ON THE ENERGY TRANSFER IN LUMINESCENT MIXED SOLUTIONS*

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The problem of the determination of "true" fluorescence intensities and emission anisotropy, not disturbed by the radiative excitation energy transfer has been considered for mixed systems. The expression describing the recorded flux of light has been modified by taking into account the spatially anisotropic distribution of the fluorescence intensity. Mixed systems of Na-fluorescein (donor) and rhodamine B (acceptor), characterized by high concentrations of donor molecules, were investigated. Substantial discrepancies were observed between the efficiencies of the excitation energy transfer from the donor to the acceptor, determined from the measurements of concentration dependent changes of the donor quantum yield and the acceptor fluorescence intensities.

1. Introduction

The transfer of the electronic excitation energy in mixed systems leads to the quenching of the donor fluorescence and intensification of the acceptor radiation if this component is also fluorescent. At the same time an increase of the polarization degree of the donor molecules' fluorescence and decrease of their lifetime in the excited state are observed [1].

It was pointed out in some papers [2–4] that the fluorescence intensity of the acceptor molecules was lower than could be expected from the changes of the donor quantum yield. This discrepancy is especially marked in mixed systems of high concentration. Its explanation could be of interest in connection with e.g. photosynthesizing systems.

Supposedly, an additional process of excitation energy degradation accompanying acts of energy transfer between active molecules of the solution may be responsible in part for the discrepancy [5, 6]. Results obtained for dimerizing systems confirm this opinion. By taking into account this process consistent explanation of the effects of concentration dependent quenching and depolarization observed in these systems was made possible [7–10].

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For luminescent systems of high concentration the radiative excitation energy transfer is, also beside the nonradiative one, an important process. The influence of the energy migration due to the radiative transfer on the fluorescence spectrum in mixed systems has been examined in detail in [11, 12] under the assumption of isotropic spatial fluorescence distribution. Actual distributions are, in general, anisotropic and strongly affected by the state of polarization of the solution investigated [13, 14]. The transfer of the excitation energy from the donor molecules to those of the acceptor results in a rise of the polarization degree of the first component and decrease of its value for the second component. This effect induces an additional deformation of the measured emission spectra of the mixed systems. This leads to some changes in the nonradiative excitation energy transfer efficiency.

2. *Theoretical*

Consider a rigid (viscous) solution containing fluorescent molecules of two kinds, namely energy donors at concentration c_1, and energy acceptors, of concentration c_2. Assume that the donor emission and acceptor absorption spectra overlap, while no overlapping takes place for the acceptor emission and donor absorption spectra. The last condition means that no return of the energy from the acceptor molecules to those of the donor, either by radiative or by nonradiative transfer process, is possible [1].

For such solutions Dombi [12] found, taking into account both radiative and nonradiative energy transfer, the relation between the directly measured fluorescence spectrum $B(\lambda')$ of the mixed solution and the normalized fluorescence quantum-spectra $f_1(\lambda')$, $f_2(\lambda')$ of the solution components. The expression obtained there for $B(\lambda')$ can be written as

$$B(\lambda') = C(\lambda, \lambda') \cdot \{[\eta_1'(\lambda)/(1-\kappa_{11})] f_1(\lambda') + [\eta_1'(\lambda) \cdot \kappa_{12}/(1-\kappa_{11})(1-\kappa_{22}) + \eta_2'(\lambda)/(1-\kappa_{22})] \cdot f_2(\lambda')\}, \tag{1}$$

$$C(\lambda, \lambda') = (\varrho/4\pi n^2) \cdot E_0(\lambda) \cdot [\alpha/(\alpha+\beta)] \cdot [1-\exp(\alpha+\beta)],$$

where λ and λ' are the wavelengths of the exciting and luminescent light, respectively; $\eta_i'(\lambda)$ is the apparent yield of the i-th component of the mixed solution associated with the nonradiative energy transfer, defined as the quotient of the number of photons emitted by the i-th component of the mixed solution and that of photons absorbed by the mixed solution in the same volume and time interval; $\alpha = k(\lambda) \cdot l$, $\beta = k(\lambda') \cdot l$, $k(\lambda) = k_1(\lambda) + k_2(\lambda)$, where $k_i(\lambda)$ is the absorption coefficient of the i-th component; l is the solution layer thickness, $E_0(\lambda)$ — the quantum density of the exciting light, n — the refractive index of the solution, and ϱ and κ_{ij} — quantities describing the losses due to the reflection and the radiative energy transfer, respectively.

Let $r_1(\lambda, \lambda')$ and $r_2(\lambda, \lambda')$ denote the emission anisotropy of the primary fluorescence for the respective components of the mixed solution. If we assume that the fluorescence of the second and higher orders is not polarized [15], then it follows from the Weber addition law [16, 17] that the measured anisotropy of emission of mixed solution, $r_{12}'(\lambda, \lambda')$,

is given by

$$r'_{12}(\lambda, \lambda') = [C(\lambda, \lambda')/B(\lambda')] \cdot [\eta'_1(\lambda) f_1(\lambda') \cdot r_1(\lambda, \lambda') + \eta'_2(\lambda) f_2(\lambda') \cdot r_2(\lambda, \lambda')]. \quad (2)$$

It was shown [13, 14] that even for solutions excited by unpolarized light the spatial distribution of the emitted fluorescence is anisotropic and depends on the fluorescence degree of polarization.

For any particular kind of fluorescence characterized by the emission anisotropy r the following relation holds true [14]

$$I' = I \cdot (1 + a \cdot r), \quad (3)$$

where I' is the fluorescence intensity detected, I is the fluorescence intensity averaged over the full solid angle, and a is a constant depending on the geometry and polarization of the exciting light beam.

As equation (1) was derived under the assumption of spatial isotropy of the fluorescence emitted, $B(\lambda')$ should be regarded as the mean fluorescence intensity for the mixed system. Taking into account the anisotropy of the fluorescence distribution we arrive at the relation

$$B'(\lambda') = B(\lambda') \cdot [1 + a \cdot r'_{12}(\lambda, \lambda')] \quad (4)$$

which is analogous to (3). Here $B'(\lambda')$ is the intensity of the mixed system fluorescence detected by the measuring system. Relation (4) has some practical importance, because the value of the correction factor $1 + a \cdot r'_{12}(\lambda, \lambda')$ can vary as much as 20% [14].

Reference [12] gives a detailed description of the methods of determination of the quantum yields of the donor and acceptor molecules due to the nonradiative excitation energy transfer, together with their phenomenological relations to $\eta'_i(\lambda)$ and κ_{ij}. The general relations obtained there remain valid when values of $B(\lambda')$ resulting from (4) are substituted into formula (1). Thus, if the quantum yields $\eta'_1(\lambda)$ and $\eta'_2(\lambda)$ are known it is possible, in turn, to find from relation (2) the experimental values of the emission anisotropy r_1 and r_2. These depend on the nonradiative energy transfer only. It is especially easy to determine the value of the emission anisotropy r_1 for the donor molecules if the measurements for a mixed solution are carried out in a range λ' where no fluorescence of the acceptor is observed (i.e. $f_2(\lambda') = 0$). Then the following simple relation holds true

$$r_1(\lambda, \lambda') = r'_{12}(\lambda, \lambda')/(1 - \kappa_{11}), \quad (5)$$

analogous for that valid for a single-component system.

The concentrational changes of the quantum yield and emission anisotropy observed for mixed solutions are determined by elementary processes occurring in these solutions and leading to the excitation energy degradation. If the donor absorption and acceptor emission spectra do not overlap the following elementary processes should be taken into account

a) spontaneous emission, with the rate constant s_i for the molecules of the i-th species ($i = 1, 2$),

b) internal quenching, characterized by the rate constant b_i,

c) nonradiative energy transfer between molecules of the same kind, with the rate constant a_{ii},

d) nonradiative energy transfer from the donor to the acceptor, characterized by the rate constant a_{12}.

Besides these processes some authors [5–8] allow for the possibility of the energy deactivation via an additional channel, in which the excitation energy transfer to a molecule does not cause its electronic excitation.

This effect, when accounted for in a manner analogous to that presented in [12] leads to the following relations

$$K_i = (s_i/e_i)/[s_i/(s_i+b_i)], \tag{6}$$

$$K_{12} = (s_2/e_2)\cdot\alpha_0^{(12)}\cdot(a_{12}/e_1)/[s_1/(s_1+b_1)], \tag{6'}$$

$$e_i = s_i+b_i+(1-\alpha_0^{(ii)})a_{ii}, \tag{7}$$

where K_i ($i = 1, 2$) has the meaning of the relative quantum yield of the i-th kind molecules in a mixed solution, resulting from the nonradiative excitation energy transfer between these molecules; K_{12} is the quantum yield of acceptor molecules due exclusively to the energy transfer from the donor to the acceptor; $\alpha_0^{(ij)}$ is the probability of excitation of a j-th kind molecule to an excited electronic state after an act of energy transfer from an i-th kind molecule.

Quantum yields K_1, K_2 and K_{12} can be found experimentally from the concentrational changes of the mixed system fluorescence intensities. Thus probabilities of occurrence of the elementary processes, s_i/e_i, b_i/e_i and a_{12}/e_1 can be determined from relations (6) and (6'). The remaining probabilities, a_{11}/e_1 and a_{22}/e_2, can be found from concentrational changes of the emission anisotropy.

Consider excited donor molecules in a mixed solution illuminated by a light beam of constant intensity $E_0(\lambda)$. Denote the concentration of these molecules by c_1 and assume that the concentration of the donor molecules which are primary light absorbers is $c_1^{(0)}$. For such a system the following equation is satisfied

$$\alpha_0^{(11)}a_{11}c_1^{(0)}-\alpha_0^{(11)}a_{11}c_1^{(b)}[1-F(c_1)]-(s_1+b_1+a_{11}+a_{12})c_1^{(b)} = 0. \tag{8}$$

This equation describes dynamic changes of the concentration, $c_1^{(b)} = c_1-c_1^{(0)}$ of those donor molecules that are not primary absorbers of light. The first term in Eq. (8) is the number of donor molecules (per unit time) which become excited as a result of the energy transfer from the primary light absorbers; the second term describes the number of donor molecules which are not absorbers of the external radiation and gain the excitation energy from molecules of the same type — here the quantity $F(c_1)$ is the probability of energy remigration to the primary absorbers. The third term denotes the number of molecules of type (b) which lose the energy in degradation processes.

Under the assumption that fluorescence of the primary light absorbers only contribute to the polarization, the following relation is obtained

$$r_1 = (c_1^{(0)}/c_1)\cdot r_{g1}, \tag{9}$$

where r_{g1} is the limit value of the donor emission anisotropy when no acceptor molecules (e.g. $c_2 = 0$) and the concentration $c_1 \to 0$.

From equations (8) and (9) we obtain

$$\alpha_0^{(11)} \cdot (a_{11}/e_1) = [r_1/(r_{g1} - r_1) - F(c_1)]^{-1}. \tag{10}$$

This relation can be used for the evaluation of the probability a_{11}/e_1. From an analogous dependence for the acceptor the value of a_{22}/e_2 can be estimated. The energy remigration coefficients $F(c_1)$ and $F(c_2)$ appearing in both relations cannot be measured experimentally but can only be determined from theoretical considerations regarding the concentrational depolarization of photoluminescence [18, 19].

3. *Experimental results*

To investigate the efficiency of the excitation energy transfer in mixed systems of high concentration two sets of solutions have been prepared, each having a fixed concentration of donor molecules ($c_1 = 3.16 \times 10^{-3}$ M/l for system I; $c_1 = 10^{-2}$ M/l for system II),

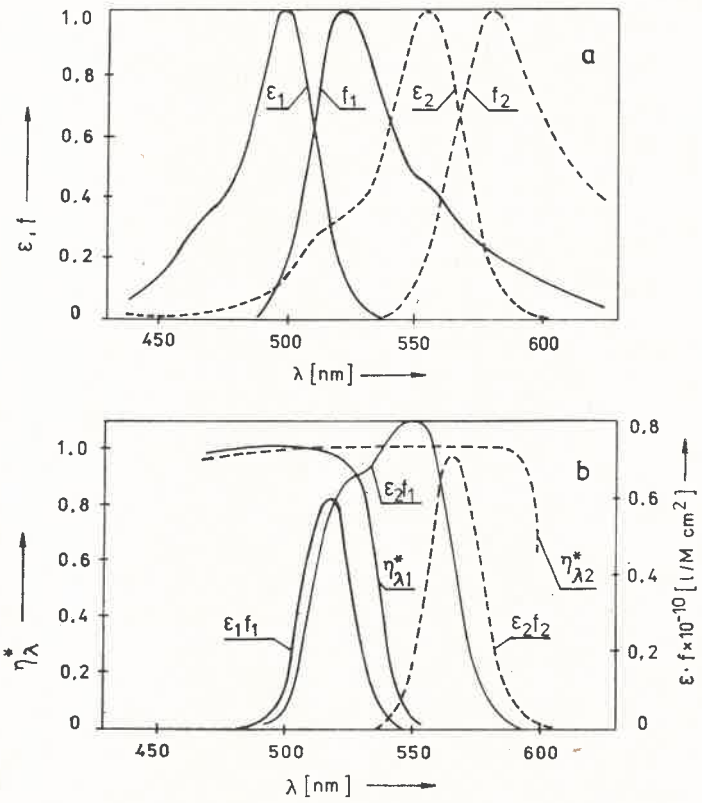

Fig. 1. a — Absorption (ε) and emission (f) spectra of Na-fluorescein (1) and rhodamine B (2); b — quantum yield vs exciting light wavelength, normalized to 1.0 at the maximum, for the donor ($\eta^*_{\lambda_1}$) and acceptor ($\eta^*_{\lambda_2}$); spectral distributions of products $\varepsilon_1 \cdot f_1$, $\varepsilon_2 \cdot f_2$ and $\varepsilon_2 \cdot f_1$

while the acceptor concentration was variable over the range $c_2 = 10^{-6} \div 10^{-3}$ M/l. Na-fluorescein was the donor, rhodamine B served as the acceptor. Dehydrated glycerin with 5% (w/w) of methanol, 0.4% H_2O and a small amount of NaOH was used as a solvent in both systems. The viscosity of this solvent was $\eta_{293K} = 6.2$ P, its pH value was 10.3.

For the mixed solutions and their components detailed investigations of the absorption, excitation, and emission spectra were carried out. Also the concentrational changes of the quantum yield and emission anisotropy were measured. Results of investigations of spectral courses for the component solutions are shown in Fig. 1. The spectra were stable within the above specified ranges of concentrations c_1 and c_2. The results obtained were used as a basis for the determination of the critical distances and concentrations for energy transfer due to the quantum-mechanical resonance [20]. The critical concentrations were equal to $c_{01} = 4.4 \times 10^{-3}$ M/l and $c_{02} = 2.5 \times 10^{-3}$ M/l for solutions of Na-fluorescein and rhodamine B, respectively, and $c_{012} = 2.1 \times 10^{-3}$ M/l for the donor-to-acceptor energy transfer. Besides, it can be seen from Fig. 1a that the donor absorption and the acceptor emission spectra were sufficiently well separated to allow neglection of the back-

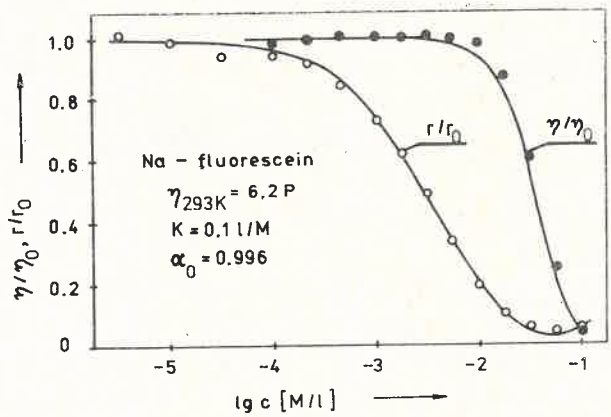

Fig. 2. Concentrational changes of quantum yield η/η_0 and emission anisotropy r/r_0 for Na-fluorescein. Continuous lines correspond to changes predicted by theories of Bojarski [7, 8]

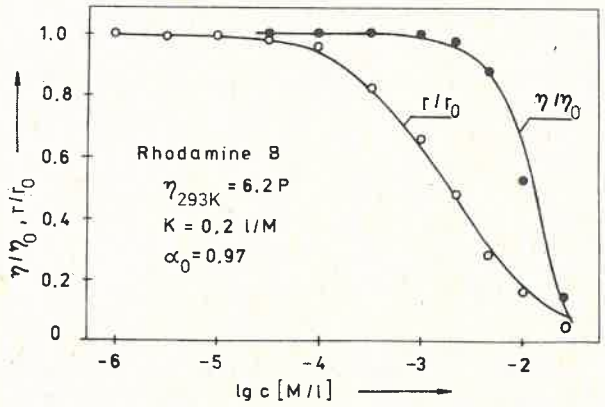

Fig. 3. The same as Fig. 2 — for rhodamine B

ward transfer from the acceptor to the donor. The results presented in Fig. 1 were also used for the determination of maximum values of the absolute quantum yield, following a procedure described in reference [21]. Values: $\eta_{01}^{m} = 0.80$ for Na-fluorescein at the wavelength $\lambda = 500$ nm, and $\eta_{02}^{m} = 0.985$ for rhodamine B, constant over the range $\lambda = 510$–580 nm, were obtained.

The experimental and theoretical results concerning the concentrational changes of the quantum yield and emission anisotropy for one-component solutions are presented in Figs 2 and 3. The theoretical curves have been plotted from the theories of Bojarski [7, 8] for the values of the dimerization constant K and probability α_0 determined using the method described in detail in [22]. Also values of the dimer concentration of Na-fluorescein in the mixed solutions I and II were obtained. Their values were 10^{-6} and 10^{-5} M/l for the system I and II, respectively. Thus the contribution of donor dimers in solutions in question was small and could be neglected. The same was valid for solutions of rhodamine B in the range of concentration $c_2 \leqslant 10^{-3}$ M/l. This was also indicated by the lack of the quantum yield changes within this range. Besides, it was observed for the mixed solutions that their absorption spectra agreed with total absorption spectra of component solutions. In conclusion we assumed that the mixed solutions under consideration contained, in practice, only monomer molecules of Na-fluorescein and rhodamine B.

Investigations of the fluorescence quantum yield and emission anisotropy for the mixed systems were carried out, as for the one-component solutions. Measurements were performed at a fixed wavelength of the exciting light $\lambda = 475$ nm, the fluorescence having been recorded at the wavelenths $\lambda_1' = 525$ nm and $\lambda_2' = 580$ nm, which corresponded approximately to the positions of maxima of the emission spectra for the donor and acceptor, respectively. Results of these measurements were subject to a processing based on relations presented in [12], modified following our considerations as presented in the theoretical part of this paper.

The measurements done at the wavelength λ_1' provided experimental values of the quantum yield $\eta/\eta_g = K_1/K_1^0$ and emission anisotropy $r/r_g = r_1/r_1^0$ of Na-fluorescein in mixed solutions (K_1^0 and r_1^0 being values of K_1 and r_1 for the donor at a constant concentration c_1 when $c_2 = 0$). They are shown in Fig. 4 by empty (system I) and full (system II) circles. The curves drawn in the same figure have been calculated from Bojarski's theories [7, 8]; in the computations the presence of dimers was neglected and the probability α_0 for the donor molecules was assumed equal to 0.996.

The recorded concentrational changes of the fluorescence intensity for mixed systems at the wavelength λ_2' were employed in the determination of experimental values of the efficiency of the excitation energy transfer from the donor to the acceptor. This efficiency, plotted against the acceptor concentration for both systems, subject to our investigations, is shown in Fig. 5 (continuous lines). Presented there are also the same relations (dashed lines) determined from measurements of the donor quantum yield at the wavelength λ_1' i.e. when the acceptor fluorescence is not registered. Fig. 6 shows, in addition, the concentration resolved emission anisotropy r_2 of rhodamine B as the acceptor in the systems I and II. The curves were obtained from measurements of polarization in these systems at the wavelength λ_2', and formula (2) of the present paper.

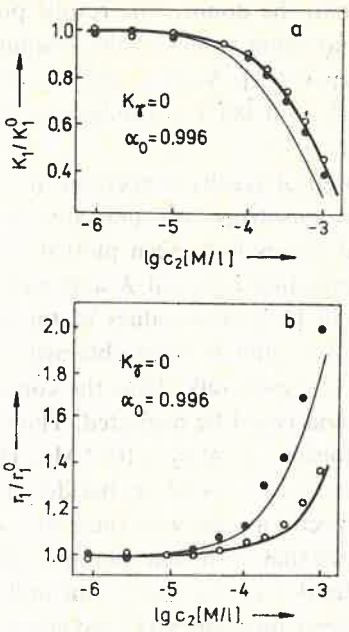

Fig. 4. Concentrational changes of (a) quantum yield and (b) emission anisotropy for Na-fluorescein in mixed systems; ○, ● — experimental results for systems I and II, respectively; continuous lines — theoretical curves according to Bojarski [7, 8]: thick — for system I, thin — for system II

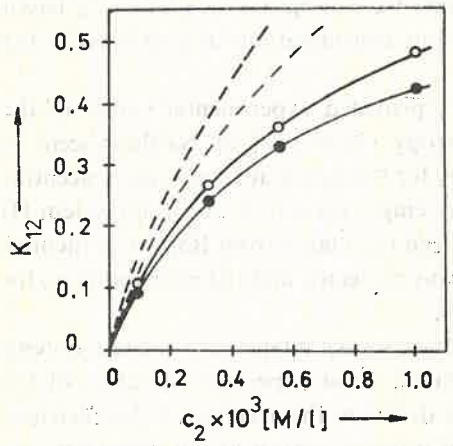

Fig. 5

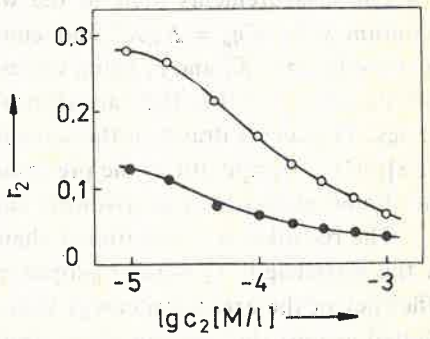

Fig. 6

Fig. 5. Efficiency K_{12} of the excitation energy transfer from molecules of Na-fluorescein to those of rhodamine B found from concentrational changes of the acceptor fluorescence intensities (solid lines and experimental points ○, ● for systems I and II, respectively) and the donor quantum yield (dashed lines, thick — for system I, thin — for system II)

Fig. 6. Values of emission anisotropy r_2 of the acceptor-rhodamine B, determined from formula (2) basing on experiments; ○ — for system I, ● — for system II

Note that the relations discussed above concerning the mixed systems have been based on experimental results only. Data obtained from investigations of component solutions were consistently used here.

4. Discussion of results. Conclusions

A theoretically coherent description of the effects of the photoluminescence concentration quenching and depolarization for one- and two-component systems has been presented in the fundamental papers by Bojarski [7, 8]. The experimental investigations fully confirmed assumptions of these theories. This is also seen, for instance, from our results shown in Figs 2 and 3.

Similarly to the results presented in [2–4], we found that the energy transfer efficiency from the donor to the acceptor, determined from changes in the acceptor quantum yield is lower than expected from concentrational dependence of the donor yield. It can be seen in Fig. 5 that the values of the transfer efficiencies K_{12} found in two ways differ from each other substantially. The differences can not be explained by the presence of dimers in the solution [4, 23] because their concentrations are low compared to those of the monomers. Also the nature of the spectra (Fig. 1a) shows that the rate constant for the energy transfer from a monomer of Na-fluorescein to its dimer is much lower than the rate constant for the energy transfer to rhodamine B.

From expression (6′), relating the energy transfer efficiency K_{12} to probabilities of elementary processes, we conclude that probability $\alpha_0^{(12)}$ is the factor responsible for the observed discrepancy. Indeed, for $\alpha_0^{(12)} = 0.7$ the transfer efficiencies determined in both

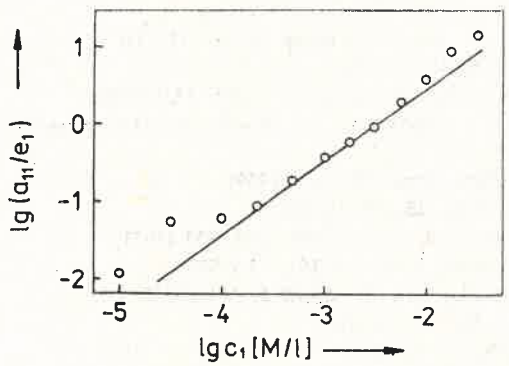

Fig. 7. Concentrational dependence of the probability a_{11}/e_1 for Na-fluorescein found at assumption $F(c_1) = 0$ (cf.Eq. (10)). A straight line with slope equal to 1.0

ways become identical. According to Galanin [5] the probability $\alpha_0^{(12)}$ is the mean value of the acceptor quantum yield, for the range where the donor emission and the acceptor absorption spectra overlap, related to its maximum value. Fig. 1b shows that for a mixed system composed of Na-fluorescein and rhodamine B the value of $\alpha_0^{(12)}$ should be nearly 1.0, as is the case with $\alpha_0^{(11)}$ and $\alpha_0^{(22)}$ in the respective one-component systems.

Fig. 7 presents experimentally determined values of $(r_{g1}-r_1)/r_1$ plotted against the concentration of Na-fluorescein. The curve reflects approximately (cf. (10)) the probability of energy transfer between donor molecules. Evidently, already for the concentration $c_1 = 3\times10^{-3}$ M/l the transfer to molecules of the same kind is the major energy degradation process. For high concentrations of the component solutions, where numerous acts of energy transfer occur, an efficient energy degradation can take place despite the fact that $\alpha_0^{(ii)}$ is near 1.0. In mixed solutions, however, the repeated energy transfer between the donor molecules is followed by a one-step transfer process to the acceptor. Thus the large deviation of $\alpha_0^{(12)} = 0.7$ for a system composed of Na-fluorescein and rhodamine B may be a result of either the existence of some unknown channel of the excitation energy transfer degradation or partial invalidity of the assumption concerning the mechanism of dipole-dipole interreaction. In high concentration systems this effect could be a result of before-relaxation excitation energy transfer. Recently, such a mechanism was proved [24], among others, for mixed solutions of Na-fluorescein and rhodamine B.

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