QUARTERLY REVIEWS

FLUORESCENCE AND FLUORESCENCE QUENCHING

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The phenomenon of the re-emission of absorbed radiation known as fluorescence was first recognised and scientifically described by Sir George Stokes in the middle of the nineteenth century. The subject has many aspects, and this review is confined to matters connected with the mechanism of the process, to "quenching," and to factors causing a lengthened "half-life."

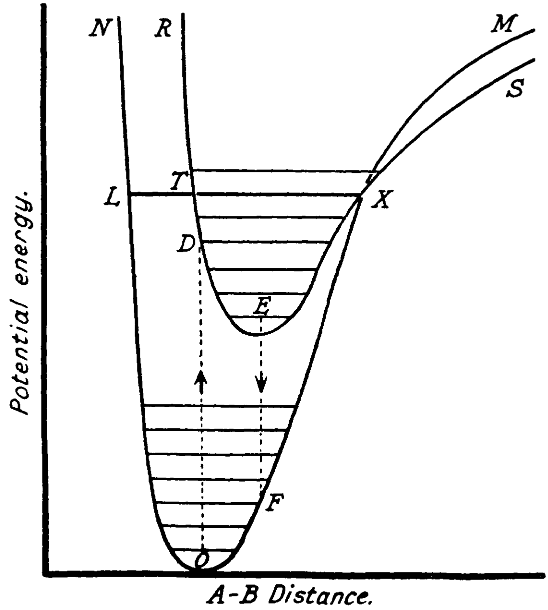

Fig. 1

Potential-energy curves for a diatomic molecule.

According to the ideas of wave-mechanics, the absorption of light is represented as the passage of an electron from one orbital to another of higher energy. Such transitions may be "allowed" or "forbidden," or be intermediate in character, depending on the "transition moment" between the energy states. The reverse process corresponding to the emission of light of the same frequency is subject to the same laws. High transition probabilities are associated with "half-lives" of the upper state before re-radiating of about 10^{-8} second, and low transition probabilities with longer "lives" up to the order of 1 second in extreme cases. Such a simple type of re-radiation, as observed in atomic sodium vapour, for example, is called a "resonance" emission.

The absorption of light by molecules is complicated by the fact that simultaneous changes of electronic, vibrational, and rotational energy occur. This is most easily understood from a "potential energy" diagram for a diatomic molecule. In Fig. 1 a molecule A—B is represented in its lowest or normal electronic state by the curve *NLOFXM*, potential energy of vibration of the two atoms being plotted against their distance apart. At the ordinary temperature practically all the molecules have only the " zero-point " energy of vibration, *i.e.*, are represented by the level at *O*. The curve *RTDEXS* relates to the molecule in an upper electronically excited state. By the Franck–Condon principle, absorption of light by a molecule at *O* gives a most probable transition vertically upwards along *OD*, producing a compressed excited molecule which vibrates with the amplitude shown by the upper curve. Vibrational excitation is thus coupled to the electronic excitation. After a " half-life " of 10^{-8} second or longer the reverse transition *DO* may occur, re-radiating the absorbed quantum by a " resonance " emission. This phenomenon is rarely observed, however, except at very low pressures in the gaseous state, because during the relatively long period of 10^{-8} second the molecule will suffer collisions and may be robbed of its energy. If it so loses *all* its energy it returns to the lower curve by a non-radiational process, the absorbed energy appearing as heat. This is what happens after light absorption by most substances, such as ordinary paints or inks. Certain molecules, however, show a different behaviour, and are capable of *fluorescence.* Examples are aqueous solutions of fluorescent dyes, which, though polyatomic, may be treated qualitatively by Fig. 1. On absorption of light of frequency proportional to the distance *OD*, transitions of molecules to the upper state at *D* occur, the " transition time " being about 10^{-15} second. Having arrived at *D*, the excited molecules vibrate with a period of about 10^{-13} second. In about 10^{-12} second, however, they have collided with water molecules and been robbed of their excess vibrational energy, so that they reach point *E*. The water molecules are unable to take away the electronic energy, and the molecules therefore return to the lower curve after a period of about 10^{-8} second along the vertical line *EF*, re-radiating light whose mean frequency is proportional to the length *EF*. Such a picture of the process enables one to visualise the following matters :

(*a*) The fluorescent light is of smaller frequency and therefore longer wave-length than the absorbed light.

(*b*) The phenomenon of fluorescence is shown only by molecules whose electronically excited state is peculiarly resistant to " deactivating " processes such as collisions.

(*c*) The fluorescence spectrum has a vibrational structure giving the vibrational frequencies of the *lower* state, since the transition *EF* leads to lower-state molecules vibrating in levels represented by the horizontal lines near *F*.

(*d*) The fluorescence spectrum is independent of the wave-length of the absorbed light (within limits), since the fluorescence transition is from *E* downwards whatever higher level such as *D* was reached on light absorption.

Potentially, any molecule is capable of fluorescence or of resonance radiation after it has absorbed a light quantum, and the comparative rarity of such radiational processes must be ascribed to the instability of the excited molecule whereby it loses its electronic energy in a period less than 10^{-8} second, by collisional effects, internal deactivation, or chemical change. Only molecules having a peculiarly resistant, stable, upper electronic level are in fact fluorescent, and these belong to two chief classes :

(1) Ions of transitional elements or of rare earths, and inorganic complexes such as uranyl, UO_2^{++}, or platinocyanide, $Pt(CN)_4^{--}$, where the electron excited by the light is a *d* or *f* electron deep in the atomic shell and not an outer valency electron. Examples are salts of bivalent manganese and of tervalent samarium, europium, dysprosium, and terbium.

(2) Organic compounds with conjugated systems of π electrons, where the excitation of a π electron to a higher orbital has only a small influence on the structure and stability of the molecule. Such are benzene and certain dyes.

Even among molecules in these classes there are great variations in fluorescent power owing to the varying efficiencies of deactivating processes.

In many of the above types of molecule the light absorption process is of an essentially " forbidden " character, owing to the symmetry relations of the electron orbitals.[1] The 2500 A. band of benzene, for example, corresponds to an electronic change in which the hexagonal symmetry of the molecule is unchanged. This is forbidden just as an atomic $s \longrightarrow s$ orbital change is forbidden, since the electric vector of the absorbed light must produce an electronic change along the vector axis as in the change $s \longrightarrow p$. The band becomes allowed in absorption when a quantum of E_g^+ vibration is simultaneously excited, this providing the necessary asymmetry in a linear direction. Since the hexagon expands uniformly as a whole on light absorption the Franck–Condon principle indicates that A_{1g} framework vibrations are also excited, *i.e.*, those corresponding to totally symmetrical movements of the carbon atoms outwards and inwards. Fig. 2 is an energy-level diagram for benzene relating the absorption and fluorescence bands to energy-level changes. The transition from the lowest vibrational level 1 of the ground state to level 1 of the excited state is forbidden ; the excited molecule must be on level 2, with one quantum of E_g^+ vibration, or on levels 3, 4, 5, . . . with additional quanta of A_{1g} vibration. Similarly, on fluorescence, the downward transitions are $1 \longrightarrow 2, 3, 4, 5, \ldots$ and not $1 \longrightarrow 1$. At first sight the absorption and fluorescence bands shown at the top of the figure appear to be mirror images of each other. This approximation is due to the small differences in vibrational frequencies between lower and upper electronic states. The E_g^+ vibration, equalling the distance between levels 1 and 2, is 606 cm.$^{-1}$ for the lower and 520 cm.$^{-1}$ for the upper state, and the A_{1g} vibration, corresponding to the distances 2—3, 3—4, . . . is 992 cm.$^{-1}$ for the lower and 923 cm.$^{-1}$ for the upper

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[1] A. L. Sklar *et al.*, *J. Chem. Physics*, 1937, **5**, 669 ; 1939, **7**, 207. See E. J. Bowen, " The Chemical Aspects of Light," 2nd edition, Clarendon Press, 1946, Chap. III ; and *Ann. Reports*, 1943, **40**, 12.

state. A small proportion of the molecules at ordinary temperatures may start from the 2 level of the ground state on absorption or return from

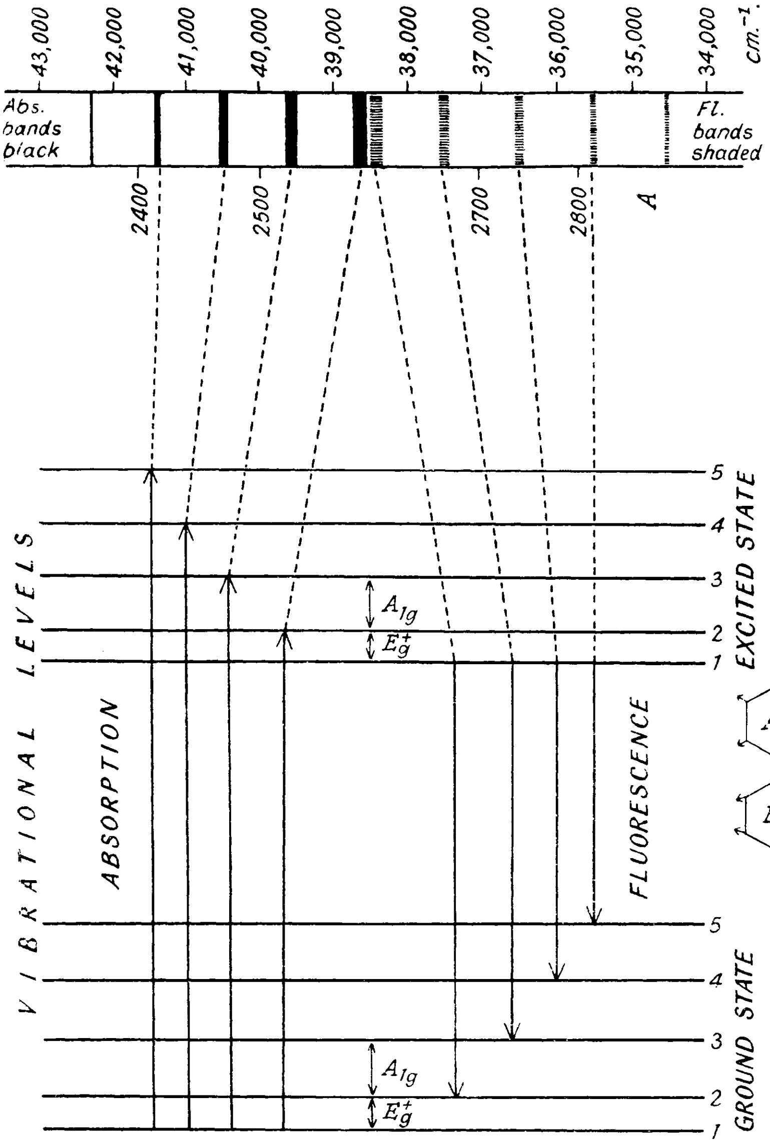

FIG. 2

Absorption and fluorescence bands of benzene, and vibrational transitions.

the 2 level of the excited state on fluorescence. This produces faint temperature-dependent bands in both processes and causes some degree of overlapping of absorption and fluorescence bands in the region where they approach one another.

Banded absorption and fluorescence spectra whose interpretation must be based on the principles described above are shown by many aromatic hydrocarbons and by uranyl salts. Most fluorescent dyes, however, show rather narrow structureless bands, often with considerable overlap between absorption and emission. Here the highly conjugated nature of the molecule leads to very small change of atomic distances on excitation. Interpreted on the simplified diagram Fig. 1, point E is almost vertically over point O. Very little " Franck–Condon " associated vibration accompanies the orbital changes, and the band width is due to the " detuning " of molecules from their ideal frequencies by the effects of their variable environments in solution.

As mentioned above, the mean life of a molecule in the gaseous or liquid state between absorption and emission is normally about 10^{-8} second. This short time interval can be measured by the use of a Kerr cell technique [2] or deduced from the depolarisation of fluorescence caused by molecular rotations.[3] In certain crystalline systems such as the well-known sulphide phosphors much longer time intervals are found.[4] The behaviour of these substances is not dealt with in this report, owing to limitations of space, but mention must be made of lengthened periods of light emission sometimes observed in liquid systems, reaching 10^{-3} and even 1 second. This phenomenon is observed sometimes at the ordinary temperature in organic crystals, as tetraphenylmethane,[5] sometimes in moderately concentrated dye solutions,[6] and sometimes in dye solutions at low temperatures, particularly in very viscous media.[7] There seem to be two distinguishable types of effect. In the first the long-life emission band is almost but not precisely identical with the fluorescence band, and is observed only in moderately concentrated solutions. The fluorescent dye molecules here form dimers or polymers,[8] an effect which largely quenches fluorescence, but the electronic energy of a small fraction of the excited molecules survives a long interval of " resonance " within the polymer and is ultimately re-radiated. This behaviour approximates to the " exciton " processes in crystals mentioned below, and probably explains

[2] E. Gaviola, *Z. Physik*, 1927, **42,** 853, 862; 1928, **48,** 397; W. Szymanowski, *ibid.*, 1935, **95,** 440; W. Kirchoff, *ibid.*, 1940, **116,** 115.

[3] F. Perrin, *Ann. Physique*, 1929, **12,** 169; *J. Phys. Radium*, 1926, **7,** 390; S. Lewschin, *Z. Physik*, 1925, **32,** 307; L. Grisebach, *ibid.*, 1936, **101,** 13; P. Pringsheim and H. Vogels, *J. Phys. Radium*, 1937, **8,** 121; S. Mitra, *Indian J. Physics*, 1939, **13,** 349; D. C. Chakravarti and S. C. Ganguly, *Trans. Faraday Soc.*, 1941, **37,** 562.

[4] J. T. Randall *et al.*, *Proc. Roy. Soc.*, 1945, *A*, **184,** 347—433.

[5] D. Clapp, *J. Amer. Chem. Soc.*, 1939, **61,** 523.

[6] H. Kautsky and H. Merkel, *Naturwiss.*, 1939, **27,** 195.

[7] P. Pringsheim, " Fluorescenz und Phosphorescenz," Springer, Berlin, 1928, p. 230; G. N. Lewis *et al.*, *J. Amer. Chem. Soc.*, 1941, **63,** 3005; 1944, **66,** 2100; 1945, **67,** 994, 1228, 1232; A. Jablonski, *Z. Physik*, 1931, **73,** 460; 1935, **94,** 38; **95,** 53; **96,** 263. See also *Kolloid-Z.*, 1944, **108,** 30.

[8] B. Söderborg, *Ann. Physik*, 1913, **41,** 381; E. Rabinowitsch and L. Epstein, *J. Amer. Chem. Soc.*, 1941, **63,** 69; T. Vickerstaff and D. R. Lemin, *Nature*, 1946, **157,** 373.

"sensitised fluorescence" in solution.[9] In the second type longer-wave emission bands are sometimes observed in addition to the normal fluorescence bands, both of lengthened duration. Such a behaviour of the cationic form of fluorescein has recently been examined,[7] and its luminescence interpreted by the electronic energy-level diagram of Fig. 3. At ordinary temperatures fluorescein dissolved in syrupy phosphoric acid, boric acid glass, or partly dehydrated aluminium sulphate shows a blue fluorescence, represented in Fig. 3 as the transition: level $F \longrightarrow$ ground level G, following light absorption $G \longrightarrow F$. At low temperatures, however, the blue fluorescence weakens and is replaced by a longer-lived green luminescence represented by a transition to G from a level P below F. The blue fluorescence remaining also grows longer-lived. The long life is explained by the assumption that the $P \longrightarrow G$ transition with radiation is a practically "forbidden" one, and the fact that the blue bands are very temperature-sensitive and the green bands much less so by the assumption of $F \longrightarrow P$ and $P \longrightarrow F$ transitions, the latter being caused by heat energy. It is further concluded that while the $G \longrightarrow F$ change is only an orbital shift of an electron between "singlet" (*i.e.*, entirely spin-paired) molecular states, the change to P involves uncoupling of electron spins as well, *i.e.*, the P level is a "triplet" state. This hypothesis was verified by the experimental proof that when P states were present in the system it became paramagnetic.

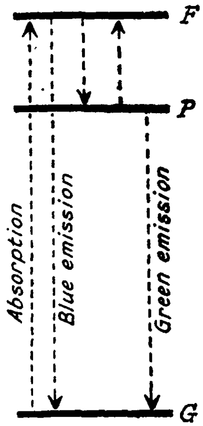

FIG 3.

Energy-level diagram of fluorescence and phosphorescence of acid fluorescein.

The term *phosphorescence* has often been used for long-life luminescence phenomenon. F. Perrin [3] has advocated the restriction of this expression to processes involving a *metastable* state. If this is accepted, the quick return from level F to level G in Fig. 3 would be called normal *fluorescence*, the same change after one or more transitions between F and P *phosphorescence*, and the $P \longrightarrow G$ transition *fluorescence of long duration.* The distinction between fluorescence and phosphorescence is then based, not on the absorption–emission time interval, but on the temperature-dependence of this quantity. Fluorescence "half-lives" are little dependent on temperature (the $P \longrightarrow G$ transition, of course, has a different "half-life" from the $F \longrightarrow G$ transition); phosphorescence "half-lives" decrease with temperature rise owing to the speeding up of $P \longrightarrow F$ transitions. When the levels P and F are near together the distinction becomes blurred. Delayed emission due to polymer formation might be included in *phosphorescence,* while an "exciton" process in a crystal would probably not.

The effect of certain added substances in extinguishing or "quenching" the fluorescence of solutions was noticed over fifty years ago,[10] and in spite

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[9] F. Perrin and (Mlle.) Choucrou, *Compt. rend.*, 1929, **189**, 1213; 1927, **184**, 1097.

[10] E. Buckingham, *Z. physikal. Chem.*, 1894, **14**, 129; G. S. Schmidt, *Ann. Physik*, 1896, **58**, 101; I. Pinnow, *J. pr. Chem.*, 1901, **63**, 239; 1902, **66**, 265.

of close study much remains to be done before the phenomenon can be said to be understood in detail. There are three types of quenching processes to be expected, *internal*, *external* or *collisional*, and *inner filter* or *compound* type.

Internal quenching may be illustrated by Fig. 1. For carbon compounds the curves *NOXM* and *REXS* approach the same asymptote ; consequently, they must " cross " at some point *X*. According to the principles of wave-mechanics, at such a " crossing point," or even at a point of near approach, the potential-energy diagram becomes " blurred," since the " vibrational " and " electronic " energies of the molecule cannot be separated from one another. This, however, does not seriously affect the conclusions to be drawn from a simple mechanical interpretation of the curves. A " vibrating " molecule can be represented by a hypothetical " marble " oscillating in the troughs. If *X* is not too high above *E*, the excited molecule, which is at *E*, may acquire thermal energy before it loses energy by fluorescence enough to raise it to *X*. The " vibrations " in the hollow *TEX* may then change over to ones in the hollow *LOX*, *i.e.*, the electronic energy may pass over into vibrational energy of the lower state, which is quickly dissipated by collisions. Such a process would explain fluorescence efficiencies of less than unity for isolated molecules ; additional quenching by foreign molecules (*e.g.*, of solvent) would be attributed either to a lowering of point *X* towards *E* by the greater distortion of the upper curve than the lower by molecular interactions or to an increased transition probability from one curve to the other. This type of quenching would have a positive temperature coefficient. A temperature-independent type might arise if point *D* were above *X*. As the newly-excited molecule at *D* loses energy by collision and moves towards *E* it passes the level *X* once and there has a fixed chance of changing to the lower curve.

External quenching is represented in Fig. 4, where potential energy is plotted against distance apart of the fluorescent and a quencher molecule. The curve *ABC* relates to the interaction of the quencher molecule with the unexcited fluorescent molecule, and *ADE* to the interaction with the excited molecule. If the curves follow the dotted lines, *i.e.*, if the molecular interactions are wholly repulsional, quenching may be pictured as the approach of two molecules from a great distance *E* up to the region *A*, followed by a change to the lower curve and separation of the molecules with extra kinetic energy corresponding to the difference of level between *E* and *C*. It is doubtful, however, whether collisional quenching ever occurs by so simple a process in solution.[22] Fluorescent molecules are usually quenched by other molecules only when strong van der Waals forces are acting between them. These forces may be either dipole attractions or " dispersion " forces.[17, 18] The latter are second-order effects predicted by wave-mechanics, giving attractions between non-polar orbitals owing to the rapid time-variation of the wave-functions, and are peculiarly powerful between molecules with the easily polarisable π electrons which characterise both fluorescent and quencher molecules. With such forces operating, the interaction curves of Fig. 4 assume the forms *AHBC* and

AGDE with shallow minima of potential energy. The various ways in which quenching may occur under these conditions are discussed below.

The most illuminating results on the mechanism of " external " quenching are obtained from the observed influence of temperature. For example, dilute solutions of rubrene in hexane emit one quantum of fluorescent light for each quantum of absorbed light, independently of temperature, while solutions in other aliphatic solvents such as acetone approach this efficiency only at low temperatures.[26] Whether this " solvent quenching " is " internal," due to solvent effects on the " crossing " of the potential-energy curves of the rubrene molecule, or " external," due to collisional effects, cannot easily be determined, but it appears that the " energy barriers " of quenching determined from the temperature coefficients are not very different for a number of solvents with oxygen in the molecule. Solutions in benzene, toluene, or xylene, however, show a less steep temperature-dependence, and the limiting value of the fluorescence at low temperatures does not reach 100% efficiency. This behaviour indicates that " dispersion force " van der Waals compounds are formed between rubrene and other aromatic hydrocarbon molecules ; these complexes share in the light absorption and, being non- or feebly fluorescent, produce the effect of quenching. Such " inner-filter " quenching is not recognisable in these instances by any gross changes in the absorption spectrum. Additions of naphthalene or diphenyl to rubrene solutions also cause fluorescence quenching which is less marked at higher temperatures, in accordance with the above hypothesis of a temperature-dependent equilibrium of complexes in the solution. Nitro-compounds exhibit this effect still more markedly.

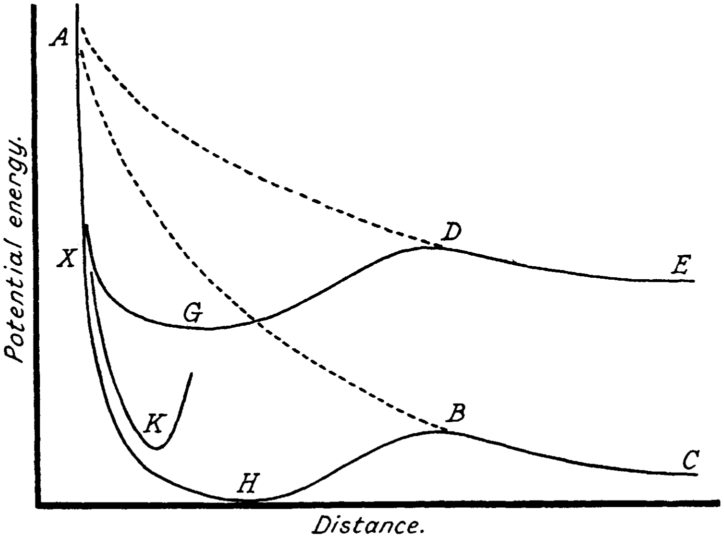

FIG. 4

" *External* " *quenching.*

Nitrobenzene added to a hexane solution of rubrene quenches the fluorescence, more strongly at low temperatures than at high, and from the temperature coefficient it may be deduced that the heat of formation of the " dispersion force " complex is 2·5 k.-cals. per mole. This is comparable with values found for this kind of linking in other ways.[18] An interesting stereochemical effect has been observed here.[26] Whereas the heats of formation of such compounds between flat hydrocarbons as benzene or anthracene with nitrobenzene, *m*-dinitrobenzene, and *s*-trinitrobenzene increase in that order,[18] the last two substances with rubrene behave as " collisional " and not as " compound " quenchers. The four phenyl side-groups of the rubrene molecule are almost at right angles to the flat naphthacene centre and interfere with the close-packing of the di- and tri-nitro-compounds. Similar quenching due to compound formation characterised by a negative temperature coefficient is found in the influence on the fluorescence of solutions of anthracene by bromobenzene, *p*-dibromobenzene, and aliphatic bromides.[30] Here the heats of complex formation are less than 1 k.-cal. per mole. Interpreted on Fig. 4, this " inner filter " type of quenching is represented as the absorption of light by a van der Waals complex at H, giving an excited complex at G, followed by a change back to the lower curve when the system reaches the region X, and transformation of the energy into thermal energy by the route $XHBC$.

Solutions of fluorescent dyes show " self " (or " concentration ") quenching at strengths above about 1 g./l. because of the above inner-filter effect exaggerated in degree.[11, 19] The van der Waals forces forming the dimers

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[11] F. Perrin, *J. Phys. Radium*, 1926, **7**, 390 ; *Compt. rend.*, 1926, **182**, 929 ; 1927, **184**, 1097, 1121 ; *J. Chim. physique*, 1928, **25**, 531.

[12] M. Privault, *Compt. rend.*, 1927, **184**, 1120.

[13] W. West, R. Müller, and E. Jette, *Proc. Roy. Soc.*, 1928, *A*, **121**, 294, 299.

[14] S. I. Wawilow, *Z. Physik*, 1928, **50**, 52 ; 1929, **53**, 665 ; A. W. Banów, *ibid.*, 1929, **58**, 811 ; 1930, **64**, 121 ; K. Weber, *Z. physikal. Chem.*, 1931, *B*, **15**, 18 ; 1932, *B*, **19**, 22 ; 1933, *B*, **24**, 68 ; J. Eisenbrand, *Z. physikal. Chem.*, 1933, *B*, **22**, 145 ; G. Kortüm, *ibid.*, 1938, *B*, **40**, 431.

[15] F. Weigert, *Naturwiss.*, 1927, **15**, 124 ; E. J. Bowen, *Trans. Faraday Soc.*, 1939, **35**, 15.

[16] J. Weiss, *Z. physikal. Chem.*, 1936, *B*, **32**, 135 ; *Trans. Faraday Soc.*, 1938, **34**, 451 ; 1946, **42**, 133 ; M. G. Evans, *ibid.*, p. 101.

[17] F. London, *ibid.*, 1937, **32**, 8 ; S. Wawilow, *J. Phys. U.S.S.R.*, 1943, 141.

[18] G. Briegleb, *Z. physikal. Chem.*, 1932, *B*, **19**, 255 ; 1934, **26**, 63 ; 1935, **27**, 474 ; 1935, **31**, 58 ; 1936, **32**, 305.

[19] W. Lewschin, *Z. Physik*, 1931, **72**, 368, 382 ; *Acta Physicochim. U.R.S.S.*, 1935, **1**, 685 ; **2**, 331.

[20] G. Schiebe, A. Schöntag, and F. Katheder, *Naturwiss.*, 1939, **27**, 449.

[21] E. J. Bowen and A. Norton, *Trans. Faraday Soc.*, 1939, **35**, 44 ; E. J. Bowen and A. H. Williams, *ibid.*, p. 765 ; H. Weil-Malherbe and J. Weiss, *Nature*, 1942, **149**, 471 ; J. A. Miller and C. A. Baumann, *J. Amer. Chem. Soc.*, 1943, **65**, 1540.

[22] J. Franck and H. Levi, *Z. physikal. Chem.*, 1934, *B*, **27**, 409.

[23] E. Schneider, *ibid.*, 1935, *B*, **28**, 311 ; W. West and W. Miller, *J. Chem. Physics*, 1940, **8**, 849.

[24] E. J. Bowen and F. Steadman, *J.*, 1934, 1098 ; W. Koblitz and H. Schumacher, *Z. physikal. Chem.*, 1937, *B*, **35**, 11.

[25] J. Franck and R. Livingston, *J. Chem. Physics*, 1941, **9**, 184.

(and higher polymers) are dispersion forces and not ordinary dipole attractions, since self-quenching of dyes is found to be greater (and not less) in solvents of higher dielectric constant. The forces are so strong that easily noticeable changes in absorption spectrum occur between dilute and concentrated solutions,[8] which means that on Fig. 4 the height *GH* differs from *CE*. Roughly speaking, we may say that the monomeric dye is fluorescent and the dimer non-fluorescent. The addition of salts to an aqueous dye solution may cause quenching by the " salting out " effect favouring polymerisation.[14] Sometimes the dimer may retain some power of fluorescence emission. The substance φ-*iso*cyanine behaves very remarkably in solution. Alcoholic solutions are monomeric, and aqueous solutions dimeric, both showing fluorescence.[20] In concentrated aqueous solution the dye becomes colloidal with its molecules stacked like coins into long chains. The polymer has an extremely narrow absorption band and a fluorescence emission almost coincident with it, the fluorescence being inhibited by exceedingly small concentrations of quenchers. It appears to behave as a single " optical unit," so that its excitation energy can flow freely throughout the complex by a " resonance " effect. More remains to be discovered of the conditions favouring quenching or resonance effects with radiation in dye aggregates.

In contrast to the above " inner-filter " type of quenching is the " collisional " type showing a *positive* temperature coefficient.[11], [27], [28], [29], [30] Collisions in liquids differ from those in gases in being " repeated," and a distinction must be drawn between " encounter frequency " and " collisional frequency." As in many cases of quenching the actual molecular process is very efficient, occurring after one or a very few collisions, rates of quenching must be related to " encounter " and not to " collisional " frequencies, and the former vary as the temperature and inversely as the viscosity. Diffusion of the molecules together is the slow stage which determines quenching rates. The mathematics of this subject have been treated by several authors,[27], [28], [29] and prove very difficult of solution. Two complications arise. Where ions are concerned, as in the quenching of quinine solutions by iodide ions, electrical interactions must be allowed for. A more serious complication is in the solution of the diffusion equations themselves. The calculation of the encounter rate starts from the diffusional equation of Smoluchowski,[32] but it is not correct to assume the usual stationary state condition owing to the disturbance of this by the

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[26] E. J. Bowen and E. Coates, *J.*, 1947, 105.

[27] S. Wawilow and J. Franck, *Z. Physik*, 1931, **69**, 100 ; S. Wawilow, *Compt. rend. Acad. Sci. U.R.S.S.*, 1944, **45**, 7.

[28] B. Svesnikow, *Acta Physicochim. U.R.S.S.*, 1935, **3**, 257 ; 1937, **7**, 755 ; R. W. Stoughton and G. K. Rollefson, *J. Amer. Chem. Soc.*, 1939, **61**, 2634 ; 1940, **62**, 2264 ; 1941, **63**, 1517 ; H. Davis and V. K. La Mer, *J. Chem. Physics*, 1942, **10**, 585 ; P. Debye, *Trans. Electrochem. Soc.*, 1942, **82**, 265.

[29] J. Q. Umberger and V. K. La Mer, *J. Amer. Chem. Soc.*, 1945, **67**, 1099 ; E. W. Montroll, *J. Chem. Physics*, 1946, **14**, 202.

[30] E. J. Bowen, A. W. Barnes, and P. Holliday, *Trans. Faraday Soc.*, 1947, to appear.

[31] J. Franck and E. Teller, *J. Chem. Physics*, 1938, **6**, 861.

[32] M. von Smoluchowski, *Z. physikal. Chem.*, 1917, **92**, 129.

process of quenching itself. An approximate solution for neutral molecules is :

$$\frac{F_0 - F}{F} = 3PV[Q]\left(\frac{Dt}{R^2} + \frac{\sqrt{Dt}}{R}\right)$$

where F_0 = fluorescence intensity *without* and F *with* quencher in the solution ;
P = probability of quenching occurring during an encounter ;
V = volume in litres per mole of the " quenching sphere," $= 4\pi R^3 N/3000$;
$[Q]$ = quencher concentration in moles per litre ;
D = diffusional constant $= \frac{kT}{6\pi\eta}\left\{\frac{1}{r_a} + \frac{1}{r_b}\right\}$, Stokes's law being assumed ;
t = half-life of fluorescent molecule, $= 10^{-8}$ second approx. ;
R = sum of radii of fluorescent and quencher molecules $= r_a + r_b$ = about 7 A. ;
η = viscosity of the solution ;
k = Boltzmann's constant ;
T = absolute temperature ;
N = Avogadro's number.

The quenching is thus dependent on two terms, the first proportional to T/η, and representing " stationary state " diffusion, and the second to $(T/\eta)^{\frac{1}{2}}$, which approximately corrects for the " non-stationary " conditions.

This equation is found to give very good agreement with results for anthracene solutions quenched by a number of substances over a range of temperature, and enables values of P to be obtained.[30] As shown in the table, such values are near to unity.

Quencher.	*P.*	Quencher.	*P.*
		(*a*) *Solvent, toluene*	
Oxygen	0·93	Bromoform	0·28
Carbon tetrabromide	0·66	Anthracene	0·25
Carbon tetrachloride	0·43	Acetylene tetrabromide	0·16
Solvent.	*P.*	Solvent.	*P.*
		(*b*) *Quencher, carbon tetrachloride*	
Ether	0·16	Benzene	0·04
Dioxan	0·09	Xylene	0·037
Acetone	0·06	*cyclo*Hexane	0·0097
Chlorobenzene	0·04	Hexane	0·005
		(*c*) *Quencher, anthracene* (*self-quenching*)	
*cyclo*Hexane	0·46	Acetone	0·22
Hexane	0·40	Xylene	0·21
Ether	0·28	Dioxan	0·16
Benzene	0·28	Chlorobenzene	0·16

The orders of the solvents in sections (*b*) and (*c*) make a significant comparison. With carbon tetrachloride as a quencher the more polar solvents give high P values, which may be attributed to their polarising effect on the molecules. With anthracene acting as its own quencher, however, the aliphatic hydrocarbons give the highest P values, and the lower values for the other solvents may reasonably be associated with their van der Waals interactions with anthracene with a consequent " blanketing " effect.

The above considerations emphasise the idea that fluorescence quenching is always due to strong molecular interactions. A further illustration of this is found for rubrene solutions.[26] The liquid system acetone–chloroform shows a well-known vapour-pressure minimum due to peculiarly strong interactions between these two molecules. Dilute rubrene solutions show a stronger fluorescence in mixtures of these liquids than in either separately. In the mixture, of course, there will be less van der Waals force " left over " to interact with the rubrene molecules. It appears probable that the well-marked experimental distinction between " compound " and " collisional " quenchers with opposite temperature coefficients may not represent a real difference of mechanism. If the average life of the " complex " is long, it will itself absorb light and " compound " quenching will appear ; but if it is very short the diffusional " collisional " type will result. The precise nature of the quenching processes occurring during the conjunction of the two molecules still remains obscure, and it is doubtful if it is profitable to attempt to distinguish between " internal " and " external " mechanisms as formally treated above.

The quenching of fluorescence in solution is often accompanied by photochemical reaction, as, for example, in the formation of dianthracene on illumination of concentrated anthracene solutions.[15] A number of investigations have been made upon the connection between the two processes. On the simple scheme :

$$\begin{aligned}
&\mathrm{A} + h\nu_1 \longrightarrow \mathrm{A}^* \\
&\mathrm{A}^* \longrightarrow \mathrm{A} + h\nu_2 \text{ (fluorescence)} \\
&\mathrm{A}^* + \mathrm{B} \longrightarrow \text{products (photochemical action)}
\end{aligned}$$

the quantum efficiency of product formation is $\frac{k[\mathrm{B}]}{1 + k[\mathrm{B}]}$ and of fluorescence $\frac{1}{1 + k[\mathrm{B}]}$.

There is no system which is known to exhibit this ideal behaviour exactly. Photochemical change involves a switch-over of the system to a new potential-energy curve corresponding to the product, *e.g.*, from curve *AGDE* of Fig. 4 to curve *K*. Consequently, even in the simplest case, photochemical reaction might be expected to be a secondary process and additional to the other modes of quenching discussed. In practice it is found that the photo-reactions of fluorescent substances are usually by no means simple. For example, the quenching by ethyl iodide of the fluorescence of naphthalene solutions, accompanied by photochemical liberation of iodine, seems to require the more complex mechanism :[23]

$$\begin{aligned}
&N + h\nu_1 \longrightarrow N^* \\
&N^* \longrightarrow N \\
&N^* \longrightarrow N' \\
&N^* \longrightarrow N + h\nu_2 \\
&N^* + E \longrightarrow N + \text{products} \\
&N' + E \longrightarrow N + \text{products} \\
&N' \longrightarrow N
\end{aligned}$$

where N = naphthalene and E = ethyl iodide. The second excited level N' may possibly represent the triplet excited level of the molecule. Here, more molecules react photochemically than are potentially able to fluoresce. The reverse is true for many systems of aromatic hydrocarbons in solution containing dissolved oxygen. The fluorescence of naphthalene solutions is strongly quenched by oxygen,[21] but scarcely any photo-oxidation occurs. With benzene the photo-oxidation is of the same order of magnitude as the quenching, but with anthracene the photo-oxidation is small and almost independent of the oxygen concentration, in contrast to the kinetics of the fluorescence quenching. Some measure of general agreement with experimental results might be attained by modifying the above scheme (N = a hydrocarbon, E = a quenching molecule) by writing

$$\begin{aligned}
&N^* + E \longrightarrow N + \textit{either}\ \text{products}\ \textit{or}\ \text{quenching only} \\
&N' + E \longrightarrow N + \textit{either}\ \text{products}\ \textit{or}\ \text{quenching only}
\end{aligned}$$

and adding

$$N' \longrightarrow N^*$$

That no simple scheme will cover the process of hydrocarbon photo-oxidation is shown by work on rubrene solutions.[24], [21] This red fluorescent hydrocarbon (R) takes up oxygen in solution photochemically to give a stable colourless peroxide RO_2. The kinetics, however, are very complex. The fluorescence quenching can be represented by the normal expression :

$$\text{Fluorescence efficiency} = \frac{1}{1 + k_1[O_2] + k_2[R]}$$

but the quantum efficiency of oxidation requires a totally different form approximating to $\dfrac{[R][O_2]}{1 + k[R][O_2]}$. A more accurate expression for the latter has been given as

$$\text{Quantum efficiency} = \frac{[R][O_2]}{\left\{\dfrac{k_2}{k_3} + [R] + \dfrac{k_4}{k_3}[O_2]\right\}\left\{\dfrac{k'_3}{k_5} + [O_2]\right\}} + \frac{[R][O_2]}{\left\{\dfrac{k_2}{k_4} + \dfrac{k_3}{k_4}[R] + [O_2]\right\}\left\{\dfrac{k_6}{k_7} + [R]\right\}}$$

derived from the hypothetical mechanism :

(1) $R + h\nu_1 \longrightarrow R^*$
(2) $R^* \longrightarrow R + h\nu_2$
(3) $R^* + R \longrightarrow R + R_1'$
(3′) $R' \longrightarrow R + h\nu_3$
(4) $R^* + O_2 \longrightarrow RO_2^*$
(5) $R' + O_2 \longrightarrow RO_2$
(6) $RO_2^* \longrightarrow R + O_2$
(7) $RO_2^* + R \longrightarrow RO_2 + R$

Owing to the difficulty of obtaining sufficiently accurate data in work of this kind it cannot be said that such a mechanism is exact in every detail. Interesting matters for further investigation are the (possibly triplet) nature of the assumed species R′ and the possibility of the dimeric form R_2 also playing a part.

The chemical effects above mentioned are closely connected with photosensitised reactions which have long been known to be brought about by light absorption in fluorescent solutions. Very complex relationships often appear between fluorescence quenching and photosensitisation.[25] Solutions of fluorescent substances such as rubrene or dyes in presence of dissolved oxygen and a reducing agent, when illuminated, may show bleaching, reversible or irreversible, or may themselves remain almost unchanged and bring about oxidation of the reducing agent, reversible or irreversible. The primary chemical reactions occurring may be " electron transfer " mechanisms.[16] Molecules which quench fluorescence of dyes strongly are often reducing agents (electron acceptors), such as aminophenol,[12] or substances with easily polarisable electronic shells, such as I^- or Br^-.[13] The quenching of the fluorescence of solutions of thionine by ferrous ions affords a particularly simple instance of an electron-transfer mechanism. The excited dye molecule receives an electron from the ferrous ion, giving ferric ion and a negative dye ion, the latter by further reactions with hydrogen ion and another dye molecule going to the leuco-dye. The process reverses itself in the dark. With the ceric ion the excited dye molecule may be robbed of an electron with quenching, giving a positive dye ion. For these processes to occur the quencher electron levels must lie between the levels of the normal and the excited dye molecule ; quenching may then occur either if the excited electron falls into the quencher level, or if the vacated electron orbital in the dye molecule is filled by an electron received from the quencher molecule. In photo-sensitised oxidation reactions mentioned above the primary processes seem to be one or other of these possibilities : either by the oxidising agent removing the excited electron of the dye, or by the acceptor supplying an electron to occupy the vacated dye orbital.[16] The observed complications arise from the subsequent further reactions of the first formed products, which may include peroxides if molecular oxygen is present. Whether the " electron-transfer " mechanism applies to apparently non-polar reactions such as the photodimerisation of anthracene is doubtful.

Reference may lastly be made to the fluorescence of solid solutions. The anthracene–naphthacene system shows an interesting feature. When excited by 3650 A. light, pure crystalline anthracene fluoresces blue-violet. A very small amount of naphthacene, in solid solution in the crystals, even as little as 1 in 10^4, largely suppresses the anthracene emission and changes the fluorescence to the green bands characteristic of the naphthacene.[33] The extinction coefficients show that this cannot be explained as a primary light absorption by the naphthacene molecules, or as a secondary absorption of the anthracene fluorescence. The excitation energy of an anthracene

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[33] E. J. Bowen, *J. Chem. Physics*, 1945, **13**, 306.

molecule after light absorption must be imagined to wander from molecule to molecule in the oriented crystal lattice by a process of resonance (" exciton " process [31]). This further explains why the half-life of the fluorescence of solid anthracene is 10^{-5} second while that of a liquid solution is of the order of 10^{-8} second. If the " exciton " reaches a naphthacene molecule before radiation occurs it is " trapped " by dropping to the lower naphthacene level, and is subsequently re-emitted as the longer-wave fluorescence of that substance. The peculiar sensitivity of the fluorescence of crystals to traces of certain substances in solid solution means that greater caution is required here than for liquid solutions in drawing conclusions of an analytical or purity nature from the observed fluorescence.