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ON THE CONNECTION BETWEEN THE EFFECTIVENESS OF THE ACTION OF CERTAIN FLUORESCENCE QUENCHERS AND THE POSITION OF THE FLUORESCENCE SPECTRUM

(Presented by Academician A. N. Terenin on 6 VI 1957)

In work (⁀1) the presence of a connection was noted, for a number of compounds, between the position of the fluorescence spectrum and the susceptibility of their fluorescence to quenching by potassium iodide and aniline. The discovery of such a connection is of fundamental interest both for the question of the mechanism of fluorescence quenching of solutions by foreign substances and for the spectroscopy of complex organic molecules. In work (⁀1), as the quantity characterizing the susceptibility of fluorescence to quenching by foreign impurities, the ratio was taken of the fluorescence yields of the unquenched solution and of the solution containing the foreign impurity at a definite concentration. In doing so, neither the influence on quenching of the lifetime of the excited state nor the influence of the viscosity of the solvent was taken into account.

In the present work a quantitative study was undertaken of the connection between the effect on the lifetime of the excited state of foreign impurities and the position of the fluorescence spectrum. As the quantity characterizing the effectiveness of the action of a quencher, the expression* was adopted

$$k = \frac{c(\tau_1 - \tau_2)\eta}{\tau_1 \tau_2 c}; \tag{1}$$

where τ_1 is the lifetime of the excited state in the absence of a quencher; τ_2 is the lifetime upon addition of the quencher at the given concentration of the quencher; c is the concentration of the quencher in mol/l; η is the viscosity of the solvent in poises.

The fluorescence lifetimes were determined on a GOI phase fluorometer (⁀2)**. Only τ_1 was measured directly. To determine τ_2, the cuvette with the quenched

solution was placed in the position of the scatterer of the exciting light usually used in determining fluorescence lifetimes. Thus, the directly measured quantity proved to be the phase difference of the modulated fluorescence light of the quenched and unquenched solutions. By subtracting this quantity from the phase difference of the modulated fluorescence light of the unquenched solution and the exciting light, the phase lag corresponding to the fluorescence lifetime of the quenched solution was found. Such a method of determining τ_2 and $\Delta\tau = \tau_1 - \tau_2$ leads to a considerable increase in the accuracy of determining the effectiveness of the action of the quencher.

The accuracy of the determination of effectiveness may be characterized by the example of 4-acetylamino-N-methylphthalimide in methyl alcohol under the action—

* The probability of radiationless deactivation of the excited state by a quencher q_2 is proportional to the concentration of the quencher and, in the first approximation, inversely proportional to the viscosity of the solvent η, i.e. $q_2 = kc/\eta$. In this expression the coefficient k determines the effectiveness of the action of the quencher; q_2 may be expressed through the lifetimes of the excited states $\tau_1 = 1/p + q$, $\tau_2 = 1/p + q_1 + q_2$, where p is the probability of a radiative transition from the excited state to the ground state, and q_1 is the probability of radiationless deactivation of the excited state in the absence of a quencher.

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···by potassium iodide. As can be seen from Table 1, over a wide range of variation of the quencher concentration the value of the efficiency is reproduced from measurement to measurement with sufficient accuracy. In the case of weak fluorescence intensities or short durations, and also in those cases when the emission is shifted too far in the spectrum into the red or ultraviolet region, within which the sensitivity of the photomultiplier used is weak, the accuracy of determining the efficiency is considerably lower.

Table 1

Efficiencies of the action of potassium iodide on the fluorescence of 4-acetylamino-*N*-methylphthalimide in methyl alcohol at various quencher concentrations
($\tau_1 = 19.8 \cdot 10^{-9}$ sec.)

c, mol/l	$\tau_2 \cdot 10^9$, sec.	$\Delta\tau \cdot 10^9$, sec.	$k \cdot 10^{-9}$	$\Delta k \cdot 10^{-9}$ from mean	Δk, % from mean
0.55	4.45	15.35	5.09	0.04	1
0.215	8.55	11.25	4.95	0.10	2
0.140	11.25	8.55	4.41	0.64	12.5
0.105	11.9	7.9	5.00	0.05	1

c, mol/l	$\tau_2 \cdot 10^9$, sec.	$\Delta\tau \cdot 10^9$, sec.	$k \cdot 10^{-9}$	$\Delta k \cdot 10^{-9}$ from mean	Δk, % from mean
0.037	15.6	4.2	5.82	0.77	15
Mean			5.05		

As extraneous quenching substances, triethylamine iodide and potassium iodide, aniline, and diethylaniline were used. The action of these impurities on the fluorescence duration of a number of 3-, 4-, and 3,6-derivatives of phthalimide was investigated in various solvents: in water, acetone, methyl, ethyl, and butyl alcohols, ethyl acetate, pyridine, anisole, benzene, chlorobenzene, and *N*-octane.

Table 2

Efficiencies of the action of aniline and triethylamine iodide on the fluorescence of phthalimide derivatives.

Numbering of solvents in quenching by aniline: 1—ethyl acetate, 2—pyridine, 3—chlorobenzene, 4—benzene, 5—*n*-octane.

Numbering of solvents in quenching by triethylamine iodide: 1—butyl alcohol, 2—ethyl acetate, 3—pyridine, 4—acetone, 5—anisole.

Substance	Solvent	Aniline: $\tau_1 \cdot 10^9$, sec.	Aniline: $\Delta\tau \cdot 10^9$, sec.	Aniline: c, mol/l	Aniline: $k \cdot 10^{-9}$	Solvent	Triethylamine iodide: $\tau_1 \cdot 10^9$, sec.	Triethylamine iodide: $\Delta\tau \cdot 10^9$, sec.	Triethylamine iodide: c, mol/l	Triethylamine iodide: $k \cdot 10^{-9}$
3-Amino-*N*-methylphthalimide	1	14.1	9.3	1.835	3.29	1	13.25	0.55	2.29	0.97
3-Amino-*N*-methylphthalimide	2	12.65	5.40	1.60	3.35	2	12.3	3.75	1.835	2.0
3-Amino-*N*-methylphthalimide	3	11.35	6.80	1.70	5.95	3	12.65	3.55	2.265	2.92
3-Amino-*N*-methylphthalimide	4	13.05	8.60	1.925	4.8	4	13.6	6.05	2.125	2.03

Substance	Solvent	Aniline: $\tau_1 \cdot 10^9$, sec.	Aniline: $\Delta\tau \cdot 10^9$, sec.	Aniline: c, mol/l	Aniline: $k \cdot 10^{-9}$	Solvent	Triethylamine iodide: $\tau_1 \cdot 10^9$, sec.	Triethylamine iodide: $\Delta\tau \cdot 10^9$, sec.	Triethylamine iodide: c, mol/l	Triethylamine iodide: $k \cdot 10^{-9}$
3-Amino-*N*-methylphthalimide	5	—	—	—	—	5	13.15	3.15	1.14	6.39
4-Amino-*N*-methylphthalimide	1	18.0	10.9	1.835	2.0	1	11.1	0.4	2.28	1.0
4-Amino-*N*-methylphthalimide	2	17.35	6.95	1.60	2.19	2	18.0	6.35	1.89	1.65
4-Amino-*N*-methylphthalimide	3	18.30	13.15	1.70	6.3	3	17.35	6.5	2.08	3.58
4-Amino-*N*-methylphthalimide	4	19.7	15.15	1.92	5.3	4	17.5	9.15	2.125	2.17
4-Amino-*N*-methylphthalimide	5	12.75	9.35	1.42	7.85	5	18.1	8.1	1.315	8.50
3,6-Diacetylamino-*N*-methylphthalimide	1	12.0	8.05	1.835	3.97	1	10.3	3.05	2.28	1.22
3,6-Diacetylamino-*N*-methylphthalimide	2	10.9	3.75	0.74	5.56	2	11.25	2.85	1.78	2.27
3,6-Diacetylamino-*N*-methylphthalimide	3	10.6	6.4	1.70	6.5	3	10.9	3.3	2.285	3.79

Substance	Solvent	Aniline: $\tau_1 \cdot 10^9$, sec.	Aniline: $\Delta\tau \cdot 10^9$, sec.	Aniline: c, mol/l	Aniline: $k \cdot 10^{-9}$	Solvent	Triethylamine iodide: $\tau_1 \cdot 10^9$, sec.	Triethylamine iodide: $\Delta\tau \cdot 10^9$, sec.	Triethylamine iodide: c, mol/l	Triethylamine iodide: $k \cdot 10^{-9}$
3,6-Diacetylamino-*N*-methylphthalimide	4	—	—	—	—	4	10.9	5.3	2.125	3.01
3,6-Diacetylamino-*N*-methylphthalimide	5	10.8	7.25	1.42	6.8	5	12.85	3.10	1.315	4.95
3-Monomethylamino-*N*-methylphthalimide	1	11.9	6.8	1.835	2.62	1	—	—	—	—
3-Monomethylamino-*N*-methylphthalimide	2	9.75	3.15	1.60	3.65	2	11.9	1.95	0.97	2.1
3-Monomethylamino-*N*-methylphthalimide	3	13.05	7.95	1.70	5.4	3	8.75	2.55	2.08	2.48
3-Monomethylamino-*N*-methylphthalimide	4	14.1	9.5	1.925	4.75	4	—	—	—	—
3-Monomethylamino-*N*-methylphthalimide	5	2.7	0.95	1.42	7.3	5	14.0	3.65	1.42	5.3

Individual results of the investigation are given in Table 2. In Figs. 1 and 2 are presented the dependences of the efficiency of the action of the quencher on the position of the fluorescence spectrum of the corresponding solutions for various phthalimide derivatives. In all cases an increase in the value of the efficiency is observed with increasing frequency of the max—

of the maximum of the fluorescence spectrum. Most of the points characterizing the relation between the efficiency and the position of the fluorescence spectrum for each substance in different solvents fall on a single curve, close to a straight line. Thus, the dependence of the efficiency on the position of the spectrum

Fig. 1 and Fig. 2: Dependences of the efficiency of quenching action on the position of the fluorescence-spectrum maximum.

Figure 1: Fig. 1 and Fig. 2: Dependences of the efficiency of quenching action on the position of the fluorescence-spectrum maximum.

not only exists, but is also determining: the influence of other properties of the solvent on the magnitude of the efficiency recedes into the background.

The values of the efficiencies of the action of potassium iodide on the fluorescence of aqueous solutions fall outside the regularities obtained (Fig. 2A). This,

Fig. 1. Dependences of the efficiency of the action of aniline on the position of the fluorescence spectrum

A. *I* –4-acetylamino-N′-methylphthalimide; *IIa, b* –4-amino-N-methylphthalimide (*b* –quenching by diethylaniline); *IIIa* –4-monomethylamino-N-methylphthalimide; *IIIb* –4-dimethylamino-N-methylphthalimide; *IV* –3-amino-N-methylphthalimide; *V* –3-monomethylamino-N-methylphthalimide

B. *I* –3,6-diacetylamino-N-methylphthalimide; *II* –3-methylacetylamino-6-acetylamino-N-methylphthalimide; *III* –3-methylacetylamino-6-amino-N-methylphthalimide; *IV* –3-acetylamino-6-amino-N-methylphthalimide; *Va* –3-dimethylamino-6-methylacetylamino-N-methylphthalimide; *Vb* –3-dimethylamino-6-acetylamino-N-methylphthalimide. Designations 1–5, see Table 2.

Fig. 2. Dependences of the efficiency of the action of potassium iodide and triethylamine iodide on the position of the fluorescence spectrum

A. Quencher –potassium iodide. *I* –3,6-diacetylamino-N-methylphthalimide; *II* –3-acetylamino-6-amino-N-methylphthalimide; *III* –3,6-diamino-N-methylphthalimide. Solvent numbering: 1 –water; 2 –methyl alcohol; 3 –ethyl alcohol; 4 –acetone.

B. Quencher –triethylamine iodide. *I* –3,6-diacetylamino-N-methylphthalimide; *II* –3-amino-N-methylphthalimide; *III* –4-amino-N-methylphthalimide; *IV* –3-monomethylamino-N-methylphthalimide. Designations 1–5, see Table 2.

probably, is explained by the considerably greater dissociation of potassium iodide in water than in the other solvents. In a number of cases the values of the efficiencies of the action of quenchers on the fluorescence of acetone solutions are lowered in comparison with the values that would be expected from the course of the dependences.

The identical character of the dependence of the action on fluorescence on the position of the fluorescence spectrum both in the case of potassium iodide and of tri-

ethylamine*, and in the case of aniline makes it possible to consider that the

quenching action on the fluorescence of aniline, just as in the case of iodide salts ([3]), consists in an increase in the probability of transition to the metastable state, despite the fact that upon addition of aniline it was not possible to observe an increase in the absolute yield of phosphorescence.

The angles of inclination of the straight lines expressing the relation between the effectiveness of the quencher' s action on the excited state and the position of the fluorescence spectrum prove to be almost the same for all 4-derivatives, for all 3-derivatives, and for all 3,6-derivatives. For the 4-derivatives the angles of inclination are the smallest, and for the 3,6-derivatives the largest. This regularity appears most distinctly in quenching by aniline, but in a less clear form it is also found under the action of triethylamine iodide. The magnitude of the effectiveness, both under the action of aniline and under the action of potassium iodide and triethylamine iodide, is of the same order.

For 3- and 4-derivatives the straight lines are situated higher as the electron-donating power of the substituent increases, i.e., at one and the same frequency the effectiveness for amino derivatives of phthalimide is greater than for acetylamino derivatives, and, further, for methyl- and dimethylamino derivatives is greater than for amino derivatives. For 3,6-substituted compounds this regularity is expressed less consistently, but in this case as well, for 3,6-diacetylamino-*N*-methylphthalimide at the given frequency the effectiveness is smaller than for 3-methylacetylamino-6-acetylamino-*N*-methylphthalimide, and for the latter it is smaller than for 3-acetylamino-6-amino-*N*-methylphthalimide. Thus, two opposite tendencies are clearly revealed: an increase in the electron-donating power of the substituent leads to an increase in the effectiveness of the quencher' s action, to an increase in the probability of transition to the metastable state; at the same time, an increase in the electron-donating power of the substituent leads to a shift of the fluorescence spectrum toward lower frequencies. This, in turn, causes a decrease in the effectiveness of the quencher' s action.

If the shift of the fluorescence spectrum is not taken into account, the impression may be created that, in passing to substances with more electron-donating substituents, the probability of transition to the metastable state decreases. For example, in the series 3,6-diacetylamino, 3-acetylamino–6-amino, 3,6-diamino–*N*-methylphthalimide in ethyl alcohol, the effectiveness of the action of potassium iodide decreases successively from the first substance to the last (Fig. 2 A). In reality this sequence expresses the dependence of the effectiveness of the quencher' s action on the spectral composition of the radiation. In other cases the influence of the spectral position of fluorescence on the effectiveness of the quencher' s action may be masked by the influence of structure. Then the sequence of change in effectiveness on passing from compound to compound in one and the same solvent may have a different character (for example, 4-derivatives of phthalimide under quenching by aniline, Fig. 1 A). Thus, in the present work it has been shown that the susceptibility of phthalimide fluorescence to the action of various foreign impurities is determined by the electron-donating power of the substituent and by the position of the fluorescence spectrum. It is

difficult to say to what extent these two factors will determine the susceptibility to quenching of the fluorescence of compounds from other chemical groups. It may only be noted that in those cases where the influence of the solvent on the position of the fluorescence spectrum is absent, distinguishing the influence of the electron-donating power of the substituent and the influence of the position of the spectrum will be a difficult task.

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* In view of the poor solubility of potassium iodide in most organic solvents, we used triethylamine iodide as the quencher.

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