744. *Decay of Fluorescence.*

By W. S. Metcalf.

If solutions that fluoresce are excited by light whose intensity is modulated, the intensity of the fluorescence is also modulated at the same frequency but there is a phase lag which is related to the rate of decay of the fluorescence. Measurements of this phase lag have been used to calculate the mean life of optically excited molecules whose concentration decays exponentially.

The decay of the fluorescence of solutions containing quenching substances, or containing more than one fluorescent species, is usually not exponential, but measurements of the phase lag can still give information about the rapid processes occurring in such solutions.

As the examples below will show, the kinetic analysis of a fluorescent system commonly leads to equations of the following form:

$$\left.\begin{aligned} \dot{x} &= \alpha x + \beta y + \gamma I \\ \dot{y} &= ax + by + cI \\ f &= mx + ny \end{aligned}\right\} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (1)$$

x, y, I, and f are time-dependent. The other symbols are functions of the rate constants and concentrations which describe the rates of the various processes by which the optically excited molecules lose their energy; x and y are the concentrations of excited species, I and f are the intensities of the exciting light and of the fluorescence, respectively. If I is modulated, it can be expressed as a Fourier series

$$I = I_1 + I_2 \sin \omega t + I_3 \sin 2\omega t \ldots$$

If I is constant only the first term is retained.

The equations (1) can be transformed by standard methods[1] into

$$\left.\begin{aligned} \ddot{x} + P\dot{x} + Qx &= R'I + S'\dot{I} \\ \ddot{y} + P\dot{y} + Qy &= R'I + S''\dot{I} \\ \ddot{f}g + P\dot{f} + Qf &= RI + \dot{S}I \end{aligned}\right\} \quad . \; . \; . \; . \; . \; . \; . \quad (2)$$

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[1] Murray, " Introductory Course in Differential Equations," Longmans, Green and Co., London, 1910, § 98.

where $-P = \alpha + b$; $Q = \alpha b - \beta a$; $R = m(\beta c - \gamma b) + n(\gamma a - \alpha c)$; $S = m\gamma + nc$; $R' = \beta c - \gamma b$; $R'' = \gamma a - \alpha c$; $S' = \gamma$; $S'' = c$.

The equations (2) are also in standard form whose solution [2] in the case of f is

$$f = c_1 e^{m_1 t} + c_2 e^{m_2 t} + \frac{1}{m_1 - m_2}\left\{e^{m_1 t}\int e^{-m_1 t}(RI + S\dot{I})\mathrm{d}t - e^{m_2 t}\int e^{-m_2 t}(RI + S\dot{I})\mathrm{d}t\right\}$$

where m_1, m_2 are the (negative) roots of $m^2 + Pm + Q = 0$.

Integration and simplification yield a Fourier series, which for each harmonic contains both sine and cosine terms. For each harmonic these are combined into terms of the form $f \sin (j\omega t - \phi)$. Then, when the transient terms $c_1 e^{m_1 t}$ and $c_2 e^{m_2 t}$ have become negligible,

$$f = f_1 + f_2 \sin (\omega t - \phi_2) + f_3 \sin (2\omega t - \phi_3) \quad . \quad . \quad . \quad . \quad (3)$$

The amplitude and phase of each frequency in the modulation of the fluorescence depends on P, Q, R, and S, and on the amplitude and phase of the corresponding frequency in the modulation of the exciting light. There are no cross terms. Consequently, it is sufficient to exclude unwanted harmonics in the detecting device, and it is unnecessary to attempt the difficult task of excluding them from the modulation of the exciting light. The terms in the series for I and for f are related as follows:

$$f_1 = RI_1/Q \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$$f_2 = I_2/[Q^2 + \omega^2(P^2 - 2Q) + \omega^4] \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

$$\tan \phi_2 = \omega(PR - QS + S\omega^2)/[QR + \omega^2(PS - R)] \quad . \quad . \quad (6)$$

For the higher terms ω is replaced by its appropriate multiple. Experimentally, the terms are separated by tuned circuits in a fluorometer.[3] Solutions for x or y are found by putting R', S' or R'', S'' for R, S.

In the particular cases which follow, only f_1 and ϕ_2 are discussed, and the subscripts are therefore discarded. The subscript 0 when it appears refers to systems in which the concentration of quencher [Q] approaches zero.

Applications.—(i) The following scheme describes the quenching of the fluorescence of some gases (A) by added substances (Q).

$\mathrm{A} + h\nu \xrightarrow{1} \mathrm{A}^*$ absorption

$\mathrm{A}^* \xrightarrow{2} \mathrm{A} + h\nu'$ fluorescence

$\mathrm{A}^* \xrightarrow{3} \mathrm{A}$ internal degradation

$\mathrm{A}^* + \mathrm{Q} \xrightarrow{4} \text{products}$ quenching

All the coefficients of the equations (1) are zero except $\alpha = -(k_2 + k_3 + k_4[\mathrm{Q}])$, and m and γ. Therefore both P and Q/R have the value $k_2 + k_3 + k_4[\mathrm{Q}]$. It follows that

$$\frac{\tan \phi_0}{\tan \phi} = \frac{f_0}{f} = 1 + \frac{k_4[\mathrm{Q}]}{k_2 + k_3} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

and

$$\tan \phi_0 = \omega/(k_2 + k_3) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

Taken together, these expressions enable the absolute value of k_4 to be found. The result, $\tan \phi_0/\tan \phi = f_0/f$, has been used [3] to calibrate apparatus for measuring $\tan \phi$. When solutions are used for this purpose the scheme above is usually inadequate and such a calibration would be inaccurate [cf. equations (10), (11) below]. The result $f_0/f = 1 + k_4[\mathrm{Q}]/(k_2 + k_3)$ is a form of the well-known law of Stern and Volmer.[4]

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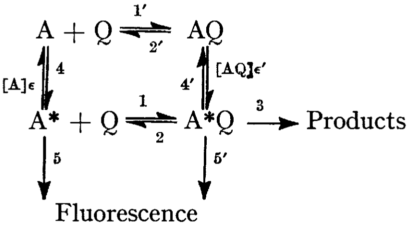
[2] Ref. 1, pp. 58, 62.

[3] Bailey and Rollefson, *J. Chem. Phys.*, 1953, **21**, 1315, where reference is made to earlier measurements.

[4] Stern and Volmer, *Phys. Z.*, 1919, **20**, 183.

(ii) In solutions, account must be taken of the different behaviour of fluorescent molecules which are excited when they are beside and when they are distant from quencher molecules. A scheme[5] which makes this distinction follows:



Processes 1 and 2 are the formation and break up of encounters, 3 is quenching, 4 internal degradation, and 5 fluorescence. Although there is no difficulty other than complexity in maintaining the distinctions implied by the primes, it is not usually necessary to do so, in which case if x and y stand for [A*] and [A*Q] then $\alpha = -(k_4 + k_5 + k_1[Q])$; $a = k_1[Q]$; $\beta = k_2$; $b = -(k_2 + k_3 + k_4 + k_5)$; $\gamma = [A]/([A] + [AQ])$; $c = [AQ]/([A] + [AQ])$; $m = n = 1$.

If k_1/k_2 is written as K, there follows

$$\frac{f_0}{f} = 1 + [Q]\frac{k_3\left\{k_1 + \dfrac{K(k_4 + k_5)}{1 + K[Q]}\right\}}{k_1[Q] + k_2 + \dfrac{k_3}{1 + K[Q]} + k_4 + k_5} \quad . \quad . \quad . \quad . \quad (9)$$

This expression is equivalent to one derived earlier[5] and tested experimentally.

The expression for tan ϕ_0/tan ϕ does not reduce readily to a simple form for the general case, but two special cases lead to simple results.

(*a*) When almost every encounter results in quenching, $k_3 \gg k_2$. Then

$$\frac{f_0}{f} = \left(1 + \frac{k_1}{k_4 + k_5}[Q]\right)(1 + K[Q]) \quad . \quad . \quad . \quad . \quad . \quad (10)$$

and
$$\frac{\tan\phi_0}{\tan\phi} = \left(1 + \frac{k_1}{k_4 + k_5}[Q]\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

and
$$\tan\phi_0 = \omega/(k_4 + k_5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

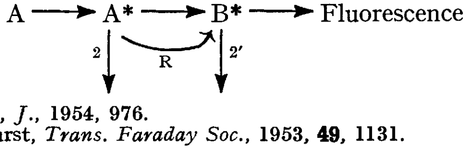
From (10), (11), and (12), k_1, k_2, and K may be calculated.

(*b*) When encounters seldom result in quenching, $k_3 \ll k_2$. In this case

$$\frac{f_0}{f} = \frac{\tan\phi_0}{\tan\phi} = 1 + \frac{k_3K[Q]/(k_4 + k_5)}{1 + K[Q]} \quad . \quad . \quad . \quad . \quad (13)$$

and
$$\tan\phi_0 = \omega/(k_4 + k_5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

(iii) This example has to do with studies of energy transfer between molecules in solution. Bowen and Brocklehurst[6] found that addition of perylene (B) to a fluorescent solution of 1-chloroanthracene (A), in circumstances where all the light was absorbed by A and emitted by B, raised the measured quantum efficiency of the fluorescence from the value 0·05 of A alone to 0·25. They attributed this rise to a fast transfer of energy from A to B before it is degraded by the process responsible for the low fluorescence efficiency of A. The scheme is



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[5] Melhuish and Metcalf, *J.*, 1954, 976.

[6] Bowen and Brocklehurst, *Trans. Faraday Soc.*, 1953, **49**, 1131.

R is the rate of transfer of quanta by the novel process proposed; 1, 1′ are fluorescence emission from A* and B*, 2, 2′ are internal degradation processes which are responsible for the fractional quantum efficiencies E of A and B, thus

$$E_{\mathrm{A}} = k_1/(k_1 + k_2) \text{ and } E_{\mathrm{B}} = k_1'/(k_1' + k_2')$$

The mean lives τ of A* and B* are given by

$$\tau_{\mathrm{A}} = (1/\omega) \tan \phi_{\mathrm{A}} = 1/(k_1 + k_2)$$
$$\tau_{\mathrm{B}} = (1/\omega) \tan \phi_{\mathrm{B}} = 1/(k_1' + k_2')$$

The coefficients in the equations (1) are $\alpha = -(k_1 + k_2 + R)$; $a = k_1 + R$; $m = 0$; $\beta = 0$; $b = -(k_1' + k_2')$; $n = k_1'$; $\gamma = 1$; $c = 0$. Whence

$$E_{\mathrm{AB}} = \frac{f_{\mathrm{AB}}}{I} = \frac{E_{\mathrm{B}}}{E_{\mathrm{A}}}\left(\frac{E_{\mathrm{A}}}{\tau_{\mathrm{A}}} + \mathrm{R}\right)\Big/\left(\frac{1}{\tau_{\mathrm{A}}} + R\right)$$

Bowen and Brocklehurst have measured E_{A}, E_{B}, and E_{AB}; τ_{A} and τ_{B} are accessible from phase-angle measurements, so R can be calculated. The theory is now able to be tested by its ability to predict $\tau_{\mathrm{AB}} = (1/\omega) \tan \phi_{\mathrm{AB}}$ by the relation below, which follows from (6).

$$\tau_{\mathrm{AB}} = \left(\frac{1}{\tau_{\mathrm{A}}} + \frac{1}{\tau_{\mathrm{B}}} + R\right)\Big/\left[\frac{1}{\tau_{\mathrm{B}}}\left(\frac{1}{\tau_{\mathrm{A}}} + R\right) - \omega^2\right]$$

(iv) A further example occurs when a fluorescent acid is in equilibrium with its conjugate base in a buffered solution. A theoretical and experimental study of such a case is reported in the following paper.

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[*Received, December* 21*st*, 1959.]