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Abstract

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ON THE QUENCHING OF SENSITIZED FLUORESCENCE IN SOLUTIONS

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In a joint work by the authors of the present article and P. I. Kudryashova ([1]), devoted to the study of sensitized fluorescence in solutions, a very curious phenomenon was noted. It was shown that in mixed solutions of trypaflavine and rhodamine, at high concentrations of the donor (trypaflavine) and the energy acceptor (rhodamine B), the values of the luminescence duration of rhodamine become smaller than the values of the fluorescence duration of a rhodamine solution of the same concentration, excited directly by the light source. On the basis of these data the authors suggested the possible existence of some kind of quenching of excited acceptor molecules by donor molecules.

One of the aims of the present work was to confirm this hypothesis under purer conditions and to obtain some information on the nature of the quenching. The second aim of the work was to investigate the quenching, by foreign substances, of the luminescence of mixed solutions in which migration of excitation energy takes place.

Quenching of the fluorescence of an energy donor in the case of sensitized fluorescence was first investigated by Förster ([2]). Förster showed that the changes in the fluorescence yield of the donor (trypaflavine) upon addition of a quencher to a mixed solution containing such concentrations of rhodamine and trypaflavine that sensitized fluorescence of rhodamine is observed are considerably smaller than the changes observed when the quencher is added to a solution of trypaflavine without rhodamine. Since changes in the fluorescence yield are sympathetic with changes in its duration, Förster' s experiments proved that the transfer of energy from a trypaflavine molecule to a rhodamine molecule is accompanied by a shortening of the duration of the excited state of the former molecule.

We used a fluorometer in order to follow directly the changes in the luminescence duration of both the donor and the acceptor when the fluorescence of solutions is quenched by foreign substances. In addition, we studied changes in the luminescence spectrum of mixed solutions of trypaflavine and rhodamine when a quencher is introduced into the solution.

The study of the quenching of rhodamine fluorescence by trypaflavine was car-

ried out under direct excitation of rhodamine fluorescence by the mercury line 546 mμ. In order to clarify the dependence of rhodamine fluorescence quenching on the viscosity of the solvent, quenching was studied both in methyl alcohol and in glycerin. In the latter case, where the duration and, apparently, the fluorescence yield are greater than in methyl alcohol, and where it is easy to prepare thin layers of solution, changes in the duration under quenching were investigated both in thick and in thin layers. Observation in all cases was made from the excitation side, at a small angle to the exciting light, through a light filter isolating a region with a maximum near 590 mμ.

Table 1

Change in the fluorescence lifetime of a rhodamine B solution as a function of the concentration of trypaflavine ($\tau \cdot 10^9$ s)

$C_R \cdot 10^3$, mol/l	Solvent	Cuvette thickness, mm	$C_T \cdot 10^2$, mol/l	$C_T \cdot 10^2$, mol/l	$C_T \cdot 10^2$, mol/l	$C_T \cdot 10^2$, mol/l	$C_T \cdot 10^2$, mol/l	$C_T \cdot 10^2$, mol/l
			0	0.125	1	1.5	1.84	2.0
5.0	Methyl alcohol	10	2.83	2.74	2.48	–	–	2.14
2.5	Methyl alcohol	10	3.12	–	2.7	–	–	–
5.0	Glycerin	0.02	3.75	3.69	2.96	2.74	2.6	–
5.0	Glycerin	10	5.87	5.6	4.42	4.0	3.6	–

The data obtained by us are given in Table 1. It is seen from the table that quenching of excited rhodamine molecules by trypaflavine molecules undoubtedly occurs. The ratio τ_0/τ, characterizing this quenching, as the table shows, depends on the fluorescence lifetime of the solution containing no trypaflavine and does not depend on the viscosity of the solution. Obviously, the type of quenching under consideration cannot be assigned either to diffusional quenching or to quenching by extraneous absorbing substances, since there is practically no overlap between the fluorescence spectrum of rhodamine and the absorption spectrum of trypaflavine. Nevertheless, from the fact of quenching it follows that the electric field created by the trypaflavine molecule acts on the excited rhodamine molecule and promotes its radiationless deactivation.

Table 2

Change in the luminescence lifetime of trypaflavine and rhodamine B in methyl alcohol as a function of the concentration of potassium iodide in pure and mixed solutions of these dyes ($\tau \cdot 10^9$ s)

Quencher concentration, mol/l	Trypaflavine, mol/l	Trypaflavine, mol/l	Rhodamine B, mol/l	Rhodamine B, mol/l	Mixture of trypaflavine and rhodamine, mol/l	Mixture of trypaflavine and rhodamine, mol/l	Mixture of trypaflavine and rhodamine, mol/l	Mixture of trypaflavine and rhodamine, mol/l
	$1 \cdot 10^{-5}$	$5 \cdot 10^{-2}$ ($d = 0.05$ mm)	$1 \cdot 10^{-5}$	$5 \cdot 10^{-3}$	trypaflavine $1 \cdot 10^{-5}$	rhodamine $1 \cdot 10^{-5}$	trypaflavine $2.5 \cdot 10^{-3}$	rhodamine $2.5 \cdot 10^{-3}$
0	4.27	4.40	3.0	3.24	4.25	5.1	1.90	3.5
$0.5 \cdot 10^{-2}$	3.2	3.12	2.55	2.73	3.1	3.68	1.55	3.0
$1 \cdot 10^{-2}$	2.37	2.5	2.3	2.37	2.37	3.2	0.96	2.56
$2 \cdot 10^{-2}$	1.8	1.85	1.9	2.07	1.75	2.58	1.08	2.19
$3 \cdot 10^{-2}$	1.50	1.46	1.78	1.85	1.46	2.3	0.95	1.85

Table 2 gives the data obtained by us in quenching, with potassium iodide, pure and mixed solutions of trypaflavine and rhodamine B in methyl alcohol. Quenching of pure solutions was studied for two cases: at dye concentrations equal to $1 \cdot 10^{-5}$ and $5 \cdot 10^{-3}$ mol/l. Quenching of mixed solutions was likewise studied for two cases: at concentrations of each of the dyes equal to $1 \cdot 10^{-5}$ and $2.5 \cdot 10^{-3}$ mol/l. In all experiments, except the case in which the concentration of trypaflavine was $5 \cdot 10^{-3}$ mol/l, cuvettes 5 mm thick were used. For the high concentration of trypaflavine a 0.05 mm cuvette was taken; this was done in order to exclude the influence of reabsorption of trypaflavine's own luminescence and thereby bring the experimental conditions closer to the case of quenching of trypaflavine fluorescence in the mixture.

Excitation of the fluorescence of the solutions in all cases, except pure rhodamine solutions, was carried out with the mercury line 436 mμ; in the case of rhodamine solutions, with the line 546 mμ. Isolation of the luminescence bands of rhodamine

and trypaflavin in mixed solutions was carried out with the aid of specially selected light filters.

It is seen from Table 2 that the quenching of trypaflavin fluorescence in pure and mixed solutions at a dye concentration equal to $1 \cdot 10^{-5}$ mole/liter is practically exactly the same. In the mixed solution at a dye concentration equal to $2.5 \cdot 10^{-3}$ mole/liter, where there is considerable migration of excitation energy from trypaflavin molecules to rhodamine molecules, and even without the addition of

Figure 1. Emission spectra of a mixed solution of trypaflavin and rhodamine B in methyl alcohol.

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potassium iodide the duration of the luminescence of trypaflavin is more than two times less than the natural duration of the luminescence of trypaflavin; the quenching of the luminescence of trypaflavin by potassium iodide $(\tau_0/\tau - 1)$* is approximately also two times less than the quenching of fluorescence of a pure trypaflavin solution.

Fig. 1. Emission spectra of a mixed solution of trypaflavin and rhodamine B in methyl alcohol ($C_T = C_R = 5 \cdot 10^{-3}$ mole/l). 1—without addition of potassium iodide; 2—with addition of potassium iodide ($C_{\mathrm{KJ}} = 2 \cdot 10^{-2}$ mole/l)

The change in the fluorescence duration of pure solutions of rhodamine B upon quenching by potassium iodide at a concentration equal to $5 \cdot 10^{-3}$ mole/liter is somewhat greater than at $1 \cdot 10^{-5}$ mole/liter, owing to an increase in reabsorption, which still plays some role in these solutions, slightly quenched because of concentration quenching of fluorescence. In mixed solutions not quenched by potassium iodide, the initial duration of the luminescence of rhodamine B is somewhat overestimated in comparison with the natural duration of fluorescence of rhodamine B. The reason for this is the fact that the luminescence of rhodamine B occurs mainly at the expense of energy received from excited trypaflavin molecules. At a concentration of the solution components of $1 \cdot 10^{-5}$ mole/liter, excitation occurs at the expense of trivial absorption by rhodamine of the fluorescence of trypaflavin; at $5 \cdot 10^{-3}$ mole/liter, mainly at the expense of nonradiative energy migration from the trypaflavin molecule to the rhodamine molecule. Since in these cases the quencher reduces both the luminescence duration of the donor and the de-excitation time of the excited acceptor molecules, measured with the aid of a fluorometer, the change in the luminescence duration of the acceptor (rhodamine B) in mixed solutions is greater than in pure rhodamine solutions**. Thus, since upon quenching by foreign substances the luminescence yield of rhodamine B in mixed solutions decreases both owing to quenching of excited rhodamine molecules and owing to a decrease in the probability of receiving excitation energy from excited trypaflavin molecules, which are also subject to quenching by potassium iodide, it is natural to expect that in the luminescence spectrum of the quenched solution the emission band of trypaflavin will be intensified. This indeed takes place (Fig. 1).

This phenomenon, in combination with the facts that upon quenching by foreign substances the luminescence yield of the donor in mixed solutions falls

* τ_0 and τ are the luminescence durations in the unquenched and quenched solutions.

** It should be emphasized that the duration of rhodamine luminescence mea-

sured on the fluorometer in the case of mixed solutions is a quantity characterizing a complex, obviously nonexponential process consisting of two stages: transfer of excitation energy from the trypaflavin molecule to the rhodamine molecule and subsequent deactivation of the excited rhodamine molecules.

smaller, and the yield of the acceptor greater, in comparison with the decrease in the yield of the pure solutions of the donor and acceptor, provides a simple way of checking the assumption that sensitized fluorescence exists in the solution under study. It is known that the assumption of the existence of sensitized fluorescence is not always put forward with sufficiently firm justification.

In conclusion, we consider it our pleasant duty to express our deep gratitude to Academician A. N. Terenin for his interest in this work.

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2. Th. Förster, *Zs. Electrochem.*, **53**, 93 (1949).

Note: Figure translations are in progress. See original paper for figures.