{2}{9}\langle P_2\rangle + \tfrac{1}{9}\phi_{00}(t) + \tfrac{1}{3}\phi_{20}(t)\,, \tag{16d}$$

$$I_{YX}(t)/F(t) = \tfrac{1}{9} - \tfrac{1}{18}\langle P_2\rangle - \tfrac{1}{18}\phi_{00}(t) - \tfrac{1}{6}\phi_{20}(t)\,. \tag{16e}$$

As an example, we shall evaluate the fluorescence polarization anisotropy ratio

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Exact intensity expressions can be obtained for any other orientation of transition moments. Here we report just another case, that of a probe with absorption and emission moments parallel to each other but perpendicular to the molecule axis (i.e. $\boldsymbol{u} \| \boldsymbol{u} \| y$), which is relevant, for example, to various condensed aromatic hydrocarbons. Working through eqs. (4)–(10), we find

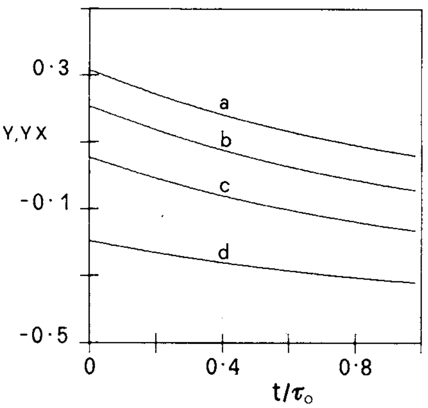

Fig. 2. The time-dependent polarization anisotropy ratio $r_{YY,YX}$ for a rod-like probe with absorption and emission moments parallel to the molecule axis. Here we assume a strong-collision model for the long-axis reorientation with a characteristic time τ_0 and order parameter $\langle P_2\rangle = 0.2$ (a), 0.4 (b), 0.6 (c) and 0.8 (d). The values of $\langle P_4\rangle$ required by the model are those expected for a Maier–Saupe distribution (see, for example, ref. [3]).

$$\begin{aligned} I_{ZZ}(t)/F(t) &= \tfrac{1}{9} - \tfrac{1}{18}\langle P_2\rangle + \tfrac{11}{288}\phi_{00}(t) + \tfrac{11}{96}\phi_{02}(t) \\ &\quad + \tfrac{1}{24}\phi_{10}(t) + \tfrac{1}{8}\phi_{12}(t) + \tfrac{1}{32}\phi_{20}(t) + \tfrac{3}{32}\phi_{22}(t)\,, \end{aligned} \tag{20a}$$

$$\begin{aligned} I_{ZX}(t)/F(t) &= \tfrac{1}{9} - \tfrac{1}{18}\langle P_2\rangle - \tfrac{7}{288}\phi_{00}(t) - \tfrac{7}{96}\phi_{02}(t) \\ &\quad - \tfrac{1}{24}\phi_{10}(t) - \tfrac{1}{8}\phi_{12}(t) + \tfrac{1}{96}\phi_{20}(t) + \tfrac{1}{32}\phi_{22}(t)\,, \end{aligned} \tag{20b}$$

$$\begin{aligned} I_{XY}(t)/F(t) &= \tfrac{1}{9} + \tfrac{1}{36}\langle P_2\rangle - \tfrac{1}{72}\phi_{00}(t) - \tfrac{1}{24}\phi_{02}(t) \\ &\quad - \tfrac{1}{24}\phi_{20}(t) - \tfrac{1}{8}\phi_{22}(t)\,, \end{aligned} \tag{20c}$$

$$\begin{aligned} I_{YY}(t)/F(t) &= \tfrac{1}{9} + \tfrac{1}{9}\langle P_2\rangle + \tfrac{1}{36}\phi_{00}(t) + \tfrac{1}{12}\phi_{02}(t) \\ &\quad + \tfrac{1}{12}\phi_{20}(t) + \tfrac{1}{4}\phi_{22}(t)\,, \end{aligned} \tag{20d}$$

$$\begin{aligned} I_{YX}(t)/F(t) &= \tfrac{1}{9} + \tfrac{1}{36}\langle P_2\rangle - \tfrac{1}{72}\phi_{00}(t) - \tfrac{1}{24}\phi_{02}(t) \\ &\quad - \tfrac{1}{24}\phi_{20}(t) - \tfrac{1}{8}\phi_{22}(t)\,. \end{aligned} \tag{20e}$$

From these expressions, we find the polarization anisotropy ratio

$$\begin{aligned} r_{YY,YX}(t) &= 6\,\{\tfrac{1}{12}\langle P_2\rangle + \tfrac{1}{72}\phi_{00}(t) \\ &\quad + \tfrac{1}{24}[\phi_{02}(t) + \phi_{20}(t)] + \tfrac{1}{8}\phi_{22}(t)\} \\ &\quad \times (2 + \langle P_2\rangle)^{-1}\,, \end{aligned} \tag{21}$$

with limiting values