151. *Some Observations on the Photochemistry of Fluorescent Substances. Part II. Concentration Quenching (Self-quenching) of Fluorescence.*

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The concentration quenching of several polycyclic hydrocarbons and of ethylchlorophyllide has been investigated in various solvents in an atmosphere of pure nitrogen and in the absence of foreign quenching substances. The primary photochemical process of self-quenching is represented by an interaction between excited and normal molecules, and the hyperbolic quenching equation was found to be valid in all the cases investigated. The relation between self-quenching and the photochemical formation of dimers has been discussed in detail. In photo-sensitised oxidations with ethylchlorophyllide self-quenching effects are of great importance, as the lifetime of the excited chlorophyll molecule is largely dependent on the concentration quenching even in the presence of other quenching substances.

It is well known that many substances show appreciable luminescence only in molecular dispersion, and that concentrated solutions of fluorescent substances often show little or no fluorescence; on the other hand, dilute solutions, and especially those in highly viscous media, show a greatly increased fluorescence yield which sometimes reaches values approaching unity (cf. Vavilov, *Z. Physik*, 1925, **31**, 750; Banow, *ibid.*, 1929, **58**, 811; Lewschin and Vinokurow, *Physikal. Z., U.S.S.R.*, 1936, **10**, 10). In all these cases there is no indication of any appreciable interaction between the molecules of the solvent and those of the fluorescent substance, the absorption spectrum of which is also only slightly changed or not changed at all. This leads to the conclusion that the fluorescence yield depends on an interaction of the molecules of the fluorescent substance with each other.

It is obvious that the phenomenon of self-quenching cannot be due to a reabsorption of fluorescence radiation, because the exciting radiation would be absorbed in a still thinner layer and one should observe surface fluorescence, which is not the case.

The importance of concentration quenching has been emphasised by Pringsheim (*Trans. Faraday Soc.*, 1939, **35**, 31), who showed that it is not due to (i) energy (resonance) transfer between the excited and unexcited molecules, as had been suggested by Perrin (*Ann. Physique*, 1929, **12**, 147), or to (ii) the simple formation of (non-fluorescent) associated molecules, because the lifetime of the excited molecules is in fact shortened.

It is clear that " pure " self-quenching can only be studied in the absence of any other foreign quenching substances such as oxygen (Weil-Malherbe and Weiss, *Nature*, 1942, **149**, 471). This condition has often not been fulfilled in previous work. The experiments now reported on polycyclic hydrocarbons and on ethylchlorophyllide (herein briefly referred to as the " dyes ") were carried out in an atmosphere of pure nitrogen, and it was ascertained that under these conditions the fluorescence efficiency was practically independent of the solvent used (Weil-Malherbe and Weiss, *loc. cit.*).

If fluorescence intensity is plotted against concentration, a characteristic curve is obtained (*idem, ibid.*). At very low concentrations only a small fraction of the incident radiation is absorbed; in this region, absorption increases linearly with increasing concentration and self-quenching is practically absent. Here the fluorescence intensity is proportional to the primarily absorbed light intensity (Beer's law). With increasing concentration the fluorescence intensity reaches a maximum, corresponding to the total (maximum) absorption of the incident irradiation which in all these cases is kept constant throughout an experiment. In the absence of concentration quenching, the fluorescence intensity should therefore remain constant. Actually, the fluorescence yield gradually decreases with increasing concentration of the dye owing to self-quenching. From this part of the curves certain deductions can be drawn about the nature of the self-quenching process, as shown below.

The processes of light absorption and fluorescence emission are described by equations (1) and (2) (Part I, preceding paper). The equation for the self-quenching, in analogy to equation (3) (Part I), is represented by (3*a*) (A = " dye," A* = excited " dye ") :

$$A^* + A \longrightarrow (A^+A^-) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3a)$$

$$\text{(reverse process)} \quad A^+ + A^- \longrightarrow 2A \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

$$\text{(stabilisation reaction)} \quad A^+ + A^- (+ M) \longrightarrow A_2 (+ M) \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

The stabilisation of the primary products leads to the photochemical formation of a dimer (reaction **8**). These equations are also in accord with Weigert's observations (*Naturwiss.*, 1927, **15**, 124) on anthracene solutions, in which the relative decrease of fluorescence is accompanied by an increase in the photochemical formation of dianthracene.

From reactions (1), (2) and (3*a*) one obtains for the fluorescence yield in the stationary state (cf. Weiss, *Trans. Faraday Soc.*, 1939, **35**, 48) :

$$\gamma = I/I_{\text{abs.}} = 1/\{1 + (k_{3a}/k_2)[A]\} \quad . \quad . \quad . \quad (9)$$

where $I_{\text{abs.}}$ and I are, respectively, the absorbed and the fluorescent light intensity, $\tau_0 = 1/k_2$ is the mean lifetime of the excited " dye " in the absence of any external quenching, and k_{3a} the (bimolecular) rate constant of reaction (3*a*). For total absorption of the (constant) primary radiation (*i.e.*, for $I_{\text{abs.}} = I_0 = \text{const.}$) one obtains for the decreasing part of the fluorescence curve the equation

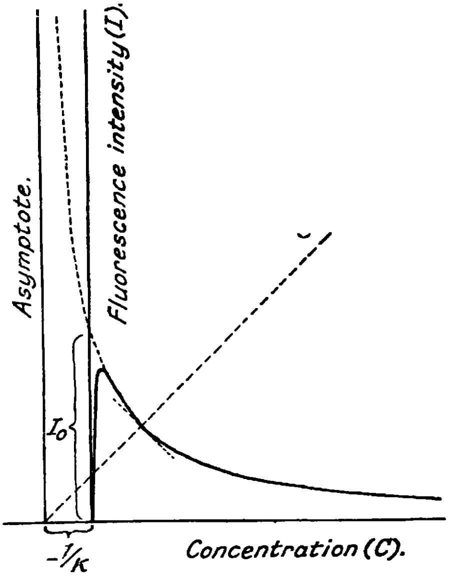
$$\gamma = I/I_0 = 1/(1 + \kappa c) \quad . \quad . \quad . \quad . \quad (10)$$

where $\kappa = \tau_0 k_{3a}$, and [A] is identified with the total concentration (*c*) of the dye, because $\{c - [A^*]\}$ is not very different from the total concentration.

Equation (10) can be written as

$$I\xi = I_0(1/\kappa) \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

where $\xi = c + 1/\kappa$, and this shows that the curve of decreasing fluorescence represents a rectangular hyperbola, one asymptote being the *c*-axis and the other parallel to the *I*-axis at a distance $c_{\xi=0} = -1/\kappa$ from the origin. The constant κ of equation (10) can thus be deduced graphically from the experimentally determined fluorescence curve, as is shown in the figure, which is self-explanatory.



Schematic representation of the graphic determination of the quenching constant (κ) *from the experimentally determined self-quenching hyperbola* (*fully drawn curve*).

The fluorescence intensities I (in arbitrary units) have been measured photoelectrically (see Experimental) for a number of substances at constant intensity of the incident radiation. The results are given in Tables I and II. All experiments were carried out at room temperature (20°).

From the value of κ determined by the above graphical method, the fluorescence yield (γ) can be calculated according to equation (10). These values are given in col. 4 of Table I. In the case of complete and constant absorption of the incident radiation of intensity I_0, these calculated values can be compared with the values derived from I/I_0 (col. 3). The value of I_0 in the same arbitrary units is given by the point of intersection of the hyperbola with the I-axis (see fig.), as follows directly from equation (11). The agreement is in many cases as good as can be expected.

Discussion.—The values of κ for different hydrocarbons and for ethylchlorophyllide are summarised in Table II. Two substances which have been found to have only a small self-quenching effect have also been included. As the mean lifetime of the fluorescent state in all these cases is presumably of the same order of magnitude ($\tau_0 \sim 10^{-8}$ sec.), the values of κ given on p. 546 are proportional to the actual rate constant of the self-quenching reaction (3*a*), as follows from equation (10). Whereas the self-quenching constants (κ) for the polycyclic hydrocarbons are of the order of 100 or smaller, that for ethylchlorophyllide is about 2000 times greater.

The self-quenching equation is essentially the same as the well-known equation of Stern and Volmer, although it is derived from a different physical point of view; it is identical with the exponential relationship proposed by Perrin (*loc. cit.*) and others if κc is small compared with unity, for in this case equation (10) can also be written as :

$$\gamma \sim \varepsilon^{-\kappa c} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

However, there is also some further support for the hyperbolic relationship from the experiments on the photochemical formation of dianthracene and from photosensitised oxidations with ethylchlorophyllide.

TABLE I.

Concentration quenching of hydrocarbons and ethylchlorophyllide.

1. Anthracene in benzene.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 290$.)	(for $\kappa = 60$.)
0·1	28	—	~1
1·0	260	—	0·99
5	270	—	0·97
10	280	0·96	0·94
20	260	0·89	0·87
40	240	0·83	0·81
60	210	0·72	0·74
80	190	0·66	0·68
100	180	0·64	0·63
200	130	0·45	0·45
300	95	0·32	0·36
400	75	0·25	0·29
500	60	0·21	0·25

2. Anthracene in hexane.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 280$.)	(for $\kappa = 100$.)
0·1	19	—	~1·0
1	212	—	0·99
5	237	—	0·95
10	250	0·89	0·91
20	230	0·82	0·83
40	200	0·71	0·71
60	170	0·61	0·63
80	150	0·54	0·56
100	140	0·50	0·50

3. Anthracene in ethanol.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 280$.)	(for $\kappa = 100$.)
0·1	17	—	~1·0
1	175	—	0·99
5	220	—	0·95
10	250	0·90	0·91
20	230	0·82	0·83
40	200	0·71	0·71
60	177	0·63	0·63
80	158	0·56	0·56
100	145	0·52	0·50

4. 1 : 2-Benzanthracene in benzene.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 300$.)	(for $\kappa = 40$.)
0·1	34	—	~1·0
1	206	—	~1·0
5	291	0·97	0·98
10	281	0·94	0·96
20	281	0·94	0·93
40	259	0·86	0·86
60	250	0·83	0·81
80	236	0·78	0·76
100	220	0·73	0·72
200	178	0·59	0·56
300	140	0·47	0·45
500	110	0·36	0·33

5. 9 : 10-Dimethyl-1 : 2-benzanthracene in benzene.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 220$.)	(for $\kappa = 83$.)
0·1	38	—	~1·0
1	205	—	0·99
5	212	0·96	0·96
10	212	0·96	0·92
20	175	0·83	0·85
40	155	0·74	0·75
60	142	0·68	0·67
80	133	0·63	0·60
100	125	0·59	0·54
200	94	0·41	0·38
300	72	0·31	0·28
500	50	0·22	0·19

6. 20-Methylcholanthrene in benzene.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 280$.)	(for $\kappa = 77$.)
0·1	37	—	~1·0
0·3	112	—	~1·0
1	220	—	~1·0
2·5	275	0·98	0·98
5	270	0·97	0·96
10	256	0·92	0·93
25	227	0·81	0·84
50	200	0·71	0·72
100	155	0·55	0·56

7. 3 : 4-Benzpyrene in benzene.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 230$.)	(for $\kappa = 77$.)
0·1	36	—	~1·0
1	174	—	~1·0
5	225	0·97	0·96
10	214	0·93	0·93
20	195	0·85	0·87
40	170	0·74	0·77
60	150	0·65	0·69
80	140	0·61	0·62
100	124	0·54	0·56
200	86	0·37	0·39
300	66	0·27	0·30
500	45	0·20	0·21

8. Ethylchlorophyllide in ethanol.

$c \times 10^4$ (mol./l.).	I.*	γ. Obs. I/I_0.	Calc.
		(for $I_0 = 700$.)	(for $\kappa = 2 \times 10^5$.)
0·01	35	—	0·89
0·05	115	—	0·50
0·075	135	—	0·40
0·1	150 (230) †	0·33 †	0·33
0·2	150	0·21	0·20
0·4	80	0·14	0·11
0·6	50	0·07	0·07
0·8	35	0·05	0·05
1·0	25	0·04	0·04

* In arbitrary units. Expts. 1—5 were carried out under identical conditions and the units are recorded on the same scale. The units of experiments 6, 7, and 8 are not comparable either amongst each other or with those of expts. 1—5.

† Extrapolated.

TABLE II.

Self-quenching constants of various fluorescent substances.

Substance.	Solvent.	Quenching constant, κ.
Ethylchlorophyllide …………	Ethanol	2×10^5
Anthracene …………………………	Benzene	60
Anthracene …………………………	Hexane	100
Anthracene …………………………	Ethanol	100
20-Methylcholanthrene ………	Benzene	77
3 : 4-Benzpyrene …………………………	Benzene	77
9 : 10-Dimethyl-1 : 2-benzanthracene	Benzene	83
1 : 2-Benzanthracene ………………	Benzene	40
Pyrene ………………………………………	Benzene	<10
1 : 2 : 5 : 6-Dibenzanthracene ………	Benzene	<10

The rate of photochemical formation of dianthracene (D) from anthracene (An) is given from equations (1), (2), and (3*a*) for the stationary state $\{[\mathrm{An}^*] = I_{\mathrm{abs.}}/(\bar{k}_2 + \bar{k}_{3a}[\mathrm{An}])$ and the barred constants now refer to anthracene in particular$\}$

$$d[\mathrm{D}]/dt = \bar{k}_{3a}[\mathrm{An}^*][\mathrm{An}] = I_{\mathrm{abs.}}[\mathrm{An}]/\{(\bar{k}_2/\bar{k}_{3a}) + [\mathrm{An}]\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

This equation is identical with that found by Bodenstein (*Z. physikal. Chem.*, 1914, **87**, 93) to represent all the experiments of Luther and Weigert (*ibid.*, 1905, **51**, 297; **53**, 385) and of Weigert and Krüger (*ibid.*, 1913, **85**, 579; 1914, **86**, 383). The value of 10 for $\bar{k}_2/\bar{k}_{3_a}$ given by Bodenstein (*loc. cit.*, p. 97) refers to anthracene in toluene at 105°, concentrations being expressed in millimols./l. The corresponding quenching constant (in mols./l.) is given by $\kappa_{\mathrm{An}} = \bar{k}_{3a}/\bar{k}_2 = 10^{-1} \times 10^3 = 10^2$ which is in good agreement with the value for anthracene derived from quenching experiments (Table II) and also with that derived from the experiments of Bowen and Williams (*loc. cit.*, Table III) and Pringsheim (*loc. cit.*).

In photosensitised oxidations, equations corresponding to an interaction between the excited and the non-excited dye have been used by several authors (cf. Gaffron, *Biochem. Z.*, 1933, **264**, 251; Koblitz and Schuhmacher, *Z. physikal. Chem.*, 1937, *B*, **35**, 11; Bowen and Williams, *loc. cit.*). These were found necessary in order to account for the kinetics of the reaction, although their full significance and their connection with self-quenching has often not been recognised.

A particularly interesting case is afforded by the behaviour of chlorophyll or ethylchlorophyllide. From the fact that in ordinary dilute solutions (10^{-3}—10^{-4} mol./l.) the fluorescence efficiency is very low and not very strongly influenced by either oxygen or reducing agents, it has been concluded that the process determining the lifetime of the excited chlorophyll molecule must be a special one. Franck and Livingston (*J. Chem. Physics*, 1941, **9**, 184) suggested that this was due to a change of the excited chlorophyll molecule into a reactive tautomer. Although this may be possible, it is not essential to explain the striking behaviour of chlorophyll, which can be accounted for quantitatively by its very strong self-quenching effect (Table II). One can easily see that the strong interaction between excited and non-excited chlorophyll molecules determines the lifetime of the excited state. The reaction is given by

$$\mathrm{Chl}^* + \mathrm{Chl} \longrightarrow 2\mathrm{Chl} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

Fluorescence emission, even in solutions of 10^{-3} M, is practically suppressed. In the presence of an acceptor molecule (Acc) a number of the excited chlorophyll molecules will interact according to the general quenching reaction :

$$\mathrm{Chl}^* + \mathrm{Acc} \longrightarrow \mathrm{Chl}^+ + \mathrm{Acc}^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (15)$$

which is the primary process and rate-determining step for the subsequent photosensitised oxidation (Weiss, *Trans. Faraday Soc.*, 1938, **34**, 451; 1939, **35**, 48; Carter and Weiss, *Proc. Roy. Soc.*, 1940, *A*, **174**, 351). In this case the stationary concentration of Chl* is given by

$$[\mathrm{Chl}^*] = I_{\mathrm{abs.}}/(k_{14}[\mathrm{Chl}] + k_{15}[\mathrm{Acc}]) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

and for the rate of the photosensitised oxidation, which is identical with the rate of reaction (15), we have

$$-\,d[\mathrm{Acc}]/dt = k_{15}[\mathrm{Chl}^*][\mathrm{Acc}] = k_{15}I_{\mathrm{abs.}}[\mathrm{Acc}]/(k_{14}[\mathrm{Chl}] + k_{15}[\mathrm{Acc}]) \quad . \quad . \quad . \quad . \quad (17)$$

For the quantum efficiency of the photo-oxidation :

$$\gamma_{\mathrm{ox.}} = -\frac{d[\mathrm{Acc}]}{dt}\left(\frac{1}{I_{\mathrm{abs.}}}\right) = \frac{(k_{15}/k_{14})[\mathrm{Acc}]/[\mathrm{Chl}]}{(k_{15}/k_{14})[\mathrm{Acc}]/[\mathrm{Chl}] + 1} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

This is identical with the empirical equation given by Gaffron (*Ber.*, 1927, **60**, 755) to represent all his results for the acceptor thiosinamine with ethylchlorophyllide as sensitiser in acetone solutions. In this particular case he found $K = (k_{15}/k_{14}) = 0{\cdot}2$, which means that the self-quenching interaction between the ethylchlorophyllide molecules is still 5 times faster than the interaction (15) with the acceptor molecules.

EXPERIMENTAL.

Fluorescence Measurements.—The apparatus used was the direct-reading, one-cell fluorimeter already described (Weil-Malherbe, J., 1943, 303; *Biochem., J.*, 1944, in the press), but modifications in its use were made : the photo-cell ("EEL" selenium barrier layer cell of 45 mm. diameter) was placed in line with the incident beam of filtered ultra-violet light. Such an arrangement is preferable when working with concentrations where the incident light is absorbed in a small depth. The beam of fluorescent light was filtered through a layer of 5% sodium nitrite solution in order to eliminate any scattered ultra-violet radiation, and through a suitable colour filter. Sensitivity of galvanometer deflection was controlled by inserting shutters of various aperture between light source and solution.

All measurements were carried out in purified nitrogen. The gas stream was saturated with the solvent used by passage through a wash-bottle before entering the solution. A zero reading with pure solvent was taken after every reading, and all figures have been corrected accordingly.

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