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DYNAMICAL THEORY OF FLUORESCENCE POLARIZATION IN A PLANAR ARRAY OF ORIENTED PIGMENT MOLECULES

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A theory is developed appropriate for the analysis of fluorescence polarization experiments with pigment molecules in a planar array (plane membrane). Especially rotatory and oscillatory dynamics of the pigment molecules are considered. Three model calculations are performed, which describe the following different situations:

(a) Rotational diffusion of molecules around the normal to the plane membrane.

(b) Oscillatory diffusion of molecules with respect to this normal.

(c) As a two-dimensional example the independent superposition of both types of motion.

Central point of these model calculations is the determination of an intensity of emission function, from which in practical applications the measured fluorescence intensities may uniquely be calculated.

1. Introduction

In the last years the use of fluorescence techniques has become an important method in the study of (biological and model) membranes. Extensive references to literature on this subjects may be found in the review article of Radda and Vanderkooi [1]. Subject of this paper are the rotatory and oscillatory dynamics of fluorescent probes in plane membranes and their effect on fluorescence polarization measurements.

It was Perrin [2–4] who first investigated the influence of Brownian *rotation* on fluorescence polarization: Regard a sample of fluorescent molecules, which are randomly oriented and excited with linearly polarized light. The degree of polarization p of fluorescence observed through analyzing polarizers is defined as

$$p = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} \tag{1}$$

where $I_\parallel$ is the polarized intensity parallel to the electric vector of incident light and $I_\perp$ perpendicular. If the molecules are rigidly held during the lifetime of the excited state, p takes the characteristic value p_0, given by [3]

$$p_0 = \frac{3\cos^2\Gamma - 1}{\cos^2\Gamma + 3} \tag{2}$$

where Γ is the angle between dipole moments of emission and absorption. For $\Gamma = 0$: $p_0 = \frac{1}{2}$. If Brownian rotation effects a partial depolarization, the observed degree of polarization p is given by the Perrin equation

$$\frac{1}{p} - \frac{1}{3} = \left(\frac{1}{p_0} - \frac{1}{3}\right)\left(1 + \frac{3\tau}{\rho}\right) \tag{3}$$

where ρ is the Brownian rotational relaxation time and τ the lifetime of the excited fluorescent state. Since that time Perrin's investigations have been extended to anisotropic Brownian rotations by several authors [5–8].

In the last years considerable work on the theoretical investigation of fluorescence polarization experiments for measuring preferred *orientation* has been done. Desper and Kimura [9] have developed a mathematical theory for biaxial and uniaxial distributions of transition moments under the restrictions that (a) the angle between the electronic transition moments of absorption and emission is zero and (b) the chromophoric group of the fluorescent molecule does not rotate appreciably during the lifetime of the excited state. Theoretical and experimental studies on molecules bound to artificial membranes have been done by Badley et al. [10,11] and Yguerabide and Stryer [12]. Considerable effort has been made by polymer chemists to investigate orientations. Nishijima and coworkers (e.g. [13]) have published extensively on the estimation of orientation distribution from the variation of fluorescence polarization with changes in the relative positions of sample and light beams. By Kimura et al. [14] the effect of restricted thermal agitation has been considered.

Recently we have studied fluorescence polarization of flavins incorporated into artificial plane membranes [15]. In line with this study we started a systematic theoretical approach to the analysis of fluorescence polarization experiments with pigment molecules in a planar array. Random orientation of molecules was assumed within the membrane plane, i.e. rotational symmetry with the normal to the membrane as axis of rotation. Quantitative calculations for different model distributions were performed. For each model distribution we assumed the existence of a characteristic relaxation time determined by the mobility of molecules and calculated two limiting cases. In the first case, the orientation relaxation time is short, in the second it is long compared with the lifetime of the fluorescent state. This paper continues the work in [15]. We present dynamic equations describing the rotatory and oscillatory motion of (fluorescent) pigment molecules. This dynamic approach admits a treatment of the especially interesting situation, where the characteristic orientation relaxation times and the lifetime of fluorescence are comparable. Explicit calculations are performed for three different model situations: First the case where the pigment molecules may do rotational diffusion about the normal to the membrane. Second oscillatory diffusion about a preferred orientation with respect to this normal. Finally, as a simple two-dimensional example, the superposition of both types of motion is treated. In these model calculations we have restricted to the case $\Gamma = 0$.

Analogous calculations on the rotational diffusion have recently been presented by Wahl [16], who has investigated the fluorescence depolarization due to local motion of chromophores attached to macromolecules in *isotropic* solutions (e.g. [16,17]). The relations (22) and (32) below are in agreement with Wahl's calculations in [16].

2. General theory

2.1. Geometry of fluorescence polarization experiments

For description of the geometry of the fluorescence polarization experiment with plane membranes we introduce appropriate angle coordinates. Essentially we will follow the notation used in [15].

The geometry of an experimental arrangement for measuring fluorescence polarization is shown in figs. 1a and b: A linearly polarized light beam excites the fluorescent sample. The fluorescence light is measured through a polarizing analyzer. We assume the sample to be small compared with the distance to the analyzer, so that the calculations of fluorescence intensities below may be done as if all fluorescent molecules are concentrated in one point (*far-field assumption*).

We define two planes: *the plane of incidence*, formed by the light beam and the normal n to the membrane, and *the plane of measurement*, formed by n and the line between the fluorescent sample and the analyzer. According to fig. 1b we call the angle between these two planes φ_0. Further angles introduced in figs. 1a and b determining the geometry are:

α angle between the incident light beam and the normal n;
β the angle between n and the line from the sample to the analyzer;
ϑ angle between the electric vector of the linearly polarized incident light and the plane of incidence;

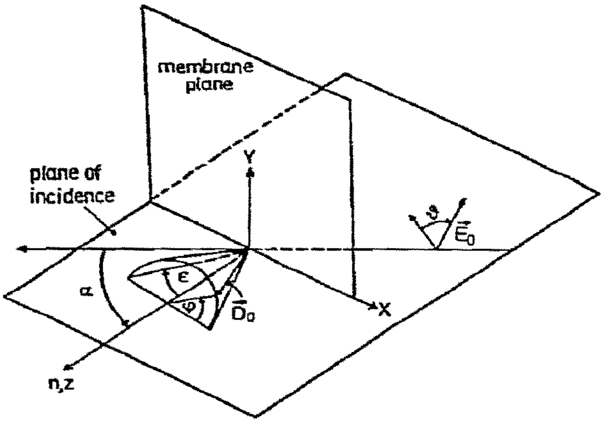

Fig. 1a.

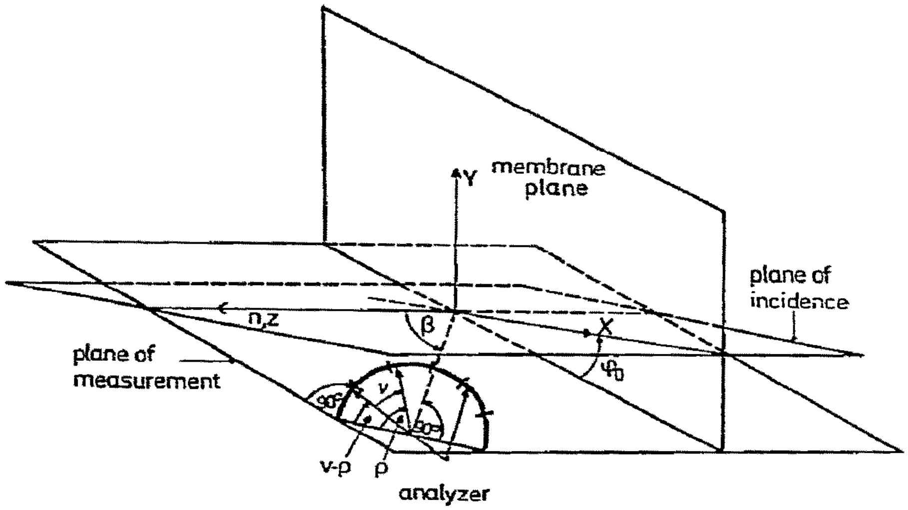

Fig. 1b.

Fig. 1. Geometry cf fluorescence polarization experiments: (a) Introduction of appropriate angle coordinates denoting the polarization direction of incident light E_0 and the orientation D_0 of a transition moment with respect to the membrane. (b) Orientation of the planes of incidence and measurement. Introduction of angles denoting the position of the analyzer.

ϵ polar angle with respect to n as polar axis

φ azimuthal angle, rotational symmetry of a distribution means independenc of φ;

ρ direction of polarization of the analyzer.

Additionally for the theoretical investigations of the experiments the following secondary angles are needed:

ψ angle between the direction of polarization (electric vector) of incident light and a dipole moment of absorption described by ϵ, φ;

μ angle between a dipole of emission described by ϵ, φ and the plane of the analyzing polarizer.

ν angle between the projection of the dipole to this plane and the direction of polarization ρ of the analyzer.

By elementary trigonometric considerations, which have been done in [15], one gets the following dependence of ψ, μ, ν on the other angles:

$$\cos\psi = -\sin\epsilon\cos\vartheta\cos\alpha\cos\varphi + \sin\epsilon\sin\vartheta\sin\varphi - \cos\epsilon\cos\vartheta\sin\alpha \tag{4}$$

$$\sin\mu = \cos\beta\sin\epsilon\cos(\varphi+\varphi_0) + \sin\beta\sin\epsilon \tag{5}$$

$$\operatorname{cotg}(\rho-\nu) = \frac{\cos\beta\cos\epsilon - \sin\epsilon\cos(\varphi+\varphi_0)\sin\beta}{\sin\epsilon\sin(\varphi+\varphi_0)}\ . \tag{6}$$

2.2. *The fluorescence process*

The whole process taking place in the experiment may be divided schematically into four sections: Absorption, rearrangement of molecules, emission and measurement.

2.2.1. *Absorption*

The intensity of absorption for an individual transition moment is proportional to $\cos^2\psi$ with $\cos\psi$ given by eq. (4). We introduce the distribution function F_A of dipole moments of absorption, being defined as a density on the unit sphere. F_A is independent of φ, because the orientation within the plane of membrane is random. Hence for the intensity of absorption $I_A(\epsilon, \varphi)$ holds

$$I_A(\epsilon,\varphi) = P_A F_A(\epsilon)\cos^2\psi(\epsilon,\varphi,\alpha,\vartheta) \tag{7a}$$

with the condition of normalization $2\pi\int_{\epsilon=0}^{\pi} F_A \sin\epsilon\, d\epsilon = 1$.

For determination of the proportionality factor P_A one needs the knowledge of different quantities, e.g. intensity of incident light, area concentration of the fluorescent molecules and an apparatus constant. We do not further specify this factor and introduce the reduced intensity of absorption $\bar{I}_A = I_A/P_A$. Hence for $\bar{I}_A$

$$\bar{I}_A = F_A(\epsilon)\cos^2\psi(\epsilon,\varphi,\alpha,\vartheta)\,. \tag{7b}$$

2.2.2. *Rearrangement*

First, the transition moments of absorption and emission may not be parallel within the molecule. The angle Γ between both moments may often be determined by auxiliary experiments with the use of eq. (2). The effect is independent of the dynamics of the molecule within its environment. Its relaxation time may be neglected. In the explicit model calculations below Γ is assumed to be zero.

Second, energy transfer between fluorescent molecules may cause a significant rearrangement. The effect of energy transfer is also not considered in this paper.

Third, a rearrangement of transition moments may occur by rotatory or oscillatory motion of molecules in the excited state. This motion is determined by the dynamical properties of the molecules in the excited state, which may be different from those in the unexcited state. It becomes important when the lifetime of fluorescence τ is at least comparable with or greater than the typical relaxation time of molecular motion.

2.2.3. *Emission and measurement*

Taking into account these possibilities of rearrangement of transitions moments, one can calculate from the intensity of absorption an intensity of emission $I_E(\epsilon, \varphi)$. The contribution of a single dipole moment of emission to the intensity of fluorescence light measured through an analyzer with a direction of polarization ρ follows from the classical relations for dipole radiation and polarization characteristics to be proportional to $\cos^2\mu$ and $\cos^2\nu$. Hence the total measured intensity I_M is given by integration over the unit sphere with I_E as weighting function

$$I_M = P_E \int_{\varphi=0}^{2\pi} \int_{\epsilon=0}^{\pi} I_E(\epsilon, \varphi) \cos^2\mu \cos^2\nu \sin\epsilon \, d\epsilon \, d\varphi . \tag{8a}$$

According to eq. (8a) I_E is defined as a density on the unit sphere. The proportionality factor is additionally depending on the quantum yield of fluorescence. Again we go over to the reduced intensity

$$\bar{I}_M = \int_{\varphi=0}^{2\pi} \int_{\epsilon=0}^{\pi} \bar{I}_E \cos^2\mu \cos^2\nu \sin\epsilon \, d\epsilon \, d\varphi . \tag{8b}$$

The central point of the model calculations below in sects. 3.1–3.3 will be the calculation of the intensity of emission I_E, from which the measured intensities I_M are uniquely determined through eq. (8).

2.3. Dynamic equations

Regard a molecule bound to a membrane. Both, the membrane as well as the surrounding medium may determine the dynamic situation of the molecule. In order to have something in view we have shown in fig. 2 a few possible examples. The molecules may be embedded within the membrane nearly completely (a, b, d), they may be attached to it (c) or they may be bound to the membrane through a "membranophilic" part (e). They may behave as rigid or as extremely deformable bodies. An example for the latter case (e) we have studied [15]: Flavins bound to artificial bilayer membranes by mediation of a lipophilic hydro-carbon chain. Long molecules may be oriented perpendicularly (a), parallely (c) or in another preferred direction (b). Molecules may have only one rotational degree of freedom, e.g., about the normal n in (a), or be nearly spherical with no preferred orientation (d). Many other possibilities are imaginable. Furthermore the orientation of transition moments of absorption and emission within the molecules must be considered.

2.3.1. One-dimensional motion

We derive dynamic equations from which in many cases one may get a description of the kinematic behaviour of molecules bound to plane structures. We assume the molecules to execute Brownian motion in a region with spatially varying potential energy. In the one-dimensional case the flux Φ of molecules is then generally determined by

$$\Phi = -D\left(\frac{\partial c}{\partial \xi} + c\frac{\partial \bar{V}}{\partial \xi}\right) \tag{9}$$

where D = diffusion coefficient; V = potential energy; $\bar{V} = V/kT$ = reduced potential energy; k = Boltzmann constant; T = (absolute) temperature.

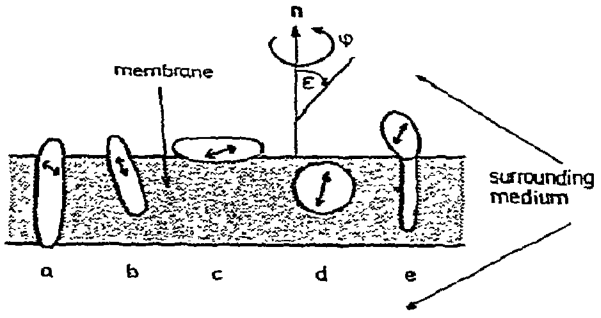

Fig. 2. Different examples for molecules bound to a planar membrane.

With regard to the balance equation

$$\frac{\partial \Phi}{\partial \xi} = -\frac{\partial c}{\partial t} \tag{10}$$

we get the diffusion equation for concentration c:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial \xi^2} + D\frac{\partial(c(\partial \bar{V}/\partial \xi))}{\partial \xi}. \tag{11}$$

Up to now we have not specified the coordinate ξ. If ξ is some angle coordinate, eq. (11) describes one-dimensional rotatory dynamics. In this case D is the *coefficient of rotational diffusion*. The first term on the right-hand side of eq. (11) is the usual term of rotational Brownian motion. The second term describes the effect of a potential on the rotatory motion. We do not specify this potential energy. It may come from electric or other chemical binding between the molecules and their surrounding. The preferred orientations of molecules are energetically most favourable and hence are given by the minima of potential energy.

2.3.2. *Multi-dimensional motion*

Up to now we have restricted to one-dimensional motion. More generally the problem is a complicated multi-dimensional one. We again consider the example in fig. 2. In case (a) the molecule may have only one rotational degree of freedom about the normal n. In (b) it may additionally to this rotation perform a second (oscillatory) motion in ϵ about a preferred orientation ϵ_0. Molecule (c) may stand for a situation with two degrees of freedom of Brownian rotation, in φ about n and in ϵ about its longitudinal axis. (d) is an example for a spherical molecule with free Brownian rotation about three angles. Finally (e) may represent a situation, where different parts of the (non-rigid) molecule make different motions, which may be independent or dependent on each other. In order to arrive at a most general set of flux equations, including the case of deformable molecules, one can decompose the molecule into n (rigid) parts. For each part an orientations distribution function may be introduced which satisfies a flux equation of the form (9), with D and $\bar{V}$ now generally depending on the situation of the whole molecule.

We do not intend to discuss such a general set up. The model calculations below are restricted to simple one-dimensional and two-dimensional problems.

2.4. *Stationary distributions*

If the distribution function c is time independent one gets from eq. (11) by simple integration

$$c = \mathrm{e}^{-\bar{V}}\left(B\int \mathrm{e}^{\bar{V}}\,\mathrm{d}\xi + A\right) \tag{12}$$

with A and B as integration constants. A sensible boundary condition for stationary solutions is the flux to be zero, so that the distribution c is in equilibrium. With eq. (9) B follows to be zero. Therefore the general equation for the distribution c in the stationary case is

$$c = A\,\mathrm{e}^{-\bar{V}}\,. \tag{13}$$

A is a factor of normalization of c. Up to now we have not specified the reduced potential $\bar{V}$. If $\bar{V}$ is independent of c and time-independent, eq. (13) represents the stationary solution for c. In case $\bar{V}$ depends on c, eq. (13) yields an equation for c (or $\bar{V}$), which still has to be solved.

According to eq. (13) for a rectangular potential we get a rectangular distribution c, for a parabolic potential a Gaussian distribution.

3. Explicit model calculations

Central point of the following model calculations is the determination of the intensity of emission function $\bar{I}_E$ defined in sect. 2.2 (see eq. (8)) for arbitrary polarization directions of incident light. From $\bar{I}_E$ the results of the polarized fluorescence experiment with a given geometrical arrangement are uniquely determined. The open parameters of the theory, which have to be fitted to the experimental data, are coefficients of rotational diffusion, position of the minimum of potential energy etc. and have a direct physical meaning.

In all calculated examples we assume the following three conditions to be valid:

(1) Vanishing angle Γ between transition moments of absorption and emission.

(2) Vanishing energy transfer between fluorescent molecules.

(3) Equality of the stationary distributions of transition moments of absorption and emission.

The third condition means, at least for the stationary equilibrium distributions, equality of dynamical properties of molecules in the excited and unexcited states, respectively.

3.1. One-dimensional Brownian rotational diffusion in φ

In the first example we investigate the situation, where the molecules make rotational diffusion about the normal n of the planar membrane. The orientation distributions of transition moments of absorption and emission are assumed to be deltashaped in ϵ. Hence the normalized stationary distribution function F_A is given by

$$F_A = \frac{1}{2\pi}\frac{1}{\sin\epsilon}\,\delta(\epsilon-\epsilon_0)\,. \tag{14}$$

In order to take into account possible rearrangements of transition moments by rotational diffusion in φ during the lifetime of the excited state, we consider the timedependent diffusion equation for the probability distribution F of one single dipole, following from eq. (10) with constant potential:

$$\frac{\partial F}{\partial t} = D_\varphi \frac{\partial^2}{\partial\varphi^2} F \tag{15}$$

D_φ = coefficient of rotational diffusion in φ.

We look for solutions $F(\varphi-\varphi', t)$ of eq. (11) satisfying the following three conditions:

normalization

$$\int_0^{2\pi} F\,\mathrm{d}\varphi = 1 \tag{16a}$$

boundary condition

$$F(\varphi-\varphi'=0, t) = F(\varphi-\varphi'=2\pi, t) \tag{16b}$$

initial condition

$$F(\varphi-\varphi', t=0) = \delta(\varphi-\varphi')\,. \tag{16c}$$

According to the initial condition (eq. 16c)) φ' denotes the position of the dipole for $t=0$.

A second boundary condition $\partial F/\partial\varphi\,(\varphi-\varphi'=0, t) = \partial F/\partial\varphi\,(\varphi-\varphi'=2\pi, t) = 0$ is automatically valid as consequence of eqs. (16b, c) and (15). If the lifetime of the excited state is τ, one gets the lifetime distribution function L

$$L = \frac{1}{\tau}\mathrm{e}^{-t/\tau}\,, \qquad \int_0^\infty L\,\mathrm{d}t = 1 \tag{17}$$

where the process of absorption is assumed to take place at $t = 0$. For an ensemble of many dipole moments, homogeneously distributed in φ, the intensity of absorption is proportional to $\cos^2\psi$. Hence the intensity of emission $\bar{I}_E$ is obviously given by integration of FL over φ' and t with $\cos^2\psi$ as weighting function

$$\bar{I}_E(\epsilon, \epsilon_0, \varphi, D_\varphi, \tau) = \frac{1}{2\pi}\frac{\delta(\epsilon-\epsilon_0)}{\sin\epsilon}\int_0^\infty \mathrm{d}t \left[L \int_0^{2\pi} \cos^2\psi(\varphi') F \,\mathrm{d}\varphi'\right]. \tag{18}$$

In case the exciting light is a short pulse, $\bar{I}_E$ in eq. (18) describes the mean value of a time-dependent intensity of emission function $L \int \cos^2\psi\, F \,\mathrm{d}\varphi'$. In this paper we are concerned with the *stationary* polarized fluorescence experiment, with incident light of time-independent intensity and polarization. Then $\bar{I}_E$ is the (stationary) intensity of emission which determines the measured fluorescence intensity according to eq. (8).

Setting

$$F = \mathrm{e}^{at} f(\varphi - \varphi') \tag{19}$$

one gets the following particular solutions of eq. (15) satisfying the boundary condition (16b)

$$F_\nu = \exp(-D_\varphi \nu^2 t)\,(A_\nu \cos\nu\,(\varphi-\varphi') + B_\nu \sin\nu\,(\varphi - \varphi')) . \tag{20}$$

The complete solution may be written as the Fourier series

$$F = \sum_{\nu=0}^{\infty} \exp(-D_\varphi \nu^2 t)\,(A_\nu \cos\nu(\varphi-\varphi') + B_\nu \sin\nu(\varphi-\varphi')) \tag{21}$$

Fourier's analysis of the initial distribution (16c) for $t = 0$ yields

$$B_\nu = 0 ,$$

$$A_0 = 1/2\pi , \qquad A_\nu = 1/\pi \quad \text{for} \quad \nu = 1, 2, \ldots$$

and hence

$$F = \frac{1}{2\pi} + \frac{1}{\pi}\sum_{\nu=1}^{\infty} \exp(-D_\varphi \nu^2 t) \cos\nu(\varphi - \varphi') \tag{22}$$

satisfying eqs. (16a–c).

The evaluation of the integrals in eq. (18) with F, $\cos^2\psi$ according to eq. (22), eq. (4) respectively is lengthy but elementary. Most integrals over the region $0 \leqslant \varphi' \leqslant 2\pi$ vanish. Only for $\nu = 0, 1, 2$ remain nonvanishing contributions. The final result is

$$\begin{aligned}\bar{I}_E(\epsilon, \epsilon_0, \varphi, D_\varphi\tau) = \frac{\delta(\epsilon-\epsilon_0)}{2\pi\sin\epsilon}\Bigg\{&\left(\frac{A^2+B^2}{2} + C^2\right) + \left(\frac{1}{1+D_\varphi\tau}\right)(2AC\cos\varphi + 2BC\sin\varphi)\\ &+ \left(\frac{1}{1+4D_\varphi\tau}\right)\left((A\cos\varphi + B\sin\varphi)^2 - \frac{A^2+B^2}{2}\right)\Bigg\}\end{aligned} \tag{23}$$

with the abbreviations

$$A = -\sin\epsilon\cos\vartheta\cos\alpha \qquad B = \sin\epsilon\sin\vartheta \qquad C = -\cos\epsilon\cos\vartheta\sin\alpha . \tag{24}$$

Eq. (23) contains the limiting cases $D_\varphi\tau \ll 1$ (no rearrangement) and $D_\varphi\tau \gg 1$, i.e. complete equipartition in φ during the lifetime of the fluorescent state:

$$D_\varphi \tau \gg 1: \qquad \bar{I}_{\mathrm{E}} = \frac{\delta(\epsilon - \epsilon_0)}{2\pi \sin \epsilon} \left(\frac{A^2 + B^2}{2} + C^2 \right) \tag{25a}$$

$$D_\varphi \tau \ll 1: \qquad \bar{I}_{\mathrm{E}} = \frac{\delta(\epsilon - \epsilon_0)}{2\pi \sin \epsilon} \cos^2 \psi \,. \tag{25b}$$

3.2. One-dimensional oscillatory motion about a preferred orientation

In case the reduced potential $\bar{V}$ as a function of the polar angle ϵ has a minimum at $\epsilon = \epsilon_0$, ϵ_0 indicates a *preferred orientation*. Generally, a good approximation for $\bar{V}$ about ϵ_0 will be the parabolic potential. But for analytic calculations of the intensity of emission function $\bar{I}_{\mathrm{E}}$ the quadratic approximation is difficult to handle. Therefore we approximate $\bar{V}$ by a square-well potential:

$$\bar{V}(\epsilon) = \begin{cases} \bar{V}_0 & \text{for } \epsilon \text{ in } [\epsilon_1, \epsilon_2] = [\epsilon_0 - \Delta\epsilon, \epsilon_0 + \Delta\epsilon] \\ \infty & \text{elsewhere} \,. \end{cases} \tag{26}$$

The probability of orientation outside $[\epsilon_1, \epsilon_2]$ vanishes.

We regard the probability distribution $E(\epsilon)$ with respect to the angle ϵ for a single dipole moment. From eq. (11) follows for E in $[\epsilon_1, \epsilon_2]$

$$\frac{\partial E}{\partial t} = D_\epsilon \frac{\partial^2}{\partial \epsilon^2} E \tag{27}$$

with D_ϵ = coefficient of rotational diffusion in ϵ. We look for solutions of eq. (27) satisfying the conditions

normalization

$$\int_{\epsilon_1}^{\epsilon_2} E \, \mathrm{d}\epsilon = 1 \tag{28a}$$

boundary condition

$$\frac{\partial E}{\partial \epsilon}(\epsilon_1) = \frac{\partial E}{\partial \epsilon}(\epsilon_2) = 0 \tag{28b}$$

initial condition

$$E(\epsilon, t = 0) = \delta(\epsilon - \epsilon') \,, \qquad \epsilon' \text{ in } [\epsilon_1, \epsilon_2] \,. \tag{28c}$$

According to eq. (9) and with $\partial \bar{V}/\partial \epsilon = 0$ in $[\epsilon_1, \epsilon_2]$ the boundary condition (28b) means vanishing flux at the edges ϵ_1, ϵ_2. On the contrary to other distribution functions discussed in this paper E is a density with respect to the angle ϵ and not to the unit sphere.

The lifetime distribution function L is given by eq. (17). Then for an ensemble of dipole moments of transition, which are homogeneously distributed between ϵ_1 and ϵ_2 with regard to the angle ϵ, one gets a (reduced) intensity of emission function $\hat{I}_{\mathrm{E}}$ analogously as eq. (18) through

$$\hat{I}_{\mathrm{E}}(\epsilon, \varphi, D_\epsilon, \tau) = \frac{1}{\sin \epsilon \, 4\pi \Delta\epsilon} \int_0^\infty \mathrm{d}t \int_{\epsilon_1}^{\epsilon_2} \cos^2 \psi(\epsilon') E \, \mathrm{d}\epsilon' \tag{29a}$$

Rotation in the azimuthal angle φ is neglected.

In eq. (29a) we have not specified the range of the interval $[\epsilon_1, \epsilon_2]$. In case $(0 \leqslant \epsilon_1 \leqslant \epsilon_2 \leqslant \pi)$, $\hat{I}_{\mathrm{E}}$ in eq. (29a) is the intensity of emission $\bar{I}_E$ being defined in eq. (8) as a density on the unit sphere. In cases $\epsilon_1 < 0$ or $\epsilon_2 > \pi$ the situation is somewhat complicated because the range of integration over ϵ in eq. (8) is only $0 \leqslant \epsilon \leqslant \pi$. The difficulty is avoided if in eq. (8) the range of integration is taken to be $\epsilon_1 \leqslant \epsilon \leqslant \epsilon_2$. Then the measured intensity $\hat{I}_M$ is given by

$$\bar{I}_M = \int_{\varphi=0}^{2\pi} \int_{\epsilon=\epsilon_1}^{\epsilon_2} \hat{I}_E \cos^2\mu \cos^2\nu \sin\epsilon \, d\epsilon \, d\varphi \,. \tag{8c}$$

Alternatively, the intensity $\hat{I}_E$ as a density on the unit sphere is easily derived from $\bar{I}_E$ through:

$$\begin{array}{ccccc} \bar{I}(\epsilon,\varphi) & = & \hat{I}(\epsilon,\varphi) & + & \hat{I}(\epsilon+\pi,\varphi) \\ (0 \leqslant \epsilon \leqslant \pi) & & (0 \leqslant \epsilon \leqslant \pi) & & (\pi \leqslant \epsilon \leqslant 2\pi) \end{array} \tag{29b}$$

where we have used that orientations (ϵ, φ) and $(\epsilon + n\pi, \varphi)$ of transition moments are undistinguishable. Without being further mentioned, these considerations are also valid in sect. 3.3 for the two-dimensional example.

Similarly as in the preceding section the total solution E of eq. (27) with eq. (28) is given by the series

$$E = \frac{1}{2\Delta\epsilon} + \sum_{\nu=1}^{\infty} A_\nu \exp\left(-\frac{\nu^2\pi^2}{4\Delta\epsilon^2} D_\epsilon t\right) \cos\left[\frac{\nu\pi}{2\Delta\epsilon}(\epsilon - \epsilon_1)\right] \tag{30}$$

where the coefficients A_ν are determined by Fourier's analysis of the initial condition (28c)

$$A_\nu = \frac{1}{\Delta\epsilon} \int_{\epsilon_1}^{\epsilon_2} d\epsilon \, \delta(\epsilon - \epsilon') \cos\left[\frac{\nu\pi}{2\Delta\epsilon}(\epsilon - \epsilon_1)\right] = \frac{1}{\Delta\epsilon} \cos\left[\frac{\nu\pi}{2\Delta\epsilon}(\epsilon' - \epsilon_1)\right] . \tag{31}$$

Hence the probability distribution $E(\epsilon, \epsilon', t)$ of a transition moment in position ϵ' for $t = 0$ is

$$E = \frac{1}{2\Delta\epsilon} + \frac{1}{\Delta\epsilon} \sum_{\nu=1}^{\infty} \left\{ \exp\left[-\frac{\nu^2\pi^2}{4\Delta\epsilon^2} D_\epsilon t\right] \cos\left[\frac{\nu\pi}{2\Delta\epsilon}(\epsilon' - \epsilon_1)\right] \cos\left[\frac{\nu\pi}{2\Delta\epsilon}(\epsilon - \epsilon_1)\right] \right\} . \tag{32}$$

For calculation of the intensity of emission $\hat{I}_E$ according to eq. (29) we first perform the time integration with the result

$$\hat{I}_E = \frac{1}{\sin\epsilon} \frac{1}{4\pi\Delta\epsilon} \int_{\epsilon_1}^{\epsilon_2} d\epsilon' \cos^2\psi(\epsilon') \left\{ \frac{1}{2\Delta\epsilon} + \frac{1}{\Delta\epsilon} \sum_{\nu=1}^{\infty} \frac{1}{\alpha_\nu^2} \cos\left[\nu\pi\left(\frac{\epsilon' - \epsilon_1}{2\Delta\epsilon}\right)\right] \cos\left[\nu\pi\left(\frac{\epsilon - \epsilon_1}{2\Delta\epsilon}\right)\right] \right\} . \tag{33}$$

The rather lengthy ϵ'-integration yields

$$\begin{aligned} \hat{I}_E = \frac{1}{\sin\epsilon} \frac{1}{2\pi} \Bigg\{ & \frac{\bar{A}^2 + \bar{B}^2}{4\Delta\epsilon} - \frac{\sin 2\Delta\epsilon}{8\Delta\epsilon^2} \left[(\bar{A}^2 - \bar{B}^2) \cos(\epsilon_1 + \epsilon_2) + 2\bar{A}\bar{B} \sin(\epsilon_1 + \epsilon_2) \right] \\ & + \cos 2\Delta\epsilon \, [2(\bar{B}^2 - \bar{A}^2) \sin(\epsilon_1 + \epsilon_2) + 4\bar{A}\bar{B} \cos(\epsilon_1 + \epsilon_2)] \, \frac{2}{\pi^2} \sum_{\nu=1}^{\infty} M_{2\nu-1} \frac{1}{\alpha_{2\nu-1}^2} \\ & + \sin 2\Delta\epsilon \, [2(\bar{A}^2 - \bar{B}^2) \cos(\epsilon_1 + \epsilon_2) + 4\bar{A}\bar{B} \sin(\epsilon_1 + \epsilon_2)] \, \frac{2}{\pi^2} \sum_{\nu=1}^{\infty} M_{2\nu} \frac{1}{\alpha_{2\nu}^2} \Bigg\} \end{aligned} \tag{34}$$

with the abbreviations

$$\bar{A} = \sin\vartheta \sin\varphi - \cos\vartheta \cos\varphi \cos\alpha\,, \qquad \bar{B} = \cos\vartheta \sin\alpha\,, \tag{35}$$

$$M_\nu = \cos\left[\nu\pi\left(\frac{\epsilon - \epsilon_1}{2\Delta\epsilon}\right)\right]\left(\frac{1}{\nu^2 - (16\Delta\epsilon^2/\pi^2)}\right), \qquad \alpha_\nu^2 = 1 + \frac{\nu^2\pi^2}{4\Delta\epsilon^2} D_\epsilon \tau\,. \tag{36}$$

Eq. (34) is simplified in the following limiting cases:

(1) $\Delta\epsilon \to 0$, delta-shaped distribution in ϵ. In the limit $\Delta\epsilon \to 0$ all contributions from the series in eq. (34) vanish (for $\nu \geqslant 1$). One gets with $\sin 2\Delta\epsilon \approx 2\Delta\epsilon$ for $\Delta\epsilon \ll 1$

$$\bar{I}_\mathrm{E} = \frac{1}{2\pi \sin\epsilon}\,\delta(\epsilon - \epsilon_0)\left[\frac{\bar{A}^2 + \bar{B}^2}{2} - \frac{\bar{A}^2 - \bar{B}^2}{2}\cos 2\epsilon - \bar{A}\bar{B}\sin 2\epsilon\right]$$

hence with eqs. (35) and eq. (4)

$$\hat{I}_\mathrm{E} = \frac{1}{2\pi \sin\epsilon}\,\delta(\epsilon - \epsilon_0)\cos^2\psi$$

in agreement with eq. (25b).

(2) $D_\epsilon \tau \to \infty$. In this case complete equipartition of transition moments in $[\epsilon_1, \epsilon_2]$ during the excited state:

$$\hat{I}_\mathrm{E} = \frac{1}{2\pi \sin\epsilon}\,\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}\left[\frac{\bar{A}^2 + \bar{B}^2}{2} - \left(\frac{\bar{A}^2 - \bar{B}^2}{2}\right)\cos(\epsilon_1 + \epsilon_2) - \bar{A}\bar{B}\sin(\epsilon_1 + \epsilon_2)\right]. \tag{37}$$

(3) $D_\epsilon \tau \to 0$. Treatment of this limiting case with the use of eq. (34) is possible but requires a troublesome evaluation of the series in eq. (34). It is more convenient to start from eq. (31).

Because of eq. (31) holds

$$\delta(\epsilon - \epsilon') = \frac{1}{2\Delta\epsilon} + \frac{1}{\Delta\epsilon}\sum_{\nu=1}^{\infty}\cos\left[\nu\pi\left(\frac{\epsilon' - \epsilon_1}{2\Delta\epsilon}\right)\right]\cos\left[\nu\pi\left(\frac{\epsilon - \epsilon_1}{2\Delta\epsilon}\right)\right]$$

and hence with eq. (33)

$$\hat{I}_\mathrm{E} = \frac{1}{\sin\epsilon}\,\frac{1}{4\pi\Delta\epsilon}\cos^2\psi \qquad \text{for } \epsilon \text{ in } [\epsilon_1, \epsilon_2]$$

as expected.

3.3. Two-dimensional motion in ϵ and φ

As a last example we discuss the situation, where the relaxation times $1/D_\epsilon$, $1/D_\varphi$ for relaxation in ϵ, φ respectively are comparable. In this case for calculation of the intensity of emission one has to consider a rearrangement of transition moments by *two-dimensional* motion.

We will investigate this problem under the assumption that rotational diffusion in φ and oscillatory diffusion in ϵ are *independent* of each other. The potential $\bar{V}(\epsilon)$ is again approximated by the square-well potential (26). Then the probability distribution G of a transition moment, which is in position ϵ', φ' at time $t = 0$, is given by the product of the distributions F and E

$$G(\epsilon, \varphi, \epsilon', \varphi', t) = \frac{1}{\sin\epsilon} F(\varphi - \varphi', t)\, E(\epsilon, \epsilon', t)\,. \tag{39}$$

From eq. (39) follows analogously as in eqs. (18) and (29) the intensity of emission $\hat{I}_\mathrm{E}$ by integration over t and all possible orientations ϵ', φ':

$$\hat{I}_{\mathrm{E}}=\frac{1}{\sin\epsilon}\,\frac{1}{4\pi\Delta\epsilon}\int_{0}^{\infty}\mathrm{d}t\left[L\int_{\epsilon'=\epsilon_1}^{\epsilon_2}\int_{\varphi=0}^{2\pi}\cos^2\psi(\epsilon',\varphi')EF\,\mathrm{d}\epsilon'\,\mathrm{d}\varphi'\right]. \tag{40}$$

The evaluation of eq. (40) leads to similar integrals as in the preceding chapters. The final result of the lengthy calculation is:

$$\begin{aligned}\hat{I}_{\mathrm{E}}=\frac{1}{\sin\epsilon}\,\frac{1}{2\pi}\Bigg\{&\frac{(1+\bar{B}^2)}{8\Delta\epsilon}-\frac{(1-3\bar{B}^2)}{8\Delta\epsilon}\cos(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}\\&-\left(\frac{1}{1+D_\varphi\tau}\right)\frac{\bar{A}\bar{B}}{4\Delta\epsilon^2}\sin(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}+\left(\frac{1}{1+4D_\varphi\tau}\right)\left(\frac{2\bar{A}^2+\bar{B}^2-1}{8\Delta\epsilon}\right)\left(1-\cos(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}\right)\\&+\frac{2}{\pi^2}(1-3\bar{B}^2)\left[-\sin(\epsilon_1+\epsilon_2)\cos 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu-1}\frac{1}{\alpha_{2\nu-1}^2}+\cos(\epsilon_1+\epsilon_2)\sin 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu}\frac{1}{\alpha_{2\nu}^2}\right]\\&+\frac{8}{\pi^2}\bar{A}\bar{B}\left[\cos(\epsilon_1+\epsilon_2)\cos 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu-1}\left(\frac{1}{\alpha_{2\nu-1}^2+D_\varphi\tau}\right)+\sin(\epsilon_1+\epsilon_2)\sin 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu}\left(\frac{1}{\alpha_{2\nu}^2+D_\varphi\tau}\right)\right]\\&+\frac{2}{\pi^2}(2\bar{A}^2+\bar{B}^2-1)\left[-\sin(\epsilon_1+\epsilon_2)\cos 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu-1}\left(\frac{1}{\alpha_{2\nu-1}^2+4D_\varphi\tau}\right)\right.\\&\left.+\cos(\epsilon_1+\epsilon_2)\sin 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu}\left(\frac{1}{\alpha_{2\nu}^2+4D_\varphi\tau}\right)\right]\Bigg\}\end{aligned} \tag{41}$$

with the abbreviations $\bar{A}, \bar{B}, M_\nu, \alpha_\nu^2$ according to eqs. (35) and (36).

As easily may be verified the one-dimensional examples treated in the preceding chapters are contained in eq. (41) as limiting cases $\Delta\epsilon\to 0$, $D_\varphi\tau\to 0$ respectively. Further marked out limiting cases are:

(1) $D_\epsilon\tau\to\infty$. *In this case a complete equipartition of transition moments in* $[\epsilon_1,\epsilon_2]$ *takes place during the excited state.*

$$\begin{aligned}\hat{I}_{\mathrm{E}}=\frac{1}{\sin\epsilon}\,\frac{1}{4\pi\Delta\epsilon}\Bigg\{&\frac{(1+\bar{B}^2)}{4}-\frac{(1-3\bar{B}^2)}{4}\cos(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}\\&-\left(\frac{1}{1+D_\varphi\tau}\right)\bar{A}\bar{B}\sin(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}+\left(\frac{1}{1+4D_\varphi\tau}\right)\left(\frac{2\bar{A}^2+\bar{B}^2-1}{4}\right)\left(1-\cos(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}\right)\Bigg\}.\end{aligned} \tag{42}$$

(2) $D_\varphi\tau\to\infty$. In this case complete equipartition in φ during the excited state:

$$\begin{aligned}\hat{I}_{\mathrm{E}}=\frac{1}{\sin\epsilon}\,\frac{1}{2\pi}\Bigg\{&\frac{(1+\bar{B}^2)}{8\Delta\epsilon}-\frac{(1-3\bar{B}^2)}{8\Delta\epsilon}\cos(\epsilon_1+\epsilon_2)\frac{\sin 2\Delta\epsilon}{2\Delta\epsilon}+\frac{2}{\pi^2}(1-3\bar{B}^2)\\&\times\left[-\sin(\epsilon_1+\epsilon_2)\cos 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu-1}\frac{1}{\alpha_{2\nu-1}^2}+\cos(\epsilon_1+\epsilon_2)\sin 2\Delta\epsilon\sum_{\nu=1}^{\infty}M_{2\nu}\frac{1}{\alpha_{2\nu}^2}\right]\Bigg\}.\end{aligned} \tag{43}$$

(3) $D_\epsilon\tau\to\infty$ and $D_\varphi\tau\to\infty$.

$$\hat{I}_{\mathrm{E}} = \frac{1}{\sin \epsilon} \frac{1}{4\pi\Delta\epsilon} \left\{ \frac{(1+\bar{B}^2)}{4} - \frac{(1-3\bar{B}^2)}{4} \cos(\epsilon_1 + \epsilon_2) \frac{\sin 2\Delta\epsilon}{2\Delta\epsilon} \right\} . \tag{44}$$

The result (44) describes the situation, where the transition moments reorient themselves completely during the lifetime of the excited state. The intensity of emission function $\hat{I}_{\mathrm{E}}$ is proportional to the total absorbed intensity. Hence eq. (44) may also be applied for the evaluation of dichroism experiments, i.e. measurements of the directional dependence of absorbed intensities (see e.g. [19]).

3.4. Discussion

In our model calculations we have restricted attention to the determination of the intensity of emission function I_{E}, from which according to eq. (8) the measured intensities for arbitrary polarization direction and positions of the analyzer may be calculated. Generally the integral in eq. (8) must be evaluated numerically. The presentation of explicit numerical results has been omitted mainly in order to restrict the volume of this paper. Furthermore, the number of possible variations of geometrical parameters is so great that the profit which the reader can derive from some special calculated examples is not high. Numerical results for a special geometrical arrangement and for the limiting cases of long and short lifetimes of fluorescence have been presented in [15], where also a method of analyzing experimental results has been proposed. The reader who wants to apply the theoretical results of this paper may easily determine numerically the measured fluorescence intensities for the special geometric parameters in his experimental arrangement from the calculated intensity of emission functions.

Recently we have discussed the general problem, how much information in principle is available from the polarized fluorescence experiment [21]. We could show, that generally the number of independent measurable polarized intensities is not exceeding 36. In the case of uniaxial symmetry, which is subject of this paper this number is not exceeding 12. Hence, the analysis of experiments with oriented systems is much more complicated than in the isotropic case, where the determination of 2 polarized intensities yields all information (cf. eq. (1)). But on the other hand the available information about mobility and orientation is much higher [21]. In the two-dimensional example in sec. 3.3 the information is contained in the parameters D_φ, D_ϵ (mobility), ϵ_0 and $\Delta\epsilon$ (orientation and strength of binding to the membrane).

Finally we mention some possible extensions of the described polarized fluorescence experiment.

First, by suitable integration over different membrane orientations, our methods may be applied e.g. for fluorescence polarization of oriented pigment molecules in spherical arrays. The results of these calculations will be published elsewhere. Recently, some work on fluorescence polarization with vesicles has been published (see e.g. [22,23]). The theoretical interpretation of these experiments has been done with a theory of anisotropic Brownian rotations, developed by Weber [6]. In general however, when orientations of molecules must be considered this theory is not applicable.

Second, time dependent fluorescence polarization can provide us with more information; especially about the mobility of pigment molecules. Our theory may be applied for the analysis of such experiments by an appropriate modification of the time integrations in eqs. (18), (29) and (40), which depends on the shape of the exciting light pulses. If the sample is excited at time $t = 0$ by a delta-shaped light pulse, the integrands of the time integrals in eqs. (18), (29) and (40) are the time-dependent intensities of emission.

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