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L. A. Kiyanskaya, P. I. Kudryashov, and B. Ya. Sveshnikov

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Abstract

Full Text

Physics

L. A. Kiyanskaya, P. I. Kudryashov, and B. Ya. Sveshnikov

On the Quenching by Foreign Substances of the Fluorescence of Solutions in the Region of High Concentrations of the Fluorescent Substance

(Presented by Academician A. A. Lebedev on 27 IX 1961)

The quenching by foreign substances of the fluorescence of solutions in the region of high concentrations of the fluorescent substance was first investigated by B. Ya. Sveshnikov ([1])*. The object of his study was the quenching of aqueous fluorescein solutions by potassium iodide. It was shown that, with a gradual increase in the dye concentration from $1 \cdot 10^{-6}$ to 10^{-2} g/cm^3, at first the quenching is observed to be independent of the change in the dye concentration; then, in the concentration region $2.5 \cdot 10^{-4}$–$1 \cdot 10^{-3}$ g/cm^3, the quenching increases noticeably; and finally, in the region of still higher dye concentrations, it decreases strongly. The cause of the first effect was evidently reabsorption by the solution of its own fluorescence. The influence of this factor was insufficiently taken into account in ([1]). The cause of the decrease in fluorescence quenching in the region of higher concentrations was the decrease in the fluorescence lifetime owing to concentration quenching of the fluorescence competing with quenching by foreign substances. In those years this experiment was the simplest way of proving that concentration quenching of the fluorescence of solutions of a given substance is quenching of the second kind.

Subsequently, quenching by foreign substances in the region of high concentrations of the fluorescent substance was studied by D. Stelmakhovich. He found a decrease in quenching by foreign substances in solutions of certain fluorescent substances when their quenching was resorptional, but at the same time he found a number of cases in which quenching of the fluorescence of solutions by foreign substances in the region of concentration quenching of the fluorescent substance proved to be stronger than quenching by the same quencher in the region of low concentrations of the fluorescent substance, although no changes in the absorption and fluorescence spectra of the solution were observed.

The latter result was soon confirmed in the work of P. P. Feofilov and B. Ya. Sveshnikov ([2]). According to the data of these three authors, concentrated solutions of fluorescein, rhodamine, trypaflavine, and eosin in glycerin are quenched by aniline more strongly than solutions containing low concentrations of the dyes. On the other hand, F. M. Pekerman ([3]) carried out a detailed study of the quenching by resorcinol of alcoholic solutions of rhodamine B, trypaflavine,

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Fig. 1

Figure 1: Fig. 1

Fig. 2

Figure 2: Fig. 2

and acridine orange and showed that, for these dyes, concentration quenching of fluorescence in the presence of quenchers proceeds more weakly.

Since the results of the investigations by Sveshnikov and Pekerman had a quite natural explanation, the results obtained by Feofilov and Sveshnikov seemed anomalous. The simplest assumption was that the explanation of the observed phenomenon should be sought in some physical quenching processes extraneous to the studied types–

* Here only such cases of fluorescence quenching are meant in which the addition of a foreign substance to the solution does not noticeably change the absorption and fluorescence spectra of the solutions.

chemical or physicochemical processes. However, this assumption was not justified. First, significant changes in the absorption and fluorescence spectra were not found in the work of Feofilov and Sveshnikov, and second, the assumption made in that work that changes in the fluorescence yield are accompanied by corresponding changes in the fluorescence lifetime was satisfactorily justified in quantitative calculations of concentration depolarization.

Fig. 1. Luminescence spectra (**L**) and absorption spectra (**P**). **A** –fluorescein in water: 1 –$C = 1 \cdot 10^{-4}$-$1 \cdot 10^{-2}$ mol/l; 2 –$C = 2 \cdot 10^{-2}$ mol/l; **a** and **c** – without quencher; **b** and **g** –upon addition of 0.3 mol/l KI. **B** –trypaflavin in glycerin: 1 –$C = 1 \cdot 10^{-4}$-$1 \cdot 10^{-2}$ mol/l; 2 –$C = 2 \cdot 10^{-2}$ mol/l; **a** and **c** – without quencher; **b** and **g** –upon addition of 0.5 mol/l aniline.

To clarify the indicated contradiction, we again investigated the quenching by foreign substances of the fluorescence of solutions containing high concentrations of fluorescent substances. Two dyes were taken (fluorescein and trypaflavin), two quenchers (potassium iodide and aniline), and three solvents (water, ethyl alcohol, and glycerin).

Fig. 2. Fluorescence quenching. **A** –fluorescein in water, quenching by potassium iodide at dye concentrations (in mol/l): 1 –$3 \cdot 10^{-5}$, 2 –$1 \cdot 10^{-3}$, 3 – $3 \cdot 10^{-3}$, 4 –$1 \cdot 10^{-2}$. **B** –trypaflavin in glycerin, quenching by aniline at dye concentrations (in mol/l): 1 –$1 \cdot 10^{-4}$, 2 –$5 \cdot 10^{-3}$, 3 –$1 \cdot 10^{-2}$, 4 –$5 \cdot 10^{-2}$.

First of all, the influence of the concentration of the fluorescent substance and of additions of quencher on the absorption and fluorescence spectra of the solutions was studied. Figure 1 presents the results of the investigation for two cases: for the quenching of aqueous fluorescein solutions by potassium iodide

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Fig. 3. Change in yield (solid curves) and change in fluorescence lifetime (dashed curves) upon addition of foreign quenchers. A—fluorescein in water: a —without quenching, b—with addition of KJ ($C = 0.3$ mole/liter). —trypaflavin in alcohol: a—without quencher, b—with addition of aniline ($C = 0.1$ mole/liter). —fluorescein in glycerine: a—without quencher, b—with addition of KJ ($C = 0.24$ mole/liter). —trypaflavin in glycerine: a—without quencher, b—with addition of aniline ($C = 0.32$ mole/liter)

Figure 3: Fig. 3. Change in yield (solid curves) and change in fluorescence lifetime (dashed curves) upon addition of foreign quenchers. A—fluorescein in water: a—without quenching, b—with addition of KJ ($C = 0.3$ mole/liter). —trypaflavin in alcohol: a—without quencher, b—with addition of aniline ($C = 0.1$ mole/liter). —fluorescein in glycerine: a—without quencher, b—with addition of KJ ($C = 0.24$ mole/liter). —trypaflavin in glycerine: a—without quencher, b—with addition of aniline ($C = 0.32$ mole/liter)

and of glycerin solutions of trypaflavin by aniline. As is seen from Fig. 1, the absorption spectra of both dyes do not change over a very large concentration range from $1 \cdot 10^{-4}$ to $1 \cdot 10^{-2}$ mol/l, and only at concentrations greater than $1 \cdot 10^{-2}$ mol/l do small changes in the spectra occur, associated, apparently,

apparently, with association of the dye molecules*. The introduction into the dye solutions of a quencher at the concentrations indicated in Fig. 1 does not change the absorption spectra of the solution in the visible region. The luminescence spectra in the region of high dye concentrations shift somewhat toward the red. This is caused by reabsorption of the fluorescence. In the layers that we used in our experiments (0.005-0.05 mm for glycerol solutions and 0.05-0.1 mm for aqueous and alcoholic solutions), we could not eliminate it at high dye concentrations. Addition of the quencher

Fig. 3. Change in yield (solid curves) and change in fluorescence lifetime (dashed curves) upon addition of foreign quenchers. A—fluorescein in water: a—without quenching, b—with addition of KJ ($C = 0.3$ mole/liter). —trypaflavin in alcohol: a—without quencher, b—with addition of aniline ($C = 0.1$ mole/liter). —fluorescein in glycerine: a—without quencher, b—with addition of KJ ($C = 0.24$ mole/liter). —trypaflavin in glycerine: a—without quencher, b—with addition of aniline ($C = 0.32$ mole/liter)

weakens the effect of reabsorption, and the luminescence spectra of the quenched solutions shift substantially less toward the red with increasing dye concentration than do the luminescence spectra of the unquenched solutions.

In Fig. 2, for the same two cases of fluorescence quenching, the dependence of the reciprocal values of the fluorescence yield on the quencher concentration is shown. From Fig. 2 it is seen that, when the fluorescence of aqueous fluorescein solutions is quenched by potassium iodide, a decrease in quenching is observed on going to higher dye concentrations**, whereas for the case of quenching by

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aniline of the fluorescence of trypaflavin solutions in glycerine

* In the case of trypaflavin, some change in pH is also possible. The fluorescein solutions contained 1% NaOH.

** In the concentration range from $3 \cdot 10^{-5}$ to $1 \cdot 10^{-2}$ mole/liter we used layers from 0.015 to 0.1 mm and therefore did not observe the small enhancement of quenching found by Sveshnikov.

an enhancement of quenching is observed in the region of high dye concentrations. The same enhancement of quenching in the region of high dye concentrations was found for the case of quenching by potassium iodide of fluorescein solutions in glycerin. By contrast, in the quenching of alcoholic solutions of trypaflavine by aniline, a decrease in quenching is observed as the dye concentration is increased.

From these data an interesting conclusion follows: for low-viscosity solutions, in agreement with the data of F. M. Pekerman, a weakening of concentration quenching of fluorescence is observed upon additional quenching by foreign substances (Fig. 3A and B), whereas for viscous solutions concentration quenching is intensified when a foreign quencher is introduced (Fig. 3C and D).

We propose the following explanation of the observed phenomena. During the time for which the excitation energy is located in the molecule excited directly by the light source, or in the molecule to which it has transferred the excitation energy in the process of energy migration, in low-viscosity solutions the position of the quencher molecules relative to the excited molecule changes continuously and rapidly, and at some sufficiently small distance between them fluorescence quenching occurs. The two quenching processes—concentration quenching and quenching by foreign substances—may in this case be regarded as independent. The situation is different in viscous media. Diffusion of quencher molecules toward the dye molecule during the time it remains in the excited state is very small; however, since the excitation energy migrates from one dye molecule to another, a case may occur in which this energy passes to a dye molecule at a short distance from which there is a quencher molecule*.

Thus, in viscous media, energy migration leads not only to concentration quenching of fluorescence, but also to an intensification of quenching by foreign substances.

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CITED LITERATURE

[1] B. Ya. Sveshnikov, *Tr. GOI*, **12**, issue 108 (1938). [2] P. P. Feofilov, B. J. Sveshnikov, *J. of Phys. USSR*, **3**, 493 (1940). [3] F. M. Pekerman, *DAN*, **52**, No. 5, 409 (1946); **52**, No. 9, 773 (1946).

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* We assume that the presence of the quencher does not affect the probability of migration of excitation energy.

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

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