

ENERGY TRANSFER PROCESSES IN LUMINESCENT MIXED SOLUTIONS

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A relation between fluorescence spectra of mixed solutions and those of the component solutions is given, by which the radiative energy transfer is taken into account more precisely than by earlier formulas. In this relation the resonance transfer of energy is characterized only by two quantities; by using the latter as well as fluorescence yields and decay times of the components, the probability of molecular processes connected with emission and energy transfer is determined. Experimental results are in good agreement with JABLONSKI's, FÖRSTER's and KETSKEMÉTY's investigations and support their theories concerning the mechanism of energy transfer. ROZMAN's method is also discussed and some corrections are suggested.

Since FRANCK and CARIO [1] first observed sensitized fluorescence, numerous investigators have tried to clarify the mechanism of intermolecular energy transfer. Earlier relations based on phenomenological considerations were difficult to apply because of the relatively numerous parameters involved; furthermore, most of these theories (e.g. [2—5]) account only for resonance transfer of energy. Relatively fewer investigations take radiative energy transfer also into account and so allow, as does the present paper, a theoretically more precise control of the different theories and a verification of their results (e.g. [6—8]).

I. Radiative energy transfer in mixed solutions

If the emission spectra and absorption spectra of luminescent solutions overlap, the primary luminescent light will excite secondary luminescence, which, in turn, excites tertiary luminescence, and so on, the intensity of these depending on the overlap of the spectra, on the wavelengths of the exciting light and of the luminescent light observed, on the layer thickness of the samples, and on other experimental conditions. In mixed solutions the overlap of absorption and emission spectra is considerable and therefore the effect of secondary, tertiary, etc. fluorescent light produced by radiative energy transfer can be very important.

By generalizing the phenomenological theory concerning radiative energy transfer in solutions of a single component [9], and taking into account only secondary fluorescence, KETSKEMÉTY [6] found the following connection

between the directly measured fluorescence spectrum $B(\lambda')$ of the mixed solution and the true, normalized fluorescence quantum-spectra $f_1(\lambda')$, $f_2(\lambda)$ of the solutions of the components:

$$\begin{aligned} B(\lambda')/C(\lambda, \lambda') &= [(1 + \kappa_{11}) \eta'_1(\lambda) + \kappa_{21} \eta'_2(\lambda)] f_1(\lambda') + \\ &+ [(1 + \kappa_{22}) \eta'_2(\lambda) + \kappa_{12} \eta'_1(\lambda)] f_2(\lambda'), \\ C(\lambda, \lambda') &= \frac{\rho}{4 \pi n^2} E_{\lambda_0} \frac{\alpha}{\alpha + \beta} (1 - e^{-(\alpha + \beta)}), \end{aligned} \quad (1)$$

where λ and λ' are the wavelengths of the exciting light and of the observed luminescent light, respectively, $\eta'_i(\lambda)$ (or $\eta'_{i\lambda}$) means the apparent yield of the i th component of the mixed solutions, *i.e.* the quotient of the number of photons emitted by the i th substance contained in a unit volume of the mixed solution and of those absorbed by the mixed solution in the same time interval, $k_i(\lambda)$ and $k(\lambda)$ are the absorption coefficients of the i th substance and the mixed solution, respectively, $k(\lambda) = k_1(\lambda) + k_2(\lambda)$, $\alpha = k(\lambda) l$, $\beta = k(\lambda') l$, l being the layer thickness of the solution, E_{λ_0} the quantum density of the exciting light,¹ n the refractive index of the solution, ρ and κ_{ik} quantities taking into account losses by reflection and radiative energy transfer,² respectively. κ_{ik} can be determined from the relation

$$\kappa_{ik} = \int_0^\infty \eta'_k(\lambda'') f_i(\lambda'') M(\lambda'') d\lambda'' \quad (i, k = 1, 2); \quad (2)$$

or the definition of the function $M(\lambda'')$ see Eq. (26) in [9]³.

In mixed solutions of low concentration

$$\eta'_i(\lambda) = \eta'_{oi}(\lambda) \equiv \eta_i(\lambda) k_i(\lambda)/k(\lambda), \quad (2a)$$

where $\eta_i(\lambda)$ (or $\eta_{i\lambda}$) is the absolute quantum yield of the i th substance measured at the wavelength λ (see Eq. (8,14) in [13]). For solutions of higher concentrations, the spectra $B(\lambda')/C(\lambda, \lambda')$ calculated on the basis of Eqs. (1) and (2) using

¹ The above quantities are expressed in the following units:

$$\begin{aligned} B(\lambda') &: \frac{\text{number of photons}}{\text{cm}^2 \text{ sec sterad}}; & f_i(\lambda') &: \frac{\text{number of photons}}{\text{nm}}; \\ k(\lambda) &: \text{cm}^{-1}; & l &: \text{cm}; & \lambda &: \text{nm}; & E_{\lambda_0} &: \frac{\text{number of photons}}{\text{cm}^2 \text{ sec}}; \end{aligned}$$

the others being dimensionless numbers.

² The physical meaning of the quotient $\kappa_{ik} f_k(\lambda')/f_i(\lambda')$ is the observed intensity of the secondary fluorescence excited in the k th substance by the primary fluorescence of the i th substance, divided by the observed intensity of the primary fluorescence of the i th substance [6].

³ Detailed tables concerning the function $M(\lambda'')$ can be found in [10].

the relation $\eta'_i(\lambda) = \eta'_{0i}(\lambda)$ considerably differ from the measured spectra (see Fig. 1); this fact, the spectra of the components being practically independent from concentration, can only be explained by supposing that the apparent yield functions are dependent on concentration. This dependence, as can be seen from the following, is to be ascribed to, and explained by, resonance transfer of energy.

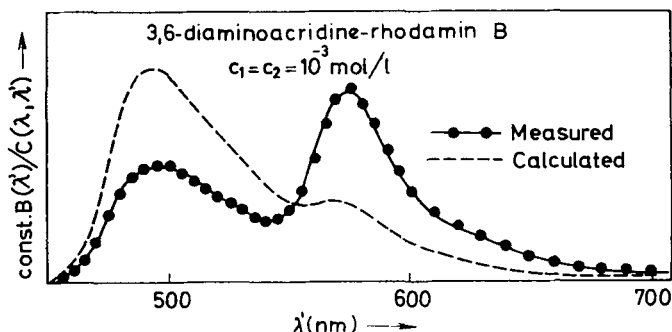


Fig. 1

II. Non-radiative energy transfer in mixed solutions

In taking into account the effect of the resonance transfer of energy, let us start from considerations given in [6], which, with some generalizations, may be summarized as follows.

Let n_i mean the number of the excited molecules of the i th substance in unit volume of the solution; a_i , b_i the probabilities of the spontaneous emission and the inner quenching of an excited molecule of the i th substance, respectively; w_k the probability of its quenching by a molecule of the k th substance, and a_k the probability of nonradiative energy transfer to a molecule of the k th substance. When exciting by light of wavelength λ and quantum density $E_{\lambda 0}$, the number of molecules excited by absorbed photons in a volume element dV during the time interval dt will be $E_{\lambda 0} \eta'_{i\lambda} k_{i\lambda} dV dt$; $\eta'_{i\lambda}$ is the probability of a molecule getting into the excited state by absorption of a photon of wavelength λ . In the case of steady state excitation the following relations will hold:

$$\begin{aligned} (E_{\lambda 0} \eta'_{1\lambda} k_{1\lambda} + a_1 n_2 - s_1 n_1 - a_2 n_1 - w_2 n_1 - b_1 n_1) dV dt &= 0, \\ (E_{\lambda 0} \eta'_{2\lambda} k_{2\lambda} + a_2 n_1 - s_2 n_2 - a_1 n_2 - w_1 n_2 - b_2 n_2) dV dt &= 0. \end{aligned} \quad (3)$$

During the time interval dt , the i th substance in a volume element dV emits $s_i n_i dV dt$ photons, and $E_{\lambda 0} k_{\lambda} dV dt$ photons will be absorbed in dV in the same time. Substituting the values n_i expressed from Eq. (3) into the quotient of the number of emitted and absorbed photons, this quotient gives $\gamma'_i(\lambda)$ according to the definition of the apparent yield. Thus, the following relations between $\eta'_i(\lambda)$ and the probabilities s_i , b_i , w_i , a_i can be obtained:

$$\begin{aligned} \eta'_{1\lambda} &= \frac{s_1 n_1}{E_{\lambda 0} k_{\lambda}} = \eta'_{1\lambda} \frac{k_{1\lambda}}{k_{\lambda}} \frac{s_1 e_2}{e_1 e_2 - a_1 a_2} + \eta'_{2\lambda} \frac{k_{2\lambda}}{k_{\lambda}} \frac{s_1 a_1}{e_1 e_2 - a_1 a_2}, \\ \eta'_{2\lambda} &= \frac{s_2 n_2}{E_{\lambda 0} k_{\lambda}} = \eta'_{1\lambda} \frac{k_{1\lambda}}{k_{\lambda}} \frac{s_2 a_2}{e_1 e_2 - a_1 a_2} + \eta'_{2\lambda} \frac{k_{2\lambda}}{k_{\lambda}} \frac{s_2 e_1}{e_1 e_2 - a_1 a_2}, \end{aligned} \quad (4)$$

where

$$e_i = s_i + b_i + a_k + w_k \quad (i, k = 1, 2; i \neq k).$$

If the concentration of one of the components (e.g. c_k) is equal to zero, then $\eta'_{i\lambda} = \eta_{i\lambda}$ and $\alpha_k = w_k = 0$. In both limiting cases $c_1 = 0$ and $c_2 = 0$, $\eta'_{i\lambda}$ can be expressed by the quantities $\eta_{i\lambda}$, s_i and b_i , and thus Eq. (4) obtains the following forms

$$\begin{aligned}\eta'_{1\lambda} &= \eta_{1\lambda} \frac{k_{1\lambda}}{k_\lambda} \frac{(s_1 + b_1) e_2}{e_1 e_2 - a_1 a_2} + \eta_{2\lambda} \frac{k_{2\lambda}}{k_\lambda} \frac{(s_2 + b_2) s_1 a_1}{e_1 e_2 - a_1 a_2}, \\ \eta'_{2\lambda} &= \eta_{1\lambda} \frac{k_{1\lambda}}{k_\lambda} \frac{(s_1 + b_1) s_2 a_2}{e_1 e_2 - a_1 a_2} + \eta_{2\lambda} \frac{k_{2\lambda}}{k_\lambda} \frac{(s_2 + b_2) e_1}{e_1 e_2 - a_1 a_2},\end{aligned}\quad (5)$$

with new symbols

$$\begin{aligned}\eta'_i(\lambda) &= \eta_{1\lambda} \frac{k_{1\lambda}}{k_\lambda} S_i + \eta_{2\lambda} \frac{k_{2\lambda}}{k_\lambda} A_i, \\ \eta'_{2\lambda} &= \eta_{1\lambda} \frac{k_{1\lambda}}{k_\lambda} A_2 + \eta_{2\lambda} \frac{k_{2\lambda}}{k_\lambda} S_2,\end{aligned}\quad (6)$$

where the meaning of S_i and A_i is clear from the comparison of Eqs. (5) and (6). In mixed solutions of very low concentration $a_i = w_i = 0$, consequently $S_i = 1$, $A_i = 0$. Only in this case the value of $\eta'_i(\lambda)$ resulting from Eq. (6) will be equal to $\eta'_{0i}(\lambda)$ defined in Eq. (2a), as is easily seen. Eq. (6), with the symbol $\eta'_{0i}(\lambda)$, can be written as follows:

$$\begin{aligned}\eta'_1(\lambda) &= S_1 \eta'_{01}(\lambda) + A_1 \eta'_{02}(\lambda), \\ \eta'_2(\lambda) &= A_2 \eta'_{01}(\lambda) + S_2 \eta'_{02}(\lambda).\end{aligned}\quad (7)$$

Using the apparent yields η'_{0i} , which also take into account non-radiative energy transfer, to calculate κ_{ik} according to Eq. (2), we have

$$\begin{aligned}\kappa_{11} &= S_1 \kappa_{11}^{\circ} + A_1 \kappa_{12}^{\circ}, & \kappa_{12} &= A_2 \kappa_{11}^{\circ} + S_2 \kappa_{12}^{\circ}, \\ \kappa_{21} &= S_1 \kappa_{21}^{\circ} + A_1 \kappa_{22}^{\circ}, & \kappa_{22} &= A_2 \kappa_{21}^{\circ} + S_2 \kappa_{22}^{\circ},\end{aligned}\quad (8)$$

$$\kappa_{ik}^{\circ} = \int_0^{\infty} \eta'_{0k}(\lambda'') f_i(\lambda'') M(\lambda'') d\lambda''.$$

By substituting the quantities η'_i and κ_{ik} given in Eqs. (7) and (8) into Eq. (1), we obtain a relation between the directly observed quantum density $B(\lambda')$ of the fluorescence of the mixed solution and fluorescence spectra of the components.

This relation, which takes into account both radiative and non-radiative energy transfer, becomes considerably less complicated if there is no overlap between the absorption spectrum $k_1(\lambda)$ of substance 1 and the emission spectrum $f_2(\lambda)$ of substance 2, which frequently occurs. In this case $A_1 = 0$, $S_2 = 1$ and $\kappa_{21} = 0$. The accuracy of this simpler expression of $B(\lambda')$ can be increased by also taking into account excited centres of higher order. Considering that the intensities of primary, secondary, etc. fluorescences decrease according to a geometrical progression, generally with good approximation [9], it is easy to obtain

$$\begin{aligned}B(\lambda')/C(\lambda, \lambda') \equiv B^*(\lambda') &= \frac{S \eta'_{01}(\lambda)}{1 - S \kappa_{11}^{\circ}} f_1(\lambda') + \\ &+ \left[\frac{\eta'_{02}(\lambda) + A \eta'_{01}(\lambda)}{1 - \kappa_{22}} + \frac{S \eta'_{01}(\lambda) (\kappa_{12}^{\circ} + A \kappa_{11}^{\circ})}{(1 - S \kappa_{11}^{\circ})(1 - \kappa_{22}^{\circ})} \right] f_2(\lambda');\end{aligned}\quad (9)$$

with the symbols $S_1 = S$ and $A_2 = A$. These quantities have a clear physical meaning, $\eta_{1m} S$ and $A \eta_{1m} / \eta_{2m}$ being the probabilities for an excited molecule of the substance 1, to lose its excitation energy by spontaneous emission, and to transmit its energy to substance 2 by non-radiative transfer, respectively [6].

III. Determination of the quantities characterizing the non-radiative energy transfer on the basis of the emission spectra

With a knowledge of the emission spectrum $B(\lambda')$ of a mixed solution, relation Eq. (9) enables us to determine the quantities S and A characterizing the non-radiative energy transfer.

a) Determination of S

Let λ'_1 be a wavelength of observation at which substance 2, the acceptor, shows no emission, i.e. $f_2(\lambda'_1) = 0$; then we obtain from Eq. (9) the following expression for S :

$$S = \frac{B^*(\lambda'_1)}{\eta'_{01}(\lambda) f_1(\lambda'_1) + \kappa_{11}^\circ B^*(\lambda'_1)}, \quad (10)$$

in which all quantities can be determined experimentally. This method is, however, cumbersome to apply because of the relatively complicated measurement of the intensity E_{λ_0} of the exciting light in the formula of $B^*(\lambda'_1)$ (see [15]).

In the case of equimolar mixed solutions, the dependence of S on concentration can also be determined by measuring at the wavelength λ'_1 the photocurrents $I_c(\lambda'_1)$ produced by the fluorescent light of solutions of different concentration c , with constant intensity of excitation, and holding the product of concentration and layer thickness at a constant value. It is easy to see from Eq. (10), that the relation

$$I_c(\lambda'_1) = C_1 \frac{S}{1 - S\kappa_{11}^\circ} \quad (11)$$

holds for the intensities of these photocurrents. Let the lowest concentration applied, c_0 , be so low that the non-radiative energy transfer may be neglected, i.e. $S = 1$. (The fulfilment of this condition can be controlled on the basis of Eq. (10).) By measuring the photocurrent $I_0(\lambda'_1)$ at c_0 , C_1 can be determined from Eq. (11), and with the symbol $I_c^*(\lambda'_1) = I_c(\lambda'_1)/I_0(\lambda'_1)$ we obtain:

$$S = \frac{I_c^*(\lambda'_1)}{1 - \kappa_{11}^\circ + I_c^*(\lambda'_1) \kappa_{11}^\circ}. \quad (12)$$

In the case of non-equimolar mixed solutions, it is not possible to choose the layer thicknesses so that κ_{11}° and the factor $\alpha(1 - e^{-(\alpha+\beta)})/(\alpha + \beta)$ in $C(\lambda, \lambda')$ can be held at a constant value for different concentrations of the acceptor. In this case, dividing the intensities of the photocurrents by the above factor and designating these quotients by $I_0(\lambda'_1)$ and $I_c(\lambda'_1)$ instead of

the intensities, we obtain, on the basis of our considerations for equimolar solutions:

$$S = \frac{I_c^*(\lambda'_1)}{1 - \kappa_{11}^\circ(c_0) + I_c^*(\lambda'_1) \kappa_{11}^\circ(c)}, \quad (13)$$

where $\kappa_{11}^\circ(c_0)$ and $\kappa_{11}^\circ(c)$ are the values of κ_{11}° corresponding to the acceptor concentrations c_0 and c , respectively.

b) Determination of A

Knowing S and the fluorescence characteristics of the solutions of the components, A can also be determined with the aid of Eq. (9). In order to determine exactly the energy of the exciting light absorbed by the sample, it is convenient to perform the measurements with greater layer thicknesses, which absorb the exciting light as far as possible; therefore we used the following experimental method, also expounded by ROZMAN et al. [7, 11], but using the relation Eq. (9) for the evaluation of the results.

Let the mixed solution be excited first with a wavelength λ_1 , absorbed mainly by the donor (substance 1), then with a wavelength λ_2 , absorbed by the acceptor (substance 2) alone, and the fluorescent light observed at a wavelength λ'_0 for which the absorption coefficient of the solution — and therefore also β — is practically equal to zero, and the measured photocurrents $I_{\lambda_1}(\lambda'_0)$ and $I_{\lambda_2}(\lambda'_0)$ are predominantly due to the emission of substance 2. Then if the intensities of the fluorescence, excited with the two wavelengths mentioned, but with the same exciting energy, are designated by I_{21} and I_{22} , respectively, these can be determined from the relations

$$I_{21} = \frac{I_{\lambda_1}(\lambda'_0) Q(\lambda'_0)}{[\int_0^\infty I_e(\lambda) Q(\lambda) d\lambda]_{\lambda_1}}, \quad I_{22} = \frac{I_{\lambda_2}(\lambda'_0) Q(\lambda'_0)}{[\int_0^\infty I_e(\lambda) Q(\lambda) d\lambda]_{\lambda_2}}, \quad (14)$$

where $I_e(\lambda)$ means the photocurrent produced by the exciting light of bandwidth λ to $\lambda + d\lambda$, and $Q(\lambda'_0)$ and $Q(\lambda)$ the sensibility of the photomultiplier for the corresponding wavelengths. Applying Eq. (9) to both exciting wavelengths, we obtain for A

$$A = \frac{\frac{1}{\alpha_{11}} \left(\frac{I_{21}}{I_{22}} \cdot \frac{1 - e^{-\alpha_1}}{1 - e^{-\alpha_2}} - \alpha_{21} \frac{\eta_{02}(\lambda_1)}{\eta_{02}(\lambda_2)} \right) \frac{\eta_{02}(\lambda_2)}{\eta_{01}(\lambda_1)} - S \left(\frac{1 - \kappa_{22}^\circ(\alpha_2)}{1 - S\kappa_{11}^\circ(\alpha_1)} \frac{f_1(\lambda'_0)}{f_2(\lambda'_0)} + \frac{\kappa_{12}^\circ(\alpha_1)}{1 - S\kappa_{11}^\circ(\alpha_1)} \right)}{1 + S \frac{\kappa_{11}^\circ(\alpha_1)}{1 - S\kappa_{11}^\circ(\alpha_1)}} \quad (15)$$

with the symbols $\alpha_1 = \alpha(\lambda_1)$, $\alpha_2 = \alpha(\lambda_2)$, $\alpha_{11} = k_1(\lambda_1)/k(\lambda_1)$, $\alpha_{21} = k_2(\lambda_1)/k(\lambda_1)$; in the functions κ_{ik}° , depending on α and β , only the dependence on α has been designated, in our case $\beta = 0$; furthermore, it is supposed that $\kappa_{22}^{\circ}(\alpha_1) = \kappa_{22}^{\circ}(\alpha_2)$, the dependence of κ_{22}° on α being very slight for large values of α . It is to be emphasized, that in determining A from Eq. (15), the greatest care is necessary in measuring the fluorescence characteristics, particularly the yield values, because they exert a sensible influence on the value of A , especially if the difference in the numerator is small.

c) Determination of the probability of the elementary processes in the molecules

The definition of quantities S and A , which can be determined experimentally, gives two simple relations between these quantities and the probabilities s_1 , s_2 , b_1 , b_2 , a_2 and w_2 of the elementary processes. Using further the relations

$$\eta_{im} = \frac{s_i}{s_i + b_i} \quad \text{and} \quad \tau_i = \frac{1}{s_i + b_i}, \quad (16)$$

which give the maximum quantum yields of the solutions of the components η_{im} and their decay times [12, 13], we easily obtain from these six equations:

$$\begin{aligned} s_1 &= \frac{\eta_{1m}}{\tau_1}, \quad s_2 = \frac{\eta_{2m}}{\tau_2}, \quad b_1 = \frac{1 - \eta_{1m}}{\tau_1}, \quad b_2 = \frac{1 - \eta_{2m}}{\tau_2}, \\ a_2 &= \frac{1}{\tau_1} \frac{A}{S} \frac{\eta_{1m}}{\eta_{2m}}, \quad w_2 = \frac{1}{\tau_1} \left(\frac{1}{S} - \frac{A}{S} \frac{\eta_{1m}}{\eta_{2m}} - 1 \right), \end{aligned} \quad (17)$$

which enable us to determine experimentally the quantities s_1, \dots, w_2 .

IV. Experimental

a) *Absorption spectra* have been measured with autocollimating grating spectrophotometers *Optica Milano*, type CF 4 and CF 4 DR.

b) For the measurement of *fluorescence spectra* an apparatus has been constructed, with which it is possible to eliminate the errors due to fluctuations in the exciting light and to obtain the conditions of excitation and observation on which the evaluation of the measurement is based (see e.g. [9]). High pressure Hg- and X-bulbs Osram type HBO 500 and XBO 450, respectively, were used as a light source, and the exciting band, which could be considered as approximately monochromatic, was selected by an interference filter or a monochromator. In order to eliminate errors of measurement due to fluctuations in the intensity of the exciting light, the following method was used. In the monochromator M resolving the luminescent light, a thin glass plate G was placed immediately behind the entrance slit (Fig. 2). This plate projected a small fraction I of the unresolved luminescent light by means of the mirror T and through the crossed filter F on the photomultiplier Ph_2 placed inside the monochromator.

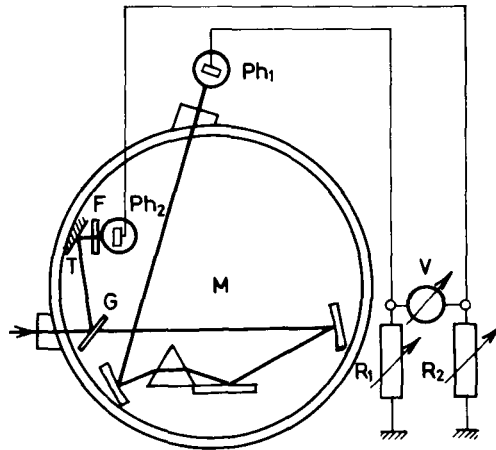


Fig. 2

whereas a fraction of the luminescent light passing the plate G fell on the multiplier Ph₂, o-known spectral sensitivity, the width $d\lambda'$ of this band of the luminescence spectrum $I(\lambda')$ being determined by the exit slit. If the resistances R_1 and R_2 of the resistors in the circuits of both multipliers are chosen so that the voltage drop on both is the same, then the quotient of the photocurrents will be equal to R_1/R_2 and independent from the intensity of the exciting light. Thus we obtain for the spectral distribution of the fluorescent light: $I(\lambda') = \text{const. } R_1/R_2$.

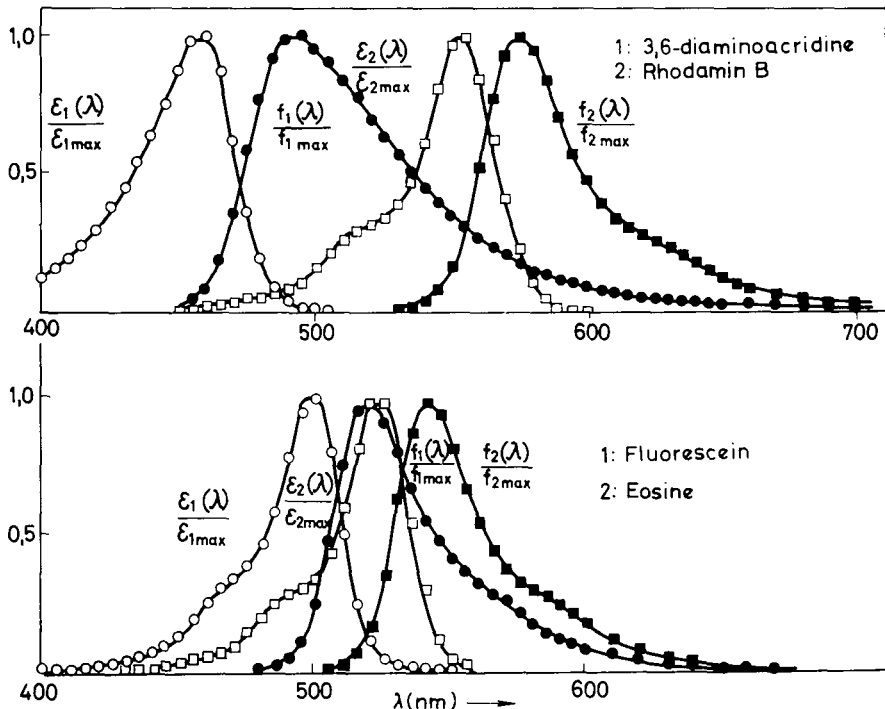


Fig. 3

The two-cells method described above, owing to the position of Ph_2 behind the entrance slit, not only also eliminates errors originating from fluctuations of the intensity of the exciting light, but also those due to the varying position of the arc projected on the sample. The linearity of the multipliers was checked separately.

c) *The true quantum yield* has been determined with our method published in [14] and [15], using the apparatus described above. In measuring the distribution of the exciting band the two cells-method has been used. The intensity of the exciting light scattered from a MgO plate and weakened by a rotating-sector disc and that of the luminescence emitted by the sample were measured with Ph_1 at wavelengths corresponding to the maxima of the exciting band and of the luminescence spectrum, respectively. The yield function $\eta(\lambda)$ was determined according to the method given in [16].

d) *The decay time* of fluorescent light was measured with a phase-fluorimeter built in our Institute, based essentially on the same principle as that described by BAUER and ROZWADOWSKI [17].

In determining the fluorescence characteristics from the measurements, the influence of secondary processes and those of higher order has always been taken into account; therefore the results obtained can be considered as true fluorescence characteristics.

e) *Dye-stuffs and solvents.* For our investigations we used mixed solutions of 3,6-diaminoacridine and rhodamin B, and of fluorescein and eosine, respectively, because the emission spectra $f_1(\lambda)$ of substance 1 and the absorption spectra $\varepsilon_2(\lambda)$ of substance 2 in these mixtures show a considerable overlap (Fig. 3). The dye-stuffs were purified by the usual chemical processes and the grade of purity checked by chromatography and absorption measurements. As solvent 96% ethanol with 1 mole/litre acetic acid was used for 3,6-diaminoacridine and rhodamin B; a mixture of 85% ethanol and 15% water with 10^{-2} mole/litre NaOH for fluorescein and eosine. Fluorescence characteristics of the components as well as of the mixed solutions were measured at the following concentrations: $1 \cdot 10^{-5}$, $2.5 \cdot 10^{-5}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $2.5 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $1 \cdot 10^{-3}$, $2.5 \cdot 10^{-3}$ mole/litre. In non-equimolar mixed solutions the concentration of the acceptor varied between the above limits, the concentration of the donor being held at the constant value of 10^{-4} mole/litre.

V. Results and discussion

a) The absorption measurements demonstrated the validity of the Bouguer—Beer—Lambert law, both for the solutions of the components and the mixed solutions, in the concentration interval investigated. The additivity of the absorption coefficients $k_1(\lambda)$, $k_2(\lambda)$ of the solutions of the components and that of the mixed solutions, $k(\lambda) = k_1(\lambda) + k_2(\lambda)$, was well fulfilled, which shows that there was no chemical interaction between the components in the mixed solution.

b) The shape of the true fluorescence spectra of the four investigated substances, presented in Fig. 3, was independent of the concentration of the solutions. Emission spectra of equimolar and non-equimolar mixed solutions, however, showed a strong dependence on concentration. As examples, the $B^*(\lambda')$ spectra of non-equimolar solutions of 3,6-diaminoacridine and rhodamin B and those of fluorescein and eosine are presented in Figs. 4 and 5, respectively.

c) The relative quantum yields $\eta(\lambda)/\eta_{\text{max}}$ of the solutions of the components and their absolute quantum yields measured at the exciting wavelengths λ_e used in the measurements of the spectra $B(\lambda')$ are given in Fig. 6 and Table I.

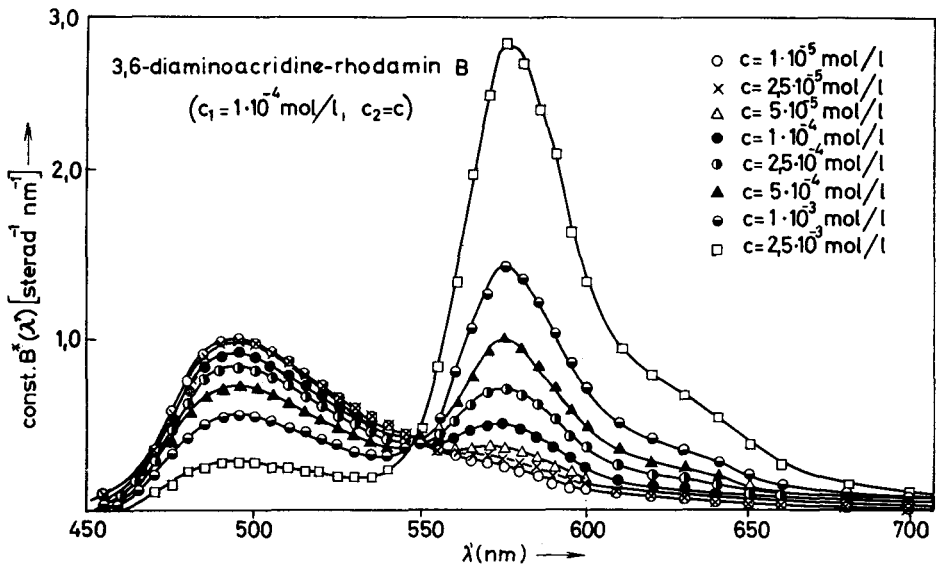


Fig. 4

d) The fluorescence characteristics of the components mentioned above being known, we determined the quantities κ_{ik} , which take into account the radiative energy transfer. If the condition $[\beta(\lambda')]_{\text{max}} = 1$ is fulfilled, κ_{ik} is

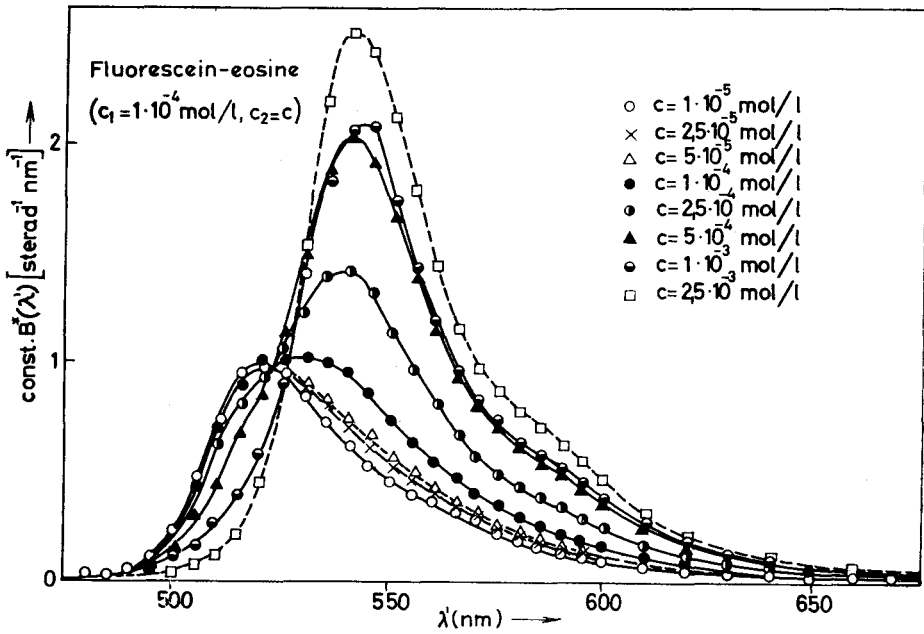


Fig. 5

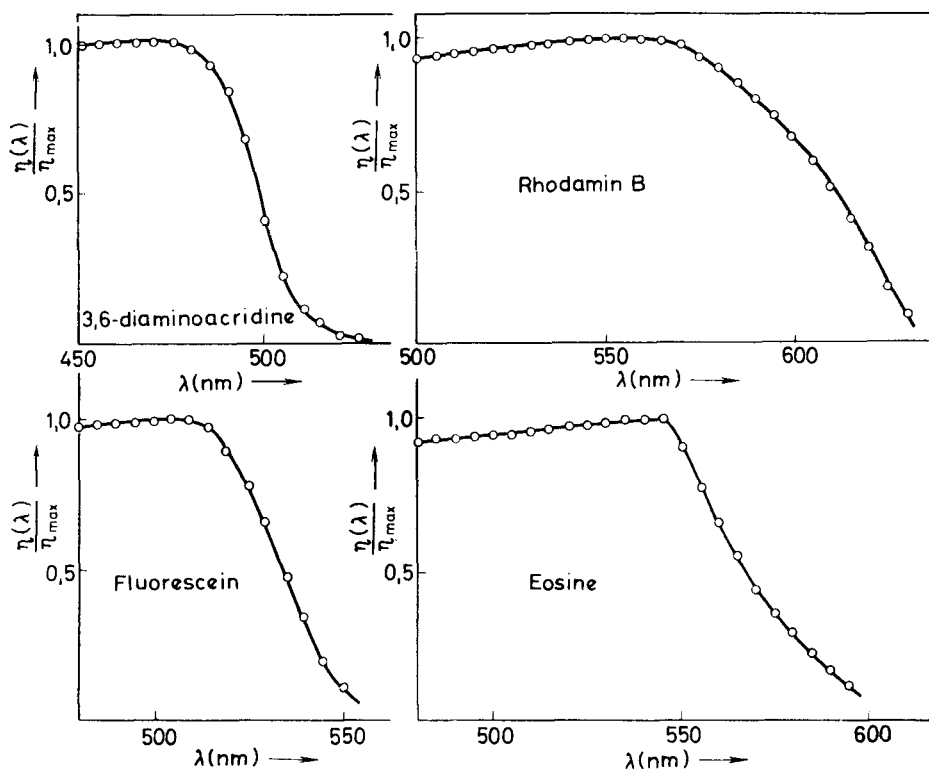


Fig. 6

independent of the wavelength λ' of the observation, and depends only on the maximum value of $\beta(\lambda')$ (Fig. 7). If this condition is not fulfilled, the dependence of κ_{ik} on λ' (and thus on $\beta(\lambda')$) is the stronger, the greater the value of $[\beta(\lambda')]_{\max}$ (Fig. 8; which, as well as Fig. 7, refers to equimolar mixed solu-

Table I
Absolute quantum yields

substance	c (mole/l)	$\eta(\lambda_e)$							
		$1 \cdot 10^{-5}$	$2.5 \cdot 10^{-5}$	$5 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$2.5 \cdot 10^{-4}$	$5 \cdot 10^{-4}$	$1 \cdot 10^{-3}$ 2.5	10^{-3}
3.6-diaminoacridine ($\lambda_e = 436$ nm)		0.61	0.60	0.61	0.58	0.60	0.60	0.56	0.51
rhodamin B ($\lambda_e = 546$ nm)		0.50	0.50	0.51	0.49	0.49	0.50	0.49	0.45
fluorescein ($\lambda_e = 436$ nm)		0.87	0.87	0.89	0.88	0.88	0.89	0.85	0.79
eosine ($\lambda_e = 436$ nm)		0.49	0.49	0.50	0.48	0.49	0.49	0.48	0.45

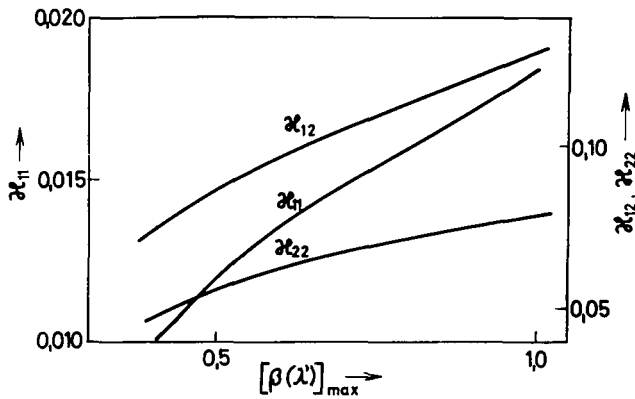


Fig. 7

tions of 3,6-diaminoacridine and rhodamin B). It follows from the above results that the intensity of the fluorescence emitted by a mixed solution is substantially affected by radiative energy transfer. Furthermore, the emission spectrum $B^*(\lambda')$ can be expressed as a linear combination

$$B^*(\lambda') = b_1 f_1(\lambda') + b_2 f_2(\lambda') \quad (18)$$

of the spectra of the components only when the product of the concentration and layer thickness of the solution is low enough; namely, in this case the factors b_1 and b_2 are independent of λ' , as can be seen from the comparison of Eq. (9) with Eq. (18) and from the statements in c).

e) The dependence of S on c has been determined for both equimolar ($c_1 = c_2 = c$) and non-equimolar ($c_1 = 10^{-4}$ mole/l, $c_2 = c$) mixed solutions of 3,6-diaminoacridine-rhodamin B and of fluorescein and eosine with the formulas (10), (12) and (13) for excitation with two different wavelengths (436 nm, 460 nm and 436 nm, 490 nm, respectively). For a given acceptor concentration the same values of S were obtained at different exciting wavelengths both for equimolar and non-equimolar solutions. Thus S practically

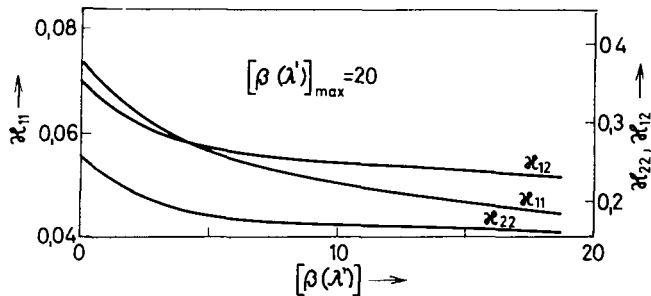


Fig. 8

depends only on the concentration of the acceptor. These results support the fundamental supposition concerning the non-radiative character of the energy transfer (see chapter II), which had not been checked carefully enough earlier.

The experimental results obtained for S render possible to control the relations resulting from JABLONSKI'S [20], FÖRSTER'S and GALANIN'S [18, 19], and KETSKEMÉTY'S [21] theories, which are the following:

$$S = \frac{1 - e^{-\nu}}{\nu} \left[\nu = \frac{4\pi}{3} (1,33 R_0)^3 n_2 \right], \quad (19)$$

$$S = 1 - 2 q e^{q^2} \int_0^q e^{-x^2} dx \left[q = 2,74 (2\pi n)^{-2} \left(\frac{\bar{\lambda}^4 \tau_0}{L \tau_e} \right)^{1/2} n_2 \right], \quad (20)$$

$$S = \int_0^\infty f_1(\lambda) \exp(-k_2(\lambda)/k^4 a^3) d\lambda \left[k = 2\pi n/\lambda; a = (1/2\pi n_2)^{1/3} \right], \quad (21)$$

where τ_0 and τ_e are the main and the natural decay times, respectively, L Loschmidt's constant, $\bar{\lambda}^4 = \int_0^\infty f_1(\lambda) \varepsilon_2(\lambda) \lambda^4 d\lambda$, n_2 the number of molecules in a cm^3 , R_0 the critical distance to be calculated from the fluorescence characteristics (R_0 is the distance between a donor and an acceptor molecule at which the probability of spontaneous emission from the donor is equal to the probability of non-radiative transfer of the exciting energy). In Figs. 9 and 10, curves 1, 2 and 3 show results calculated from JABLONSKI'S (Eq. (19)), FÖRSTER'S and GALANIN'S (Eq. (20)), and KECSEKEMÉTY'S (Eq. (21)) formulas, whereas the circles give the experimental results. As can be seen, the dependence S from concentration c is well described by all three curves. In curves 1 and 2 the deviation from the experiments increases with increasing acceptor concentration c and is somewhat greater for 1 than for 2. Curve 3 fits well to

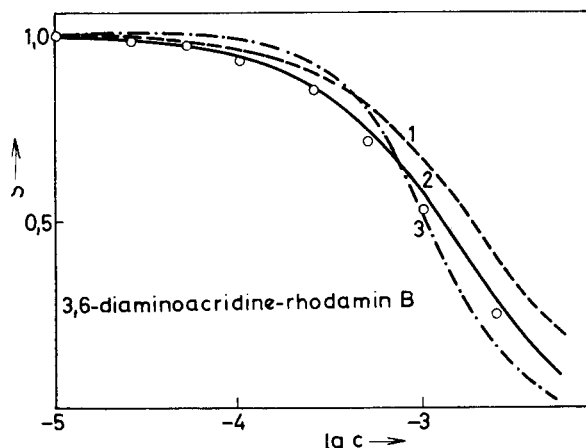


Fig. 9

the experimental results also in the region of high concentration, showing some deviation only in the region of medium concentration.

According to our experimental results the quantity a in Eq. (21), which, according to the theory, means the radius of the greatest sphere containing no acceptor molecule around a donor molecule, is to be substituted by $0.72 (1/2 \pi n_2)^{1/3}$; curve 3 has been calculated with this value. It can be stated on the basis of the results obtained that the dependence on concentration of the quantity S , characteristic for the relative yield of the donor molecules,

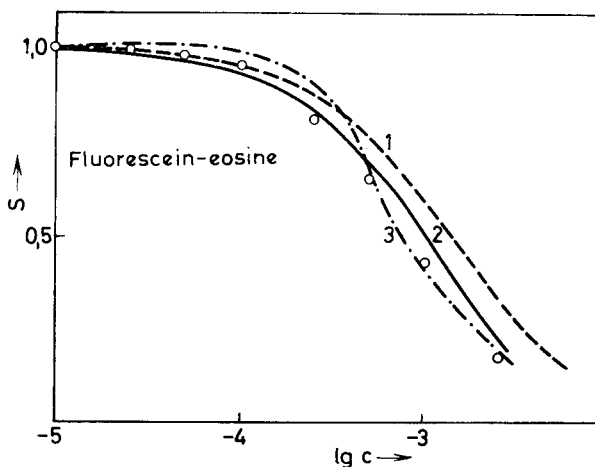


Fig. 10

can be well described with the formulas deduced on the basis of the supposed non-radiative mechanism of energy transfer.

The dependence of A on acceptor concentration is shown in Figs. 11 and 12. These results are used in the following calculations.

f) The probability of the elementary processes occurring in the molecules has been determined on the basis of the formulas given in Eq. (17). For the decay time τ of 3,6-diaminoacridine, rhodamin B, fluorescein and eosine, up to the limit of concentration quenching ($5 \cdot 10^{-4}$ mole/l), the values 4.0, 2.4, 3.5 and 2.4 nsec were obtained in turn. For the two higher concentrations ($1 \cdot 10^{-3}$ and $2.5 \cdot 10^{-3}$ mole/l) the corresponding values of τ were calculated from the relation $\tau/\tau_0 = \eta/\eta_0$ (see [13] p. 207), this relation being well fulfilled in the beginning of the region of concentration quenching. In the above sequence of the dye-stuffs, and expressed in 10^8 sec^{-1} as units, we obtained for s_i 1.51, 2.05, 2.64 and 2.68, for b_i 0.96, 2.05, 0.23, and 1.84 for all concentrations; higher values for b_i were obtained only for the concentrations 10^{-3} and $2.5 \cdot 10^{-3}$ mole/l, as a consequence of the decreasing yields of the dye-stuffs.

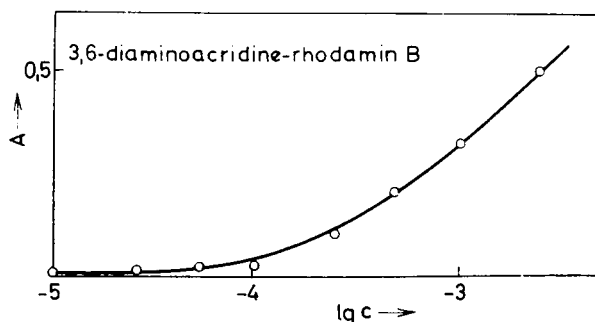


Fig. 11

While s_i and b_i are independent of the concentrations of the solutions below the region of concentration quenching, a_2 depends strongly on the acceptor concentration. According to the assumption of VAVILOV and other authors, the following relation between the probability a_2 and the molecule concentration n_2 corresponding to c should be valid:

$$a_2 = \frac{1}{k_2} n_2, \quad (22)$$

where k_2 is a constant independent of the concentration n_2 (number of molecules/cm³). On the contrary, when plotting the values $\lg a_2$ obtained from our measurements performed in a wider region of concentration as a function of $\lg c$ (see Figs. 13 and 14), we obtained straight lines with slopes of 1.1 for mixed solutions of 3,6-diaminoacridine and rhodamin B, and 1.4 for fluorescein and eosine; thus a_2 is not a linear function of c (and therefore not of n_2).

For w_2 we obtained values near zero, which are low compared with the other probabilities in the concentration interval 10^{-5} – $5 \cdot 10^{-4}$ mole/l. In the case of the two highest concentrations, however, w_2 differs markedly from zero (e.g. at $2.5 \cdot 10^{-3}$ mole/l w_2 amounts to about 10% of a_2): so the quenching

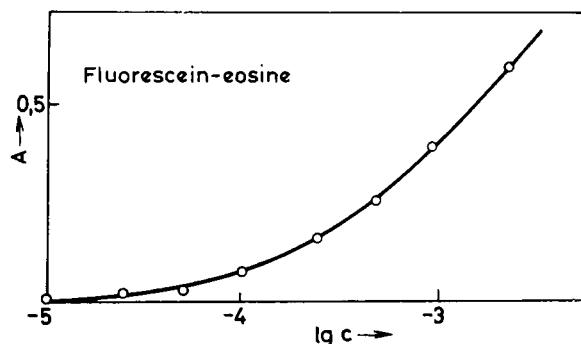


Fig. 12

effect of the acceptor on the fluorescence of the donor cannot be neglected at higher concentrations, in contradiction with ROZMAN's assumption [7].

g) In our investigations (see chapter IVb) we have used, with some modifications, a method of ROZMAN et al. to determine the probability A [7, 11]. As to the very ingenious original method of ROZMAN et al. and related considerations, we should like to make the following correcting remarks.

ROZMAN introduced the "quantum yield η_i of non-radiative energy transfer", and the "efficiency T_{12} " of the energy transfer, determinable by

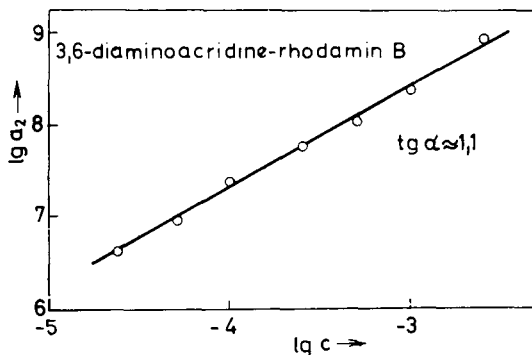


Fig. 13

measurements; according to his considerations the following relation should exist between these quantities:

$$T_{12} = \eta_i + \eta_1 R_{12}^* \quad (23)$$

the definition of which (also using our symbols) is

$$T_{12} = \frac{I_{21}}{\alpha_{11} I_{22}}, \quad \eta_i = 1 - \frac{\eta_1}{\eta_{01}} = 1 - S. \quad (24), (25)$$

The meaning of the symbols in Eq. (24) is given in connection with Eq. (15); η_{10} and η_1 are the quantum yields of the donor if $c_2 = 0$ and $c_2 \neq 0$, respectively. The term $\eta_1 R_{12}^*$ in Eq. (23), determinable on the basis of experimental data, takes into account the radiative energy transfer. In order to verify the adequacy of FÖRSTER's and GALANIN's theory, ROZMAN determined η_i experimentally from Eq. (23) and compared the dependence on concentration of this value with the dependence on concentration of η_i calculated from Eqs. (25) and (20).

Closer examination of the physical meaning of T_{12} and η_i shows that the definition of η_i has to be modified to ensure the validity of Eq. (23). Namely, let n be the number of exciting photons impinging on the sample in unit time,

n_e^0 and n_e^1 the number of the photons emitted by the donor in the same time, if $c_2 = 0$ and $c_2 \neq 0$, respectively. In case of complete absorption ($\eta_{10} = n_e^0/n$ and $\eta_{11} = n_e^1/n \alpha_{11}$)

$$\eta t = \frac{\frac{n_e^0}{n} - \frac{n_e^1}{n}}{\frac{n_e^0}{n}} \cdot \frac{n \alpha_{11}}{n} \quad (26)$$

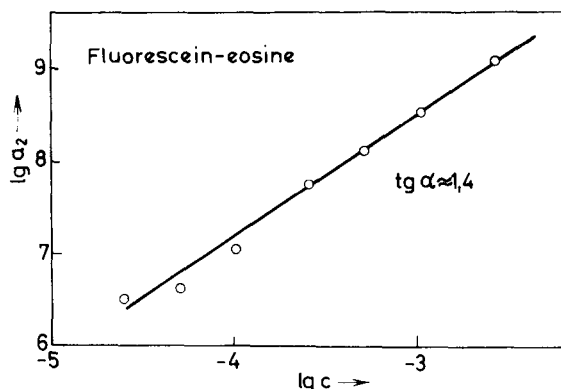


Fig. 14

Assuming that the decrease in yield of the donor is due to the efficient non-radiative energy transfer alone (i.e. $w_2 = 0$), Eq. (26) gives the number of photons transferred to the acceptor by non-radiative processes, divided by the number of the photons emitted by the “pure” donor solution under the same excitation.

Let the fluorescence intensities I_{21} and I_{22} in the definition of T_{12} be excited by n photons in unit time, of wavelengths λ_1 and λ_2 , respectively, and completely absorbed by the sample; then the number of the acceptor molecules getting into the excited state will evidently be $\alpha_{21} n + n_s + n_r$ and n for the wavelengths λ_1 and λ_2 , respectively, if n_s and n_r mean the number of the exciting photons transferred from the donor by radiative and non-radiative processes. If the quantum yield of the acceptor is η_A for both λ_1 and λ_2 , then

$$I_{21} = (\alpha_{21} n + n_s + n_r) \eta_A, \quad I_{22} = n \eta_A. \quad (27)$$

From this and Eq. (24) we obtain

$$I_{12} = \frac{n_s + n_r}{n \alpha_{11}}. \quad (28)$$

That is, T_{12} gives the number of the transferred photons not in relation to the number of photons emitted by the donor, but to the number of the exciting

quanta absorbed by the donor. Considering that the effect of the radiative energy transfer decreases with decreasing layer thickness, and in the limiting case $R_{12}^* = 0$, and therefore $\eta_t = T_{12}$, the relation (23) between T_{12} and η_t holds evidently only in the case, when in the definition of η_t the number of quanta transferred by non-radiative processes is given also in relation to the number of the exciting quanta absorbed by the donor, i.e. if η_t is defined as follows:

$$\eta_t = \eta_{10} - \eta_1 \equiv \eta_{10}(1 - S). \quad (29)$$

According to the above, the considerations of ROZMAN et al. will be valid even for $w_2 = 0$ only, if the true absolute quantum yield of the pure donor solution $\eta_{10} = 1$, which condition is generally not fulfilled, and further if the quantum yield of the acceptor is equal for both wavelengths λ_1 and λ_2 , which differ markedly.

Now, according to the more precise definition given in Eq. (29), and if $w_2 = 0$ (i.e. for low acceptor concentrations), it is easy to obtain from Eq. (17), with the acceptable supposition that $\eta_{10} = \eta_{1m}$, the following relation for η_t :

$$\eta_t = \frac{\eta_{1m}^2}{\eta_{2m}} A, \quad (30)$$

which our measurements proved to be relatively well fulfilled in the region of not too high concentrations.

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REFERENCES

1. G. CARIO and J. FRANCK, *Z. Phys.*, **11**, 161, 1922.
2. S. I. VAVILOV, M. D. GALANIN and F. M. PEKERMAN, *Abhandlungen aus der sowjetischen Physik, Folge I*, p. 9, Verlag Kultur und Fortschritt, Berlin, 1951.
3. A. F. WATSON and R. LIVINGSTON, *J. Chem. Phys.*, **18**, 802, 1950.
4. E. J. BOWEN and R. LIVINGSTON, *J. Amer. Chem. Soc.*, **76**, 6300, 1954.
5. J. B. BIRKS and J. W. CAMERON, *Proc. Phys. Soc.*, **72**, 53, 1958.
6. I. KETSKEMÉTY, *Acta Phys. Hung.*, **10**, 429, 1959.
7. I. M. ROZMAN, *Optics and Spectrosc.*, **10**, 354, 1961.
8. Á. BUDÓ and I. KETSKEMÉTY, *Acta Phys. Hung.*, **14**, 167, 1962.
9. Á. BUDÓ and I. KETSKEMÉTY, *Acta Phys. Hung.*, **7**, 207, 1957.
10. M. TÖRÖK, Doctoral dissertation, Szeged, 1967.
11. E. A. ANDREESTHEV, C. F. KILIN, I. M. ROZMAN and B. I. SHIROKOV, *Izv. Akad. Nauk. SSSR, Ser. Fiz.*, **27**, 533, 1963.
12. S. I. VAVILOV, *A fény mikrostruktúrája, III. rész*, Akadémiai Kiadó, Budapest, 1955.
13. TH. FÖRSTER, *Fluoreszenz organischer Verbindungen*, Vandenhoeck and Ruprecht Göttingen, 1951.
14. I. KETSKEMÉTY, J. DOMBI, R. HORVAI, J. HEVESI and L. KOZMA, *Acta Phys. et Chem. Szeged*, **7**, 17, 1961.

15. Á. BUDÓ, J. DOMBI and L. SZÖLLÖSY, *Acta Phys. et Chem. Szeged*, **2**, 18, 1956.
16. I. KETSKEMÉTY, J. DOMBI, J. HEVESI, R. HORVAI and L. KOZMA, *Acta Phys. et Chem. Szeged*, **7**, 88, 1961.
17. R. BAUER and M. ROZWADOWSKI, *Bull. Acad. Polon. Sci., Cl. III*, **7**, 365, 1959.
18. TH. FÖRSTER, *Z. Naturforsch.*, **4a**, 321, 1949.
19. M. D. GALANIN, *Zh. Eksper. Teor. Fiz.*, **28**, 485, 1955.
20. A. JABŁONSKI, *Bull. Acad. Polon. Sci., Cl. III*, **6**, 663, 1958.
21. I. KETSKEMÉTY, *Z. Naturforsch.*, **20a**, 82, 1965.

ПРОЦЕССЫ ПЕРЕДАЧИ ЭНЕРГИИ
В ЛЮМИНЕСЦИРУЮЩИХ РАСТВОРАХ СМЕСИ

И. ДОМБИ

Резюме

В работе дается зависимость между спектрами флуоресценции растворов смеси и компонентных растворов, которая принимает во внимание передачу излучаемой энергии с точностью, не достигнутой до настоящего времени. Резонансная передача энергии в данном выражении охарактеризуется всего двумя величинами. При помощи этих величин, далее использованием коэффициентов полезного действия и времен затухания компонентных веществ определяется частота молекулярных процессов, связанных с излучением и передачей энергии. Результаты экспериментов согласуются с данными исследований Яблонски, Фёрстера и Кечкемети, и подтверждают выдвинутую ими теорию о механизме перехода энергии. Проводится анализ метода Розмана, относящегося к переходу энергии, предлагаются коррекции к данному методу.