

INFLUENCE OF QUENCHERS' DIPOLE MOMENT ON FLUORESCENCE QUENCHING

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The influence of the dipole moment of organic quenchers (isomers of dibromobenzene and dichlorobenzene) on quenching of fluorescence from anthracene in benzene solutions was investigated. Quenching was found to decrease with increasing dipole moment of the quencher. The interpretation suggested attributes the primary role to the probability of collision between the quenching substituent and the excited anthracene molecule, which is higher in *para*- than in *meta*- or *ortho*-derivatives.

In a previous paper (Głowacki 1965), one of us proved that the dipole moment of quencher molecules plays an important role in the process of fluorescence quenching in solutions. Part of the results could be rendered by the simple experimental formula

$$q = A\Pi^4 + B, \quad (1)$$

where

$$\Pi = \alpha - \frac{\mu_Q^2}{3kT}, \quad (2)$$

relating the quenching constant q (*i.e.* the probability of quenching during an encounter between a luminescent and a quencher molecule) with the polarizability α of the quencher and its permanent dipole moment μ_Q at the temperature of the solution T , whereas A and B are constants. Other results beyond the scope of formula (1) also indicated that the dipole moment of the quencher molecule lowered the quenching constant. Since those results could still be considered as not being the rule, it was felt that their confirmation by more data would be of importance for the understanding of the complicated mechanism of fluorescence quenching in solutions by foreign substances, as stressed earlier (Głowacki 1964).

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There is all the more need for such investigation as the role of the dipole moment is very great here and can by no means be overlooked in the interpretation of fluorescence quenching.

Basing on the results of the paper cited (Głowacki 1965), we came to the conclusion that investigation of the isomers of benzene dihalogen derivatives should be most fruitful. These compounds were chosen because they are the simplest in the case under consideration, yielding no perturbing side-effects such as *e.g.* associate formation. If the effect described above (Głowacki 1965) is of a general nature, it should thus become apparent in new experimental results.

Figs 1 and 2 represent the results obtained in experiments on the quenching of fluorescence from anthracene (at concentration 5×10^{-5} g/cc) in benzene by isomers of dichloro-

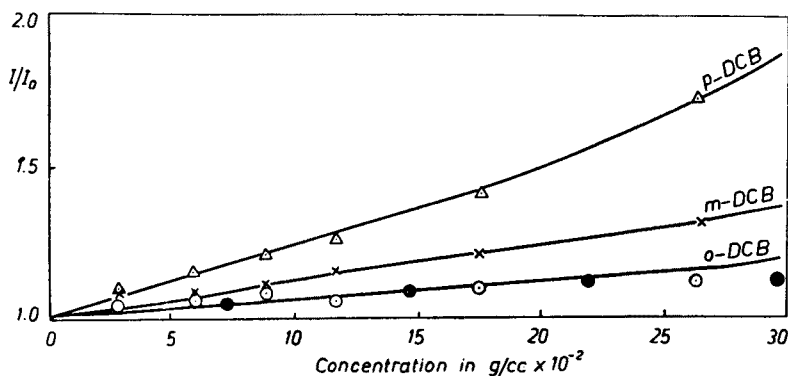


Fig. 1. Reciprocal relative fluorescence light intensity for anthracene in benzene, versus the concentration of dichlorobenzene (DCB) • — experimental results for chlorobenzene

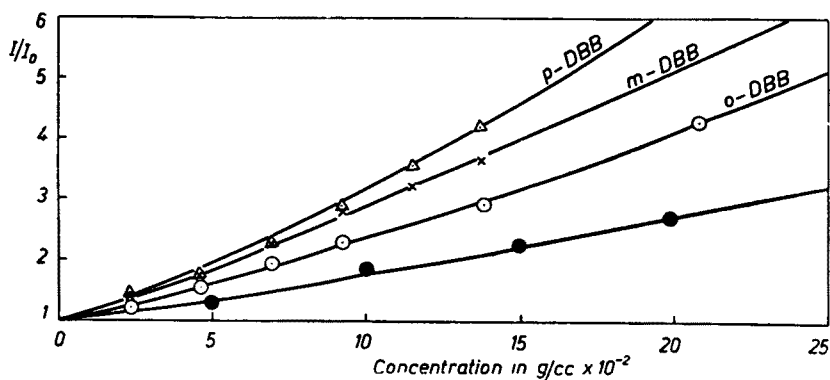


Fig. 2. Reciprocal relative fluorescence light intensity for anthracene in benzene, versus the concentration of dibromobenzene (DBB) • — experimental results for bromobenzene

and dibromobenzene. The compounds and solvents used were purified as for spectroscopic work. The results show that the influence of the dipole moment of the quencher on the effectiveness of quenching is considerable and increases for progressively weaker quenchers. For comparison, the quenching of anthracene by chloro- and bromobenzene is also given.

Thus, our suppositions based on earlier results (Głowacki 1965) are confirmed, and there may be ground for stating that the presence of a permanent dipole moment in a quencher molecule reduces its quenching power. Since in the paper cited the role of the permanent dipole moment in quenchers was merely announced, we will now proceed to an explanation of the effect.

Fluorescence quenching by foreign non-absorbing substances consists of two steps:

1. Formation of a Van der Waals collision complex, and
2. Desactivation of the excited molecule in the collision complex.

The formation of a collision complex is the necessary condition for desactivation of the excited molecule as a result of acceptor-donor interaction (Głowacki 1964). In the first step, the decisive role belongs to London dispersional forces whereas in the second step — to acceptor-donor interaction forces, which are entirely different in nature. The simultaneous presence of these two kinds of entirely different forces was justified by Mulliken (1952) in his theory of charge-transfer complexes.

Now, Van der Waals' forces are largest for such orientations of molecules for which polarization is the largest. Hence, reasonably, the quenching constant ought to depend on $P = \alpha + \frac{\mu_Q^2}{3kT}$ and not on $\Pi = \alpha - \frac{\mu_Q^2}{3kT}$ (2). This different, specific behaviour of the permanent dipole moment in the process of fluorescence quenching shows that one has to attribute to the permanent dipole moment of the quencher an entirely different role than that observed in the forces of Van der Waals.

It results (Głowacki 1965) that the chief role in the quenching process is played by the substituent, whereas the effectiveness of the quencher is determined by the polarizability of its bonding. In the case of two identical substituents in the molecule (the present case), the probability that a substituent shall encounter an excited molecule depends on the way in which the substituents are disposed within the quencher molecule. For *parabisisubstitute* molecules, the probability for an excited molecule to come up against a quenching substituent is almost double that of encountering a quenching substituent in the *orto*-position. Hence the importance of the structural disposition of substituents.

In order to calculate Π we assume that the solution is isotropic and that the probability of an encounter between a substituent and an excited molecule is determined by the Boltzmann factor $\exp\left(-\frac{\vec{\mu}_Q \cdot \vec{E}}{kT}\right)$, wherein \vec{E} is the strength of the spherically symmetric electric field of the excited molecule in the point of the dipole moment μ_Q of the quencher. With α denoting the mean polarizability of the quencher's electron shell, the mean value of Π is given by the expression

$$\Pi = C(T)\alpha e^{-\frac{\langle \vec{\mu}_Q \rangle \cdot \vec{E}}{kT}}. \quad (3)$$

The constant $C(T)$ depends on the temperature of the solution. For $\langle \vec{\mu}_Q \rangle \cdot \vec{E} \ll kT$, Eq. (3) takes the form

$$\Pi = C(T)\alpha \left(1 - \frac{\langle \vec{\mu}_Q \rangle \cdot \vec{E}}{kT}\right). \quad (4)$$

$\langle \vec{\mu}_Q \rangle = \overline{\vec{\mu}_Q \cos \theta}$ and denotes the mean value of the projection of $\vec{\mu}_Q$ on the field direction E . It will be remembered that

$$\overline{\cos \theta} = L(x) = \operatorname{ctgh} x - \frac{1}{x} \quad (5)$$

is given by Langevin's function $L(x)$, where $x = \frac{\mu_Q E}{kT}$. For $x \gg 1$ we have $L(x) = 1$ i.e. $\vec{\mu}_Q$ is parallel to E . For $x \ll 1$ we have $\overline{\cos \theta} = x/3$. This then is the condition fulfilled in Eq. (4). This yields finally

$$H = C(T) \left(\alpha - \gamma \frac{\mu_Q^2}{3kT} \right), \quad (6)$$

where $\gamma = \alpha E^2/kT$. Eq. (6) resembles (2) if $\gamma = 1$. This condition allows to evaluate the field strength \vec{E} directly. At $\alpha \sim 10^{-24} \text{ cm}^3$, one has $E \sim 5 \times 10^7 \text{ Volt/cm}$. Such fields exist only when molecules are very near to one another. This result can mean that the quencher has to come very close to the excited molecule and may well corroborate our hypothesis concerning the formation of collision complexes.

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