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CONCENTRATION QUENCHING OF FLUORESCENCE IN MIXED DYE SOLUTIONS*,**

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Fluorescence concentration quenching and sensibilization of mixed dyes in solution were measured. The experimental results were compared with numerical values calculated from expressions derived from the active sphere model theory of migration given by Jabłoński. From the difference of experimental results obtained, due to different excitation wavelengths, conclusions regarding possible migration mechanism are drawn.

1. *Introduction*

Quantitative measurements of concentration depolarization and quenching of like and mixed dyes in solution provide information about the mechanism of migration of excitation energy. Since "in vivo" mostly dye mixtures with usually strong overlapping absorption bands exist, it seems specially interesting to study energy migration between such molecules.

In previous papers [1–3] concentration depolarization in mixed dye solutions as a function of increasing absorption band overlap and excitation wavelength were studied. The experimental results were compared with theoretical curves evaluated from Jabłoński's concentration depolarization theory [4] only in the case of ν_{0-0} excitation and small absorption band overlap. To explain the discrepancies between experimental results and theoretical predictions a possible before relaxation back-transfer from acceptor to donor molecules was assumed.

"Before-relaxation" energy transfer is a well known phenomenon in crystals [5], gases [6] and solutions [7]. A possible energy migration, before a Boltzmann distribution of vibrational energy in the S_1 state is established, was suggested also in the case of aromatic hydrocarbon solutions [8] (a dependence of the concentration depolarization on excitation wavelength in hydrocarbon solution was found also in [9] although in a much lesser

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* Dedicated to our Master and Teacher Professor Aleksander Jabłoński on the occasion of his 80th birthday.

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degree) and other conventional dye solutions [1–3, 10, 11, 16]. The effect of possible energy migration from higher vibrational levels on the measured concentration depolarization is very weak and to study it one must perform careful and accurate measurements. The aim of this paper is to study the concentration quenching and sensibilization of dyes with strongly overlapping absorption bands, and to compare the results with theoretical evaluation based on formulas derived from Jabłoński's theory of active sphere model of migration.

2. *Theory*

A new approach to Jabłoński's earlier [17] active sphere model of excitation energy transfer was given by him in later papers [4, 12, 13]. This improved version is more general and introduces directly the migration rate constant.

The most general expressions for probabilities of emission in the case of mixed unlike dyes are given in [4] (Eqs. (21) to (23)). They allow us to evaluate formulas for practically every case of concentration depolarization as well as quenching. Even before-relaxation energy transfer may be taken into account (although this is very troublesome since usually one does not know the numerical values of parameters of before-relaxation energy transfer). First such evaluation of specific formulas which enable one to make numerical calculations for the case of concentration depolarization were performed in [14] and for concentration quenching in [15]. Wróbel et al. [15] calculated the efficiency of energy transfer for the simplest case of two unlike kinds of molecules with very low donor concentrations.

To find a more general equation for the case of equimolar solutions of two different kinds of molecules one has to take into account centres of type I (1 donor of dye I, $k-1$ acceptors of dye I and l acceptors of dye II) as well as of type II (1 donor of dye II, $l-1$ acceptors of dye II and k acceptors of dye I). The probability of occurrence of these centres in solution is given by Smoluchowski's distribution which in our case reads

$$P_{kl} = \frac{\alpha\chi}{\alpha\chi+\alpha'\chi'}\, e^{-(\chi+\chi')}\, \frac{\chi^{(k-1)}}{(k-1)!}\, \frac{\chi'^{l}}{l!}. \tag{1}$$

Here α and α' are coefficients proportional to the extinction of the appropriate wavelengths exciting dye I or dye II, whereas $\chi = VN$ and $\chi' = VN'$ (V is the volume of the active sphere and N, N' are the number densities of dye I and dye II, respectively).

The simplest mixed dye centre of type I is the one containing one donor I and one acceptor II. The probabilities of finding the excitation with donor DI ($p_{\mathrm{I}}(t)$) and acceptor AII ($p_{\mathrm{II}}(t)$) at time t is governed by the equations

$$\frac{dp_{\mathrm{I}}(t)}{dt} = -(\gamma_{\mathrm{I}}+q_{\mathrm{I}}+\mu_{\mathrm{I\,II}})p_{\mathrm{I}}(t)+\mu_{\mathrm{II\,I}}p_{\mathrm{II}}(t),$$

$$\frac{dp_{\mathrm{II}}(t)}{dt} = \mu_{\mathrm{I\,II}}p_{\mathrm{I}}(t)-(\gamma_{\mathrm{II}}+q_{\mathrm{II}}+\mu_{\mathrm{II\,I}})p_{\mathrm{II}}(t), \tag{2}$$

where γ, q and μ are the rates of fluorescence, quenching and migration from molecules of type I to molecules of type II ($\mu_{\mathrm{I\,II}}$) and reverse ($\mu_{\mathrm{II\,I}}$), respectively. The probability of photon emission by DI is

$$F_{\mathrm{D1}1} = \gamma_{\mathrm{I}} \int_0^\infty p_{\mathrm{I}}(t)dt = \eta_{0\mathrm{I}} \frac{1+\mu_{\mathrm{II\,I}}\eta_{0\mathrm{II}}\tau_{0\mathrm{II}}}{1+\mu_{\mathrm{I\,II}}\eta_{0\mathrm{I}}\tau_{0\mathrm{I}}+\mu_{\mathrm{II\,I}}\eta_{0\mathrm{II}}\tau_{0\mathrm{II}}}. \tag{3}$$

To average this expression over all possible orientations and distances we introduce the following expressions

$$\mu_{\mathrm{I\,II}} = \frac{\kappa^2}{\tau_{0\mathrm{I}}}\left(\frac{R_0^{\mathrm{I\,II}}}{R}\right)^6 = \frac{\kappa^2}{\tau_{0\mathrm{I}}}\beta_{\mathrm{I\,II}}\left(\frac{R_0^{\mathrm{I\,I}}}{R}\right)^6,$$
$$\mu_{\mathrm{II\,I}} = \frac{\kappa^2}{\tau_{0\mathrm{II}}}\left(\frac{R_0^{\mathrm{II\,I}}}{R}\right)^6 = \frac{\kappa^2}{\tau_{0\mathrm{II}}}\beta_{\mathrm{II\,I}}\left(\frac{R_0^{\mathrm{I\,I}}}{R}\right)^6, \tag{4}$$

where $\beta_{\mathrm{I\,II}}$ and $\beta_{\mathrm{II\,I}}$ characterize the ratios of the rate of migration

$$\beta_{\mathrm{I\,II}} = (\mu_{\mathrm{I\,II}}/\mu_{\mathrm{I\,I}}) = \frac{J_{\mathrm{I\,II}}}{J_{\mathrm{I\,I}}}, \qquad \beta_{\mathrm{II\,I}} = \frac{\mu_{\mathrm{II\,I}}}{\mu_{\mathrm{I\,I}}} = \frac{J_{\mathrm{II\,I}}}{J_{\mathrm{I\,I}}} \tag{5}$$

(here J's are the overlap integrals). It the overlap integral $J_{\mathrm{II\,I}}$ of the emission band (dye II) and absorption band (dye I) is equal to zero (this is almost the case for the dyes rhodamine 6G and rhodamine B) a back transfer from dye II to dye I is assumed to be impossible after a Boltzmann distribution of vibrational levels is established. The following equation describes the probability of emission without taking before-relaxation energy transfer into account and is simpler in form than Eq. (3)

$$F_{\mathrm{D1}1} = \eta_{0\mathrm{I}} \frac{1}{1+\beta_{\mathrm{I\,II}}\mu_{\mathrm{I\,I}}\eta_{0\mathrm{I}}\tau_{0\mathrm{I}}}. \tag{6}$$

By averaging and summing $\langle F_{\mathrm{D}kl}\rangle$ over all centres in the solution and introducing the Smoluchowski distribution of these centres one obtains

$$\overline{F}_{\mathrm{D}kl} = \sum_{k=1}^{\infty}\sum_{l=0}^{\infty} P_{kl}\langle F_{\mathrm{D}kl}\rangle. \tag{7}$$

The same operation for $\langle F_{\mathrm{A}kl}\rangle$ leads to

$$\overline{F}_{\mathrm{A}kl} = \sum_{k=1}^{\infty}\sum_{l=0}^{\infty} P_{kl}(k-1)\,\langle F_{\mathrm{A}kl}\rangle. \tag{8}$$

The sum of Eqs. (7) and (8) gives the whole probability of light emission by molecules of type I in the presence of molecules of type II. This probability is also equal to

$$\overline{F}_{\mathrm{D}kl}+\overline{F}_{\mathrm{A}kl} = \eta_{0\mathrm{I}}(1-f) = \eta_{0\mathrm{I}}\frac{\eta_{\mathrm{I}}}{\eta_{0\mathrm{I}}}, \tag{9}$$

where f is the quantum efficiency of energy transfer. Eqs. (8) and (9) lead to

$$\frac{\eta_{\mathrm{I}}}{\eta_{0\mathrm{I}}} = \frac{1}{\eta_{0\mathrm{I}}}\left[\sum_{k=1}^{\infty}\sum_{l=0}^{\infty} P_{kl}(\langle F_{\mathrm{D}kl}\rangle + (k-1)\langle F_{\mathrm{A}kl}\rangle)\right]. \tag{10}$$

The mean value of $F_{\mathrm{D}kl}$ and $F_{\mathrm{A}kl}$, which appear in Eq. (10) are for $k+l > 2$ evaluated in a somewhat simplified way [12]. In numerical calculations it was assumed that $\alpha' = 0$, what means that molecules II are excited only via energy transfer from molecules I. In fact, one excites directly both kinds of molecules and only our experimental procedure allows us to consider just the quenched and sensibilized light intensity.

If there exists a transfer mechanism which allows a migration from higher vibrational levels of molecule II to molecule I then $\beta_{\mathrm{II\,I}} \neq 0$. In this case the assumption $\alpha' = 0$ is fully justified only if molecules of type II are not directly excited and therefore we derived in a similar way as above the following expression with the assumptions that both $\alpha' \neq 0$ and $\beta_{\mathrm{II\,I}} \neq 0$:

$$\frac{\eta_{\mathrm{I}}}{\eta_{0\mathrm{I}}} = \frac{1}{\eta_{0\mathrm{I}}}\Bigg\{e^{-(\chi+\chi')}\sum_{k=1}^{\infty}\sum_{l=0}^{\infty}\frac{\chi^{(k-1)}}{(k-1)!}\,\frac{\chi'^{l}}{l!}\left[\langle F_{\mathrm{D}kl}\rangle + (k-1)\langle F_{\mathrm{A}kl}\rangle\right]$$

$$+\frac{\alpha'}{\alpha}\,e^{-(\chi+\chi')}\sum_{k=0}^{\infty}\sum_{l=1}^{\infty}\frac{\chi^{k}}{k!}\,\frac{\chi'^{(l-1)}}{(l-1)!}\,k\langle F'_{\mathrm{A}kl}\rangle\Bigg\}. \tag{11}$$

In Eq. (11) the second term describes the migration of energy from directly excited molecules II to molecules I. This term is equal to zero if $\alpha' = 0$ and then in Eq. (11) only back-transfer is considered. In the case of direct excitation of both kinds of molecules (even if the experimental procedure allows for exclusion of the part of directly excited light intensity) there exists a migration from directly, as well as transfer, excited molecules of type II if $\beta_{\mathrm{II\,I}} \neq 0$.

3. *Experimental*

The studied dyes (rhodamine 6G and rhodamine B) were chosen because they have a considerably high overlap of absorption bands (Fig. 1). The solvent was ethylene glycol which at room temperature (all the measurements were performed at this temperature) has a sufficiently high viscosity not to consider diffusion controlled energy transfer. In this solvent dimer formation of rhodamine 6G up to a concentration of 5×10^{-3} M is negligible and the optical density of mixed dyes is equal to the sum of optical densities of pure solutions in the spectral range of interest. The optical density for all the measured samples (even for mixed equimolar solutions) was kept lower than 0.05. The excitation light source was a quartz-halogen bulb with a stabilized current source. The excitation wavelenghts were 500, 525 and 547 nm isolated by means of a "Zeiss-Jena" SPM-2 monochromator. The emitted light was viewed through a second SPM-2 monochromator and detected by a calibrated EMI 9558 QB photomultiplier. The photocurrent was measured using a "lock-in" system and a recorder. The whole mechanical set-up was very stable and accurate to ensure a geometrical reproducibility while changing the samples. This somewhat complicated method was necessary since the accuracy of measurements has to be very high to use the measured light intensities in the method of calculating the transfer efficiencies in the case of strongly overlapping emission bands. The basic idea of this method was described earlier [16].

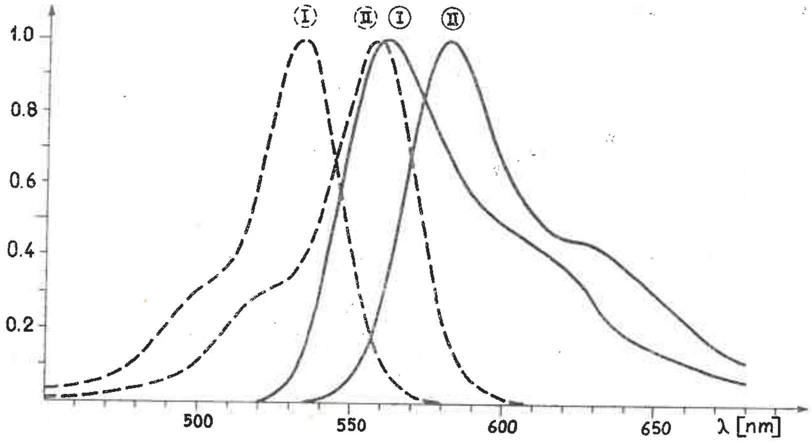

Fig. 1. Absorption (------) and emission (———) spectra of rhodamine 6G (I) and rhodamine B(II)

We assume here that our samples have an equal thickness for a set of measurement, the geometry of the measuring system is constant and the concentration exactly the same. Under these conditions

$$1-f_D = ({}^2I_D/{}^1I_D) = (\eta_I/\eta_{0I}) \tag{12}$$

and

$$f_A = \frac{\varepsilon_A}{\varepsilon_D}\left[\left(\frac{{}^2I_A}{{}^1I_A}\right)-1\right], \tag{13}$$

where f_D and f_A are the efficiencies of energy transfer gained from quenching and sensibilization measurements, respectively, and ε are the appropriate extinction coefficients. The meanings of the super and subscripts indicating the light intensities I of fluorescence are given elsewhere [3, 16]. From fluorescence light intensity measurements of pure donor and acceptor solutions (superscript 1) as well as mixed solutions (superscript m) under steady geometrical conditions one can calculate intensities of the donor and acceptor fluorescence themselves (superscript 2). A straightforward calculation gives thus

$$\frac{\eta_{\mathrm{I}}}{\eta_{0\mathrm{I}}} = \frac{{}^{\mathrm{m}}I_{\mathrm{D}} - b\,{}^{\mathrm{m}}I_{\mathrm{A}}}{{}^{1}I_{\mathrm{D}}(1-b\beta)}, \tag{14}$$

$$f_{\mathrm{A}} = \frac{\varepsilon_{\mathrm{A}}}{\varepsilon_{\mathrm{D}}}\left[\frac{{}^{\mathrm{m}}I_{\mathrm{A}} - \beta\,{}^{\mathrm{m}}I_{\mathrm{D}}}{{}^{1}I_{\mathrm{A}}(1-b\beta)} - 1\right]. \tag{15}$$

The results of measurements are plotted in Fig. 2.

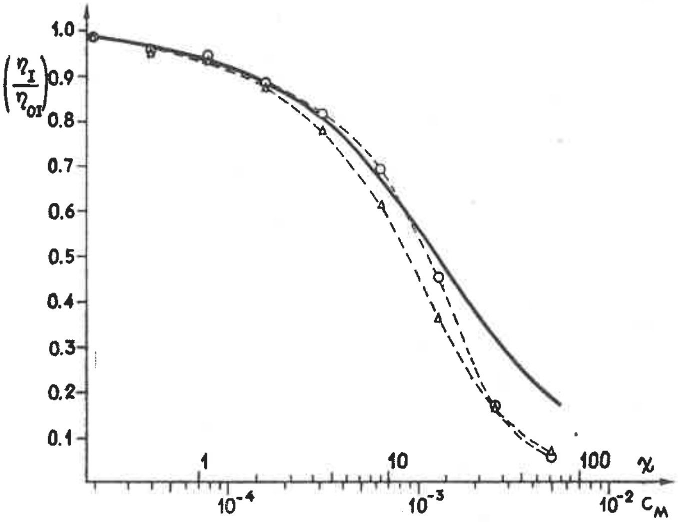

Fig. 2. Concentration quenching of fluorescence of rhodamine 6G due to energy transfer to rhodamine B. ○, △ — experimental data for excitation wavelengths 547 nm and 500 nm, respectively; ——— — theoretical curve calculated from Eq. (10)

4. *Discussion*

Although the measurements were performed by exciting the molecules with three difference wavelengths, we present in Fig. 2 results for only two such wavelengths. The excitation wavelength $\lambda = 547$ nm corresponds to the 0–0 transition, the experimental results for the shorter wavelengths are practically equal. The solid line represents the theoretical curve evaluated from Eq. (10), i. e. with no back-transfer considered. Therefore a comparison of this curve should rather be performed with experimental results gained by λ_{0-0} excitation. The best fit of theoretical and experimental curves is achieved if in Eq. (10) R_0 equals 6.77 nm. This fit could be made much more accurate for low concentrations up to 8×10^{-4} M if one puts $R_0 = 6.52$ nm then, however, the discrepancy in the concentration region up to 5×10^{-3} M rises considerably. Such a discrepancy between experimental results and the theoretical curve for high concentrations results either from the simplification in calculating $\langle F_{\mathrm{D}kl} \rangle$ and $\langle F_{\mathrm{A}kl} \rangle$ for $k+l > 2$ [12] or because there exists a strong overlap of absorption bands influencing the distance dependence of the transfer rate.

From the results shown in Fig. 2 it follows that if one excites higher vibrational levels of the donor the energy loss from the donor to acceptor molecules is more probable. Only for very high concentrations (2×10^{-3} M and higher) the dependence of $\eta_{\mathrm{I}}/\eta_{0\mathrm{I}}$ on excitation wavelength is reversed. In order to search for a possible explanation of the experimental results we present them in Fig. 3 in a somewhat different way. Due to the mentioned simplification in $\langle F_{\mathrm{D}kl} \rangle$ and $F_{\mathrm{A}kl} \rangle$ calculation we compare these results with theoretical curve only for concentrations up to about 10^{-3} M and therefore put for the upper curve (λ_{0-0} excitation) $R_0 = 6.52$ nm and for the lower curves (500 nm excitation) $R_0 = 7.26$ nm. The two lower curves are calculated from Eq. (11) assuming α'/α equal to the ratio of the extinction coefficients for 500 nm, and $\beta_{\mathrm{II\,I}}$ equal 0.02 and 0.1. As one sees the shape of the calculated curves is quite independent of the $\beta_{\mathrm{II\,I}}$ value in the mentioned concentration region. The calculated $\eta_{\mathrm{I}}/\eta_{0\mathrm{I}}$ values depend however on $\beta_{\mathrm{II\,I}}$ the stronger, the higher the dye concentration. The measured change of the slope of the $\eta_{\mathrm{I}}/\eta_{0\mathrm{I}}$ dependence on concentration for $\lambda_{\mathrm{exc}} = 547$ nm does not, therefore, necessarily mean that the energy transfer for the latter wavelength gets less pronounced.

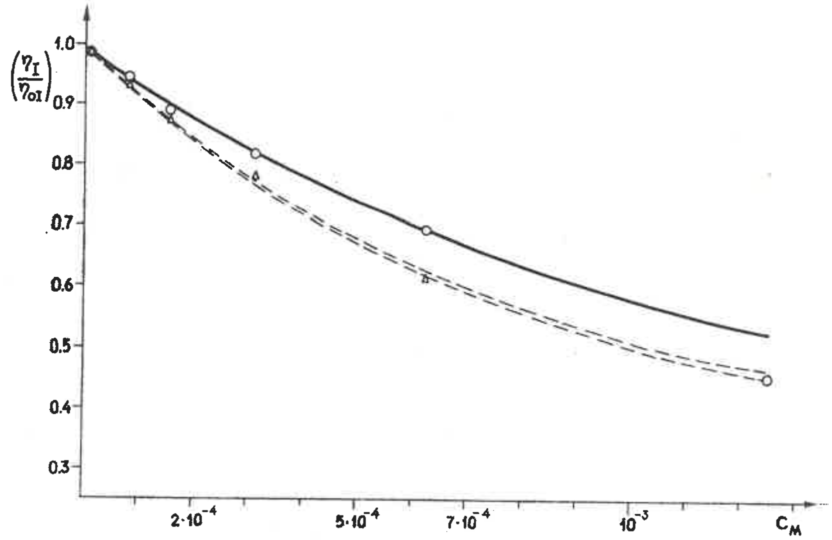

Fig. 3. Concentration quenching of fluorescence of rhodamine 6G due to energy tranasfer to rhodamine B. ○, △ experimental data for excitation wavelengths 547 nm and 500 nm, respectively; —— theoretical curve calculated from Eq. (10). ----- theoretical curve calculated from Eq. (11) for $\beta_{\mathrm{II\,I}} = 0.1$ (upper curve) and for $\beta_{\mathrm{II\,I}} = 0.02$ (lower curve)

It can mean also that there exsists a back-transfer from acceptor to donor molecules. Such a point of view was expressed earlier [16] explaining similar results for the case of a Chl **a** — Chl **b** mixture.

The present experiments confirm these earlier results also for the more conventional dyes studied here, and prove the existence of some kind of before-relaxation energy transfer mechanism. In our opinion there exists a possibility of energy transfer from higher vibrational levels of the donor to the acceptor and also a back-transfer from sufficiently high vibrational levels of the acceptor to the donor.

Such energy transfer requires a very high rate since from picosecond spectroscopy studies it is well known that the redistribution time of vibrational levels (intramolecular vibrational relaxation) is equal to about 5 ps [18]. A high rate would in turn require a stronger than "very weak" interaction (R^{-6} distance dependence) as discussed in [19]. Such stronger interaction, although arguable, could be possible if one takes into account the high overlap of the "virtual" emission band of the donor with the absorption band of the acceptor [7]. A weak or intermediate interaction takes place if molecules are much closer to each other than the mean distances in solution even for concentration of the order of 10^{-3} M, one has, however, to remember that real intermolecular distances may be much smaller (Poisson distribution!) than the mean ones.

A somewhat different mechanism of a before relaxation energy transfer should also be considered. According to Vries [6] in anthracene vapour one observes a hot fluorescence with a decay time equal to the normal fluorescence, what excludes a direct emission from higher excited vibrational levels. In that case the initially high vibrational energy is in the very fast (few picosecond) process of redistribution (intramolecular relaxation) spread over great number of low energy vibrational modes. The distribution of the whole vibrational energy fluctuates and during the lifetime of the molecule in the excited electronic state high vibrational levels may get populated and the "hot" emission occurs from these levels. A similar situation may occur in solutions of dyes if one assumes that the relaxation of vibrational energy from the molecule to the thermal bath of the sample is of the order of the decay time. To our knowledge this relaxation time was not measured yet. If fluctuation of the redistributed vibrational energy does result in the population of higher levels, the energy transfer takes places from a broader manifold of such levels increasing the transfer rate.

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