The Quenching of the Fluorescence of Anthracene. The Transition from Strong to Weak Quenching.

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Quenching of fluorescence in solution occurs when quencher molecules and excited molecules occupy contiguous positions in a liquid. Strong quenchers are limited by the viscosity-controlled rate of formation of neighbouring pairs, while weak quenchers are limited by the concentration of such pairs and the efficiency of collisions within them. An equation is derived from this model which describes the observed behaviour of quenchers of intermediate strength. A photometer is described which is suitable for the precise measurement of relative fluorescence intensities.

A mechanism which describes the quenching of the fluorescence of solutions of anthracene by substances which deactivate the excited molecules on every encounter has been given by Bowen and Metcalf (*Proc. Roy. Soc.*, 1951, *A*, **206**, 437). This scheme may now be extended to cases where encounters are less effective.

As before, quenching is considered to occur only in collisions between nearest neighbours. In the present extension there is included the possibility of an excited molecule and a quencher molecule vacating contiguous positions before quenching occurs. Let α be the fraction of anthracene in encounters. The relevant processes and their rates are

		Rates, etc.
Absorption	$A + h\nu \longrightarrow A^*$	$(1 - \alpha)I_{abs.}$
	$AQ + h\nu \longrightarrow A^*Q$	$\alpha I_{abs.}$
Fluorescence	$A^* \longrightarrow A + h\nu'$	$k_f[A^*]$
	$A^*Q \longrightarrow AQ + h\nu'$	$k_f[A^*Q]$
Quenching	$A^*Q \longrightarrow$ Products or energy degradation	$k_3[A^*Q]$
Encounter equilibria	$A + Q \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AQ$	$[AQ] = K[A][Q]$
	$A^* + Q \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A^*Q$	$K = k_1/k_2$

A and Q represent anthracene and quenching substance, respectively.

It is assumed that excitation does not appreciably alter the rate constants for formation and dissociation of encounters, and encounter formation does not sensibly alter the rate constant k_f for fluorescence emission.

Putting $d[A^*]/dt = d[A^*Q]/dt = 0$ and noting that $(1 - \alpha) = 1/(1 + K\,[Q])$, we have

$$\frac{f_0}{f} = \frac{k_2' + (1 + k_3')(1 + k_1'[Q])}{k_2' + (1 + k_1'[Q]) + k_3'/(1 + K[Q])} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

A prime denotes division by k_f; f and f_0 are respectively the fluorescence intensities with and without quencher added.

This expression applies only to dilute solutions, since no account is taken of the existence of encounters containing more than one quencher molecule.

k_f is of the order 10^8 and is presumed to be independent of the composition of the paraffin mixtures used as solvents. Units of time are taken as seconds, and of concentration as moles/l.

K, the equilibrium constant for the formation of encounters, is of the order of unity if the molecules are equal-sized spheres randomly arranged on a close-packed hexagonal lattice. For real solutions, no large change is expected if excited and quencher molecules show no strong preferential interaction. Since $K = k_1/k_2$, k_1 and k_2 are of the same order and depend similarly on the mean diffusion coefficient D of the interacting species. They are approximately $10^{15}D$, which for common organic solvents ($\eta \sim 0{\cdot}01$ poise) is about 10^{10} (*idem, loc. cit.*; $k_1 = k_e$); k_1 and k_2 are not expected to differ greatly for various quenchers, because diffusion coefficients are not sensitive to small changes in the size of molecules.

Equation (1) may be further reduced for comparison with experimental values of the quenching constant k defined by $k = (f_0/f - 1)/[Q]$. As $[Q]$ approaches zero, k approaches k_0, given by

$$k_0 = k_3'\,(k_1' + K)/(1 + k_2' + k_3') \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The application of this equation to a number of quenchers of the fluorescence of anthracene is shown in Fig. 1, *a* and *b*, Fig. 1*b* being an enlargement of that part of Fig. 1*a*

FIG. 1*a*.

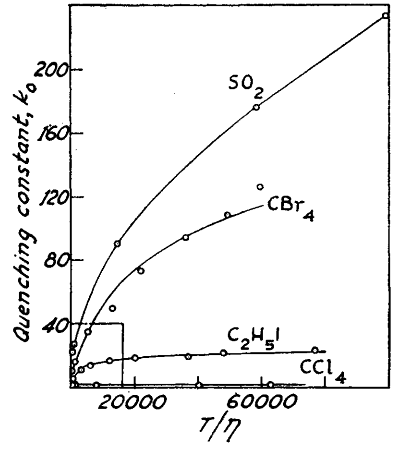
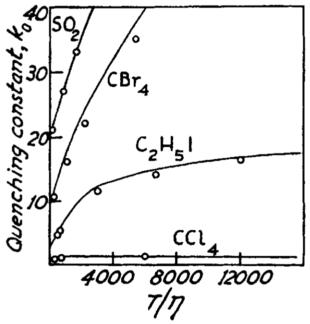


FIG. 1*b*.



shown at the bottom left-hand corner. The points are experimental. The curves are calculated from equation (2), the following values of K and k_3' being used:

	SO_2	CBr_4	C_2H_5I	CCl_4		SO_2	CBr_4	C_2H_5I	CCl_4
K	20	9	3·2	2·5	k_3'	>500	40	7·5	0·44

k_1' is as found for SO_2 at each fluidity (*idem, loc. cit.*) and the same value is used for the other quenchers. The deviations from the predicted values are not greater than can be attributed to this approximation.

For *weak* quenchers such as carbon tetrachloride in light petroleum, the dissociation of encounters is much more probable than the quenching process: $k_2 \gg k_3$. Equation (2) becomes

$$k_0 = k_3'K \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

which contains no terms dependent on the viscosity. As the viscosity is increased by the addition of medicinal paraffin to the solvent, the rates of formation and dissociation of encounters are reduced until k_2 is no longer much larger than k_3. Quenching then becomes dependent on viscosity and at infinite viscosity ($k_1 = k_2 = 0$), the quenching constant falls to

$$k_0 = k_3'K/(1 + k_3') \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

k_3' and K may thus be calculated from the experimental values of k_0 in mixed paraffin solvents. The product $k_3'K$ is obtainable with accuracy from the horizontal part of the curve, but the nature of the equations (3) and (4) and the extrapolation to infinite viscosity allow considerable latitude in the separate values of k_3' and K.

For *strong* quenchers of the fluorescence of anthracene such as oxygen or sulphur dioxide, quenching is more probable than the dissociation of encounters at all accessible viscosities : $k_3 \gg k_2$. Hence

$$k_0 = (k_1' + K)k_3'/(1 + k_3')$$

At infinite viscosity ($k_1' = 0$)

$$k_0 = Kk_3'/(1 + k_3')$$

If, as is usual in these cases, $k_3' \gg 1$, the term $k_3'/(1 + k_3')$ may be omitted. If the equation is extended to substances other than anthracene this last condition may not always apply.

When $[Q] = 0$, equation (1) reduces to

$$k = k_1' + K + k_1'K[Q]$$

for strong quenchers of anthracene fluorescence ($k_3' \gg 1$ and $k_3 \gg k_2$). This equation has been shown to describe the effect of quencher concentration on the quenching of anthracene fluorescence by carbon tetrabromide (*idem, loc. cit.*) although it is not certain that $k_3 \gg k_2$ in the less viscous solvents.

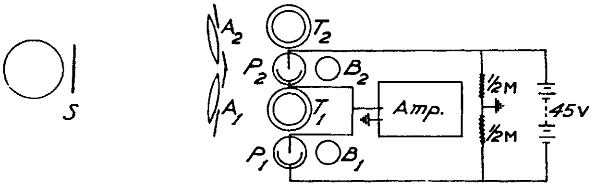
Ethyl iodide is a quencher of *intermediate* type, and its conformity with equation (2) is shown in Fig. 1.

EXPERIMENTAL

The data for sulphur dioxide and carbon tetrabromide at 20° are from Bowen and Metcalf (*loc. cit.*).

The solvents used were mixtures of light petroleum and medicinal paraffin. They were shaken with fuming sulphuric acid until no more brown material was formed. Fluorescent material remaining was removed by a silica-gel column, without a preliminary washing with water which gives troublesome emulsions. The other materials were colourless. Fractional distillation (10 theoretical plates) of the reagent-grade materials gave middle fractions whose quenching power did not differ from that of the combined fractions.

FIG. 2.



The solutions were freed from oxygen by boiling under reflux, and entered the photometer without contact with air. They could be returned for further boiling if necessary. The concentration of anthracene was that at which maximum fluorescence reached the photo-cell, namely, 0·056 g./l. The quenching coefficient $k = (f_0/f - 1)/[Q]$, measured at 18° and over a range of low concentrations of carbon tetrachloride and ethyl iodide, was extrapolated to $[Q] = 0$.

The photometer used is shown in Fig. 2. Solutions whose relative intensities are required are placed in turn in the temperature-controlled tube T_1, and the aperture A_1 is adjusted till the fluorescence intensity matches that of a reference solution in a similar tube T_2. The apertures A_1 are then inversely as the fluorescence intensities at constant illumination.

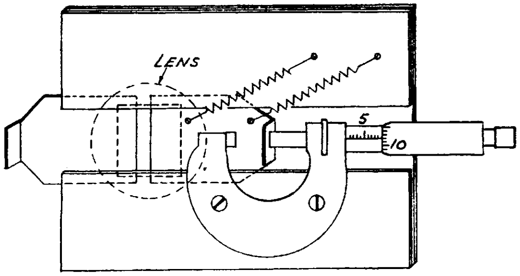
Matching is achieved when the potential at the junction of the photocells P_1 and P_2 (Philips 90 AV) connected in series is at earth potential and does not change when the shutter S is opened, as shown by the D.C. amplifier. Large changes in the intensity of the source do not affect the aperture required for matching if the dark currents are equal, as can be arranged by passing a small adjustable current through one of the bulbs B_1, B_2 near the photo-cells. This adjustment is important because the dark current is frequently larger than the photo-current.

Movement of the arc in the 80 w high-pressure mercury lamp used as source is a serious and frequent cause of irreproducibility. By lateral adjustment of the lenses a position may be found where large movements of the lamp in any direction do not affect the balance. Presumably, the fluorescent images are identically related each to its respective photo-cell, when this adjustment is complete. None of the several other optical arrangements tried permitted such complete compensation of lamp movements.

Fluctuations accompanying arc movements were still noticeable. They were traced to irregularities of the lamp bulb in the respective light paths. By rotation of the lamp a sufficiently uniform portion of the quartz and glass envelopes can usually be found.

The amplifier should be sensitive to about 1 mv, and should be stable to this extent for a few minutes. The input impedance should exceed the dynamic resistance of the photo-cells, which is of the order 10^{10} ohms. Hill's circuit (Dole, " Glass Electrode," Wiley, New York, 1951, p. 58) is satisfactory. An amplifier, based on Nielsen's work (*Rev. Sci. Instr.*, 1947, **18**, 18), also satisfies these requirements, as does the amplifier described by Elmore and Sands (" Electronics," McGraw-Hill, New York, 1949, p. 190). The photo-cells and the first stage of the amplifier require careful insulation, which is improved by drying with silica gel or treating the glass surfaces with silicone fluid.

FIG. 3.



The aperture is controlled by a micrometer, aperture area being proportional to the scale reading. The sensitivity and stability are more than is required to set the micrometer to 0·01 mm. The maximum aperture is 2·5 cm. An aperture of 0·01 mm. corresponds to a photocurrent of about 2×10^{-12} amp. in the present experiments. The optical equivalence of each portion of the scale is tested and the scale is corrected by noting the aperture in various regions of the scale required to balance a fixed aperture in the other beam. The micrometer aperture is constructed to facilitate this test as shown in Fig. 3. (This design permits all edges whose straightness is essential to be cut with a guillotine.)

The spring fitting slider on the left is pushed in and both sliders advanced across the lens face by the micrometer to any desired position. The micrometer may then be reversed to adjust the aperture to balance. Should there by any lack of optical equivalence for various parts of the lens face, a correction table may be prepared, or a strip of thin metal may be shaped and attached to the upper edge of the aperture to make the scale uniform.

The quality of the optical parts is not important, because the calibration above allows for any imperfections in them. The tubes T_1 and T_2 and their water jackets are of ordinary glass tubing rigidly fixed. Light scattered from the source (3650 Å) was removed from the fluorescence by a filter of anthracene in bromobenzene.

The rate of formation of encounters, their equilibrium concentration, and the frequency and efficiency of collisions between nieghbouring molecules are all relevant to the quenching of fluorescence in solution. In favourable cases, some of these quantities can be separately determined. A study of their variation with pressure, temperature, and, in the case of electrolytes, with dielectric constant and ionic strength, may be expected to yield results of considerable interest.

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