Measurements of the Fluorescence Decay Time in Mixed Solutions Using Pulse Fluorometery*

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The mean fluorescence decay time for rhodamine 6G (donor) in the presence of malachite green (acceptor) has been investigated using a pulse fluorometer. The experimental results have been compared with theoretical ones obtained earlier [1].

One of the commonly investigated luminescence quantities is the mean fluorescence decay time (MFDT) τ:

$$\tau \equiv \int_0^\infty t\,\Phi(t)\,\mathrm{d}t \Big/ \int_0^\infty \Phi(t)\,\mathrm{d}t\,, \tag{1}$$

where t is time and $\Phi(t)$ is the fluorescence decay function after a sudden excitation at time $t=0$.

Recently [1] we discussed the influence of excitation energy migration among donors on MFDT of donor molecules in a donor-acceptor system. Assuming that the interaction between an excited donor molecule and a non-excited donor or acceptor molecule (trap) is of dipole-dipole type [2], we obtained the following expression for τ/τ_0:

$$\frac{\tau}{\tau_0} = \frac{1+\gamma^2-(\frac{3}{2}+\gamma^2)f+\alpha f(f+\gamma^2-\frac{1}{2})-\alpha\gamma^2}{(1-f)(1-\alpha f)}\,, \tag{2}$$

where

$$f = f(\gamma) \equiv \pi^{1/2}\gamma\exp(\gamma^2)\,[1-\operatorname{erf}(\gamma)]\,, \tag{3}$$

$$\gamma = \gamma_\mathrm{D}+\gamma_\mathrm{A} \equiv (\pi^{1/2}/2)\,(c_\mathrm{D}/c_\mathrm{0D}+c_\mathrm{A}/c_\mathrm{0A})\,. \tag{4}$$

c_D, c_A are concentrations and c_0D, c_0A critical concentrations of the donor and acceptor, respectively. τ_0 is MFDT of the donor in the absence of the acceptor and $\alpha=\gamma_\mathrm{D}/\gamma$. When $\alpha\approx 0$, that is the migration of excitation energy among donors is negligible, (2) becomes Galanin's formula [3]

$$\frac{\tau}{\tau_0} = \frac{1+\gamma_\mathrm{A}^2-(\frac{3}{2}+\gamma_\mathrm{A}^2)f(\gamma_\mathrm{A})}{1-f(\gamma_\mathrm{A})}\,. \tag{5}$$

MFDT of rhodamine 6G (donor) in the presence of malachite green (acceptor) was investigated with pulse fluorometer using the sampling method. The device used in the measurements was described earlier [4]. Rhodamine 6G ($C_{26}H_{27}O_3N_2Cl$ m.w. 450.98) and malachite green ($C_{25}H_{26}N_2O_4$ m.w. 418.53) were purified by repeated crystallization from ethyl alcohol under vacuum. The measurements were carried out for three series of ethanol solutions. For each series the acceptor concentration was kept constant while the donor concentration was changed. The samples were excited by the green line ($\lambda=514$ nm) of an argon laser. In view of the very low absolute yield of malachite green in liquid solutions [5] one needs an OS-13 cut-off filter ($\lambda>570$ nm) only for the observation of the rhodamine 6G fluorescence. In order to minimize the effect of reabsorption thin layers of solution were used so that the optical density reached 0.1. In the light of the discussion presented by Porter and Tredwell [6], the influence of material diffusion on the non-radiative energy transfer can be neglected in this system.

The measured signal $F_\mathrm{M}(t)$ averaged by means of a multichannel analyser was a convolution of the excitation function $A(t)$ with the decay function $\Phi(t)$

$$F_\mathrm{M}(t) = \int_0^t A(t-t')\,\Phi(t')\,\mathrm{d}t'\,. \tag{6}$$

Due to the complex form of the decay function $\Phi(t)$ in this case we could not apply conventional methods in which it is assumed that $\Phi(t)$ is, for example, exponential [7]. The method worked out by Bauer and Wolniewicz [8] was applied here. In this method it is assumed that

$$\Phi(t) = \exp(-\beta t)\sum_{j=1}^{J} k_j\,\omega_j(t)\,, \tag{7}$$

where β is a parameter with no physical meaning, k_j are coefficients, $\omega_j(t)$ are polynomials of the jth degree,

$$\omega_{j+1}(t) = a_{j+1}\,t\,\omega_j(t) + \sum_{l=1}^{j} b_l^{(j+1)}\,\omega_l(t)\,, \tag{8}$$

and J is the highest degree of these polynomials.

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Table 1. Comparison of experimental and theoretical results.

γ_A	0.5			0.75			1.5		
α	$\left(\frac{\tau}{\tau_0}\right)_E$	$\left(\frac{\tau}{\tau_0}\right)_T$	β [10^9 s]	$\left(\frac{\tau}{\tau_0}\right)_E$	$\left(\frac{\tau}{\tau_0}\right)_T$	β [10^9 s]	$\left(\frac{\tau}{\tau_0}\right)_E$	$\left(\frac{\tau}{\tau_0}\right)_T$	β [10^9 s]
0	—	0.65	—	—	0.53	—	—	0.30	—
0.167	0.57	0.63	0.4	0.43	0.50	0.6	0.23	0.26	0.9
0.5	0.54	0.55	0.55	0.39	0.39	0.7	0.16	0.16	1.4
0.833	0.36	0.29	0.8	0.15	0.16	1.5	—	—	—

Having put this $\Phi(t)$ into (6) one obtains a calculated signal $F_c(t)$. Defining

$$G_j(t) = \int_0^t A(t-t')\, e^{-\beta t'}\, \omega_j(t')\, dt' \tag{9}$$

one has

$$F_c(t) = \sum_{j=1}^{J} k_j G_j(t). \tag{10}$$

Assuming $\omega_1 =$ constant and putting

$$\tilde{G}_j(t) = \int_0^t A(t-t')\, t'\, e^{-\beta t'}\, \omega_j(t')\, dt' \tag{11}$$

one can calculate the coefficients a and b of the polynomials (8) from the condition of orthonormality of $G_j(t)$. Minimalizing the expression $\Delta = \sum_i [F_M(t_i) - F_c(t_i)]^2$ one gets

$$k_l = \sum_i F_M(t_i)\, G_l(t_i), \quad l = 1, 2, \ldots, J. \tag{12}$$

Thus, for the so obtained coefficient

$$\Delta_J = \sum_i [F_M(t_i)]^2 - \sum_{j=1}^{J} k_j^2. \tag{13}$$

Values of the coefficients k were not printed out by the computer. The best fit of $F_c(t_i)$ to the experimental values $F_M(t_i)$ was judged by the magnitude of Δ_J. For the eight samples measured the best fit, i.e. the lowest Δ_J value, was obtained for $J = 8$ and the individually selected β values given in Table 1. Values of Δ_J are of the order of 10^{-3}. Selecting β for small α values ($\alpha = 0.167$) we took into consideration that the decay should be close to a Förster type [2, 6]

$$\Phi(t) \sim \exp[-t/\tau_0 - 2\gamma_A (t/\tau_0)^{1/2}]. \tag{14}$$

To calculate β we compared this decay with the first term of (7) $\Phi(t) \sim \exp(-\beta t)$ for

$$t = \tau_0 = 4.2\,\text{ns}\,[6]: \beta \approx (1 + 2\gamma_A)/\tau_0.$$

For larger α the fluorescence decays faster, and we increased β appropriately. The choice of the β value is not critical in obtaining a good fit, although a proper selection of β reduces the J value and computation time. The detailed discussion of this deconvolution method will be presented in [8]. Comparison of the values of $\Phi(t_i)$ obtained with theoretical results concerning the influence of the energy migration among donors on the fluorescence decay will be the subject of another paper [9].

From (1) we found MFDT using known values of $\Phi(t)$. τ_0 was obtained from investigations of the fluorescence decay of the donor (rhodamine 6G) in the absence of acceptor. Values of $\Phi(t)$ obtained in this case by the above method form a straight line on a $\ln \Phi(t)$ vs t plot. By the method of least squares $\tau_0 = 4.2$ ns was found. In Table 1 there are presented values of $(\tau/\tau_0)_E$ obtained from the pulse measurements and compared with theoretical results $(\tau/\tau_0)_T$ from (2). Values of γ_A and γ_D have been computed for $c_{0A} = 1.77 \times 10^{-3}$ M/l and $c_{0D} = 2.54 \times 10^{-3}$ M/l [10]. Also presented in Table 1 are values of $(\tau/\tau_0)_T$ from (5), where the migration of excitation energy among donors was not taken into consideration ($\alpha \approx 0$).

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