

FLUORESCENCE OF ISOTROPIC SOLUTIONS EXCITED BY INTENSITY MODULATED PRIMARY LIGHT

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A general expression is given for "fluorescence curves" (*i. e.* for curves describing the time dependence of the intensity of fluorescence, or that of its components) of isotropic solutions excited by harmonically intensity modulated primary light. The course of such curve depends *ceteris paribus* on the law according to which the decay following a pulse excitation proceeds. On ground of the theoretical and observed fluorescence curves the mean lifetime fluorescence (or its components) can be determined. Some examples of application of the general expression to particular cases are given. The fluorescence curves obtained are equivalent to those resulting from the treatment of the same problem by Spencer and Weber, but the conclusions concerning the determination of the mean lifetime drastically differ. According to Spencer and Weber *e. g.* "the difference in lifetime between the polarized components of the emission" is "a ... function of the ratio of the modulation frequency ω to the emission rate...". No such dependence on ω results from the present treatment. An explanation of this discrepancy is given. Let it be mentioned that in the particular case of a simple exponential decay both treatments lead to identical, and ω -independent, values of the mean lifetime.

1. Introduction

In this paper the problem is discussed of the time-dependence of the intensity of fluorescence (and its components) of isotropic solutions excited by intensity modulated primary light. The intensity of the primary light is assumed to be sufficiently low to produce no stimulated emission of fluorescence and to lead to number densities of excited luminescent molecule (LM) very low compared to the total number density of LM in the solution. In such conditions there exist no phase relations between the phase of the exciting light and that of fluorescence, as well as between phases of light emitted by particular LM.

If a solution is excited by intensity modulated primary light the intensity of the emitted fluorescence is also modulated with the same frequency but with the phase of modulation shifted and the degree of modulation altered. Let us call a "fluorescence curve" a function describing the time dependence of the intensity of the emitted fluorescence. In the case of polarized fluorescence different fluorescence curves belong to different

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components of fluorescence. The course of a fluorescence curve depends *ceteris paribus* on the decay function (defined in the next section) concerned. On ground of the observed fluorescence curves the mean lifetime τ of fluorescence and/or its components can be determined by means of a phase fluorometer¹. Usually the period T of modulation much longer than the measured lifetime τ is used in such devices ($T \gg \tau$). Recently Spencer and Weber [11], [12] and Spencer [13] have discussed the case in which the condition $T \gg \tau$ is not fulfilled, and obtained promising results. They arrived, however, to a paradoxical conclusion that the measured lifetime depends in some cases on the frequency of modulation of the exciting light.

Below another approach to the problem of fluorescence excited by modulated light is proposed, and the "Spencer-Weber-paradox" explained.

2. Definitions and general considerations

Let us begin with some definitions. Let fluorescence of a solution be excited at the moment $t = 0$ by an infinitely short light pulse. The intensity of fluorescence (as well as that of any of its components) as a function of time t for $t \geq 0$ may be written as

$$I(t) = P(t)f, \quad (1)$$

where $P(t)$, called here "decay function", is

$$P(t) = I(t) / \int_0^{\infty} I(t) dt, \quad (2)$$

and f the total energy emitted from $t = 0$ to $t = \infty$.

Obviously

$$\int_0^{\infty} P(t) dt = 1. \quad (2a)$$

The mean lifetime of $I(t)$ is defined as

$$\tau = \langle t \rangle = \int_0^{\infty} P(t)t dt. \quad (3)$$

In the particular case of a purely exponential decay, *i. e.* when

$$P(t) = \Gamma e^{-\Gamma t}, \quad (\Gamma = \text{Const}), \quad (4)$$

$$\tau = \frac{1}{\Gamma}. \quad (4a)$$

For

$I(t) = \sum a_i e^{-\frac{t}{\tau_i}}$ (a_i and τ_i being constants) the decay function becomes

$$P(t) = \frac{\sum a_i e^{-\frac{t}{\tau_i}}}{\sum a_i \tau_i}, \quad (5)$$

¹ Description of various types of fluorometers may be found in [10], [4], [2], [11], [13] and [8]. The theory of measurements of τ by means of fluorometers was given by Duschinsky [3].

and the mean lifetime

$$\langle \tau \rangle = \frac{\sum a_i \tau_i^2}{\sum a_i \tau_i}, \quad (6)$$

thus not the arithmetical mean of τ_i .

We now consider the case in which the excitation by primary light lasts a finite time. The intensity $I(t)$ observed at the time t is now equal to the sum of intensities of fluorescence emitted by groups of LM excited at various times $t' \leq t$:

$$I(t) = \int_{-\infty}^t P(t-t')f(t')dt', \quad (7)$$

where $f(t')$ is proportional to the intensity of the primary light at t' (the proportionality factor, irrelevant in our case, depends on experimental conditions and on the quantum yield of fluorescence).

By putting $t-t' = s$ Eq. (7) may be written in a form more convenient for applications:

$$I(t) = \int_0^{\infty} P(s)f(t-s)ds. \quad (8)$$

This is the general expression for the fluorescence intensity (or that of its components) as function of time t for all possible forms of $f(t')$. Obviously $P(s)$ must be normalized (like $P(t)$ of Eq. (2a)) as to give

$$\int_0^{\infty} P(s)ds = 1. \quad (9)$$

We now turn to the case of fluorescence excitation by primary light whose intensity is harmonically modulated, *i. e.*, when *e. g.* $f(t)$ has the form

$$f(t) = A + B \cos \omega t, \quad (10)$$

where $A \geq B > 0$, and ω is the (angular) frequency of modulation. Putting $f(t-s)$ from Eq. (10) into Eq. (8) one obtains

$$\begin{aligned} I(t) &= \int_0^{\infty} P(s) [A + B \cos \omega(t-s)] ds = \\ &= A + B \int_0^{\infty} P(s) (\cos \omega s \cos \omega t + \sin \omega s \sin \omega t) ds = \\ &= A + B [\langle \cos \omega s \rangle \cos \omega t + \langle \sin \omega s \rangle \sin \omega t], \end{aligned} \quad (11)$$

where

$$\langle \cos \omega s \rangle = \int_0^{\infty} P(s) \cos \omega s ds \quad (12)$$

and

$$\langle \sin \omega s \rangle = \int_0^{\infty} P(s) \sin \omega s ds \quad (13)$$

are the mean values of $\cos \omega s$ and $\sin \omega s$, respectively, averaged over the entire decay time.

Eq. (11) can be written as

$$I(t) = A + b \cos(\omega t - \Phi), \quad (14)$$

where

$$\Phi = \text{arc tg} \frac{\langle \sin \omega s \rangle}{\langle \cos \omega s \rangle} \quad (15)$$

and

$$b = B \sqrt{\langle \cos \omega s \rangle^2 + \langle \sin \omega s \rangle^2}. \quad (16)$$

Eq. (14) has the form similar to that of Eq. (10), but with the phase of modulation shifted by $-\Phi$, and with the degree of modulation

$$M = \frac{b}{A} = \frac{B}{A} \sqrt{\langle \cos \omega s \rangle^2 + \langle \sin \omega s \rangle^2} \quad (17)$$

instead of B/A .

3. Some applications to particular cases of the general expressions derived in the Section 2

We begin with the simplest case, *viz.* that in which the decay of fluorescence is a purely exponential one, and thus the decay function becomes

$$P(s) = \frac{e^{-\frac{s}{\tau}}}{\tau}, \quad (18)$$

where

$$\tau = \int_0^{\infty} P(s) s ds = \frac{1}{\tau} \int_0^{\infty} e^{-\frac{s}{\tau}} s ds = \langle s \rangle. \quad (19)$$

By means of Eqs (12), (13), (15) and (17) one obtains

$$\langle \cos \omega s \rangle = \frac{1}{\tau} \int_0^{\infty} e^{-\frac{s}{\tau}} \cos \omega s ds = \frac{1}{1 + \omega^2 \tau^2}, \quad (20)$$

$$\langle \sin \omega s \rangle = \frac{1}{\tau} \int_0^{\infty} e^{-\frac{s}{\tau}} \sin \omega s ds = \frac{\omega \tau}{1 + \omega^2 \tau^2}, \quad (21)$$

$$\Phi = \text{arc tg} \omega \tau \quad (22)$$

and

$$M = \frac{b}{A} = \frac{B}{A\sqrt{1+\omega^2\tau^2}}. \quad (23)$$

Eqs (22) and/or (23) may serve for determination of the mean lifetimes $\tau = \langle s \rangle$ from the observed values of Φ and/or M in the particular case considered, but not generally. Eq. (22) may be used for determination of τ also in other cases (although only approximately), provided that the frequency of modulation is so low that practically

$$\int_0^{\infty} P(s) \sin \omega s ds \approx \int_0^{\infty} P(s) \omega s ds = \omega \langle s \rangle, \quad (24)$$

thus

$$\text{tg } \Phi \approx \sin \Phi \approx \Phi \approx \omega \langle s \rangle \approx \omega \tau. \quad (25)$$

As far as I know the condition $\omega \tau \ll 1$ is almost always fulfilled (although, perhaps, in some cases not sufficiently well) in practical determinations of τ from the observed phase shifts (*cf. e. g.* [1]).

Let us now consider the application of Eq. (14) to the components of polarized fluorescence of isotropic solutions in which fluorescence depolarization by some factors occurs. We assume that fluorescence is excited by a parallel beam of plane polarized primary light, and thus the emission anisotropy (EA) is given by

$$r = \frac{I^{\parallel} - I^{\perp}}{I} \quad (26)$$

where I^{\parallel} and I^{\perp} are the intensities of the components of fluorescence parallel and perpendicular to the electric vector of the primary light, respectively, and $I = I^{\parallel} + 2I^{\perp}$.

It follows from Eq. (26) that

$$I^{\parallel} = \frac{I}{3}(1+2r) \quad (27)$$

and

$$I^{\perp} = \frac{I}{3}(1-r). \quad (28)$$

The intensity of a component I^{α} making an angle α with I^{\parallel} is:

$$I^{\alpha} = I^{\parallel} \cos^2 \alpha + I^{\perp} \sin^2 \alpha = \frac{I}{3}(1+D_{\alpha}r), \quad (29)$$

where $D_{\alpha} = 2 \cos^2 \alpha - \sin^2 \alpha$; ($2 \geq D_{\alpha} \geq -1$).

We now assume that r decreases with time t from the moment $t = 0$ of excitation with an infinitely short light pulse according to

$$r(t) = r_0 e^{-\varphi t}, \quad (30)$$

where $\varphi = \text{const.}$, and $r_0 = r(0)$ the limiting value of r , *i. e.* that for $t = 0$.

Putting $I = I_0 \exp(-t/\tau)$ and $r = r_0 \exp(-\varphi t)$ into Eq. (29) yields

$$I^\alpha(t) = \frac{I_0 \exp(-t/\tau)}{3} (1 + D_\alpha r_0 \exp(-\varphi t)), \quad (31)$$

hence the decay function

$$\begin{aligned} P^\alpha(s) &= I^\alpha(s) / \int_0^\infty I^\alpha(s) ds = \\ &= e^{-s/\tau} \frac{(1 + D_\alpha r_0 e^{-\varphi \tau}) (1 + \varphi \tau)}{\tau(1 + \varphi \tau + D_\alpha r_0)}, \end{aligned} \quad (32)$$

τ being the mean lifetime of the total fluorescence I .

The mean lifetime τ^α of the component I^α is

$$\tau^\alpha = \int_0^\infty P(s) s ds = \tau \frac{(1 + \varphi \tau)^2 + D_\alpha r_0}{(1 + \varphi \tau)^2 + D_\alpha r_0 (1 + \varphi \tau)}. \quad (33)$$

Obviously $\tau^\alpha = \tau$ for $D_\alpha = 0$.

By means of Eqs (12), (13) and (32) we get

$$\langle \sin \omega s \rangle = \frac{(1 + \varphi \tau) \omega \tau}{1 + \varphi \tau + D_\alpha r_0} \left[\frac{1}{1 + \omega^2 \tau^2} + \frac{D_\alpha r_0}{(1 + \varphi \tau)^2 + \omega^2 \tau^2} \right] \quad (34)$$

and

$$\langle \cos \omega s \rangle = \frac{1 + \varphi \tau}{1 + \varphi \tau + D_\alpha r_0} \left[\frac{1}{1 + \omega^2 \tau^2} + \frac{D_\alpha r_0 (1 + \varphi \tau)}{(1 + \varphi \tau)^2 + \omega^2 \tau^2} \right]. \quad (35)$$

Hence

$$\text{tg } \Phi^\alpha = \omega \tau \frac{(1 + \varphi \tau)^2 + \omega^2 \tau^2 + D_\alpha r_0 (1 + \omega^2 \tau^2)}{(1 + \varphi \tau)^2 + \omega^2 \tau^2 + D_\alpha r_0 (1 + \varphi \tau) (1 + \omega^2 \tau^2)}. \quad (36)$$

² Such time-dependence of $r(t)$ results. *e. g.* when the depolarization of fluorescence is produced by thermal movements of LM, and rotations of LM are treated as Brownian rotations of spherical particles consisting of LM together with their solvation shells. r_0 differs from the fundamental EA owing to torsional vibrations of LM ([9], [7]).

Since

$$1 + \varphi\tau = \frac{r_0}{\langle r \rangle}, \quad (37)$$

$\langle r \rangle$ being the EA of fluorescence excited by steady illumination of the solution with light of constant intensity (*cf.* [9], [6], [7]) the above equations may be written as

$$\tau^\alpha = \tau \frac{r_0 + D_\alpha \langle r \rangle^2}{r_0 + D_\alpha r_0 \langle r \rangle}, \quad (33a)$$

$$\langle \sin \omega s \rangle = \frac{\omega\tau}{1 + D_\alpha \langle r \rangle} \left[\frac{1}{1 + \omega^2 \tau^2} + \frac{D_\alpha r_0 \langle r \rangle^2}{r_0^2 + \langle r \rangle^2 \omega^2 \tau^2} \right], \quad (34a)$$

$$\langle \cos \omega s \rangle = \frac{1}{1 + D_\alpha \langle r \rangle} \left[\frac{1}{1 + \omega^2 \tau^2} + \frac{D_\alpha r_0^2 \langle r \rangle}{r_0^2 + \langle r \rangle^2 \omega^2 \tau^2} \right], \quad (35a)$$

and

$$\text{tg } \Phi^\alpha = \omega\tau \frac{r_0^2 + \langle r \rangle^2 \omega^2 \tau^2 + D_\alpha r_0 \langle r \rangle^2 (1 + \omega^2 \tau^2)}{r_0^2 + \langle r \rangle^2 \omega^2 \tau^2 + D_\alpha r_0^2 \langle r \rangle (1 + \omega^2 \tau^2)}. \quad (36a)$$

Let us remark that for $\omega\tau \ll 1$ $\text{tg } \Phi^\alpha \approx \omega\tau^\alpha$.

The degree of modulation of the corresponding fluorescence curve (*cf.* Eq. (17)) is

$$M = \frac{b}{A} = \frac{B}{A \sqrt{1 + \omega^2 \tau^2}} \sqrt{\frac{r_0^2 + \omega^2 \langle r \rangle^2 \tau^2 (1 + D_\alpha r_0)^2 (1 + D_\alpha \langle r \rangle)^{-2}}{r_0^2 + \omega^2 \langle r \rangle^2 \tau^2}}. \quad (37)$$

Similar expressions can be easily obtained for the case of natural light excitation. It is sufficient to replace $D_\alpha = 2 \cos^2 \alpha - \sin^2 \alpha$ by $D_\alpha = \cos^2 \alpha - 2 \sin^2 \alpha$, and r_0 and r by their values for natural light excitation.

Eqs (36), (36a) and (38) can be obtained from expressions derived by Spencer and Weber [11], [12] and by Spencer [13] if their notations of different quantities appearing in their expressions are replaced by those used in the present paper. One thus could think that both treatments are fully equivalent. There appears, however, a drastic difference in conclusions concerning the measured mean lifetime of fluorescence. This point needs elucidation.

Spencer and Weber [11], [12] and Spencer [13] arrived in particular at a paradoxical conclusion that the mean lifetime of fluorescence components depends on the frequency of modulation, and, moreover, the lifetime determined from the phase shift $\langle \tau_p \rangle$ differs in general from that determined from the degree of modulation $\langle \tau_M \rangle$. They obtained this results on assumption that $\text{tg } \Phi = \omega \langle \tau_p \rangle$, and the degree of modulation is that given by Eq. (23) (in my notation), *i. e.* on the assumption of general validity of expressions obtained for the particular case of the simple exponential decay function. Inspection of

³ See [7], [1], [5].

Eqs (32)–(37) shows that such assumption is not justified. Although Φ and M depend *ceteris paribus* on ω , but τ^α does not, unless a new definition of the mean lifetime differing from that given by Eq. (3) is adopted. Thus the values of τ^{\parallel} and τ^{\perp} obtained by Spencer and Weber [12] should be not compared to those resulting from Eq. (33) or Eq. (33a), and their remark [12] that although “the theory of Jabłoński is qualitatively correct, but ... there are obvious quantitative discrepancies with the experiment” appears to be groundless.

Let me mention that the problem of the decay of compents of polarized fluorescence was lately also treated in a paper by Merkelo *et al.* [8]. Their treatment is restricted to the case of time independent depolarization rate, as also is the treatment of Spencer and Weber as well as that of the present paper. The assumption of time-independent depolarization rate is the simplest one, and constitutes in most cases a fairly good approximation. The application of general expressions derived in Section 2 is not restricted to this simplest case.

REFERENCES

- [1] R. Bauer, *Z. Naturforsch.*, **18a**, 718 (1963).
- [2] R. Bauer, M. Rozwadowski, *Bull. Acad. Polon. Sci. Sér. Sci. Math. Astron. Phys.*, **7**, 365 (1959).
- [3] F. Duschinsky, *Acta Phys. Polon.*, **4**, 435 (1935); **5**, 255 (1936).
- [4] T. Förster, *Fluoreszenz organischer Verbindungen*, Vandenhoeck und Ruprecht, Göttingen 1951.
- [5] J. Heldt, *Acta Phys. Polon.*, **30**, 3 (1966).
- [6] A. Jabłoński, *Z. Naturforsch.*, **6a**, 1 (1961) and in *Luminescence of Organic and Inorganic Materials*, edited by H. P. Kallmann and G. M. Spruch, John Wiley and Sons, Inc. (1962), p. 110.
- [7] A. Jabłoński, *Acta Phys. Polon.*, **28**, 767 (1965).
- [8] H. Merkelo, J. H. Hammond, S. R. Hartman, Z. I. Derzko, *J. Luminescence*, **1–2**, 502 (1970).
- [9] F. Perrin, *J. Phys. (France)*, **5**, 497 (1934); **7**, 1 (1936); *Acta Phys. Polon.*, **5**, 333 (1936).
- [10] P. Pringsheim, *Fluorescence and Phosphorescence*, Interscience Publishers, INC, New York 1949.
- [11] R. D. Spencer, G. Weber, *Ann. New York Acad. Sci.*, **158**, 361 (1969).
- [12] R. D. Spencer, G. Weber, *J. Chem. Phys.*, **52**, 1654 (1970).
- [13] R. D. Spencer, *Ph. D. Theses*, Urbana 1970.