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## The Dependence of the Intensity of Fluorescence on the Composition of a Fluorescing Solution

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The fluorescence of solutions of fluorescein and acridone has been studied as a function of (1) the concentration of iodide added with a constant concentration of the fluorescer and (2) the concentration of the fluorescent substance. It is shown that the results are fitted much better by the equation of Stern and Volmer than by that of Perrin. In the absence of a quencher the intensity of the fluorescence observed is given by a function of the concentration which takes into account the following three effects: (1) the absorption of the exciting light by the solution, (2) the absorption of the fluorescent light, and (3) the quenching by the molecules of the fluorescent substance. No other effects needed to be considered for the substances studied.

MANY measurements have been made of the dependence of the intensity of fluorescence in solution on the concentration of the fluorescing substance and also on the concentration of various foreign substances which decrease the intensity. The results have been expressed by means of various equations. Bouchard<sup>1</sup> has used an equation proposed by F. Perrin.<sup>2</sup> For solutions of a fluorescent substance alone this equation is

$$I_0/I = e^{ks}, \quad (1)$$

in which  $I$  and  $I_0$  are the intensities of the fluorescence per unit mass of fluorescent substance at concentrations of  $s$  and infinite dilution, respectively, and  $k$  is a constant. If a quencher is also present the equation is written

$$I_0/I = e^{ks+k'c}, \quad (2)$$

in which the  $k'$  is a constant and  $c$  is the concentration of the quencher. Stern and Volmer<sup>3</sup> assumed that the quenching depended only on the number of collisions of the quencher molecules with the photoactivated molecule and derived the equation

$$I_0/I = 1 + kc. \quad (3)$$

This equation did not attempt to account for any change in intensity with change in the concentration of the fluorescing substance. Frank

and Vavilov<sup>4</sup> derived an equation, also dealing only with the action of quenchers, which may be written as

$$I_0/I = (1 + kc)e^{k'c}. \quad (4)$$

The exponential factor is introduced to allow for the quenching action of those molecules which happen to be within the necessary sphere of action at the time that the fluorescing molecule is activated. The other factor is determined by the diffusion of the quencher. At low concentrations of the quencher any of these equations will fit the data, but they yield distinctly different results for high concentrations.

In some earlier papers<sup>5</sup> it has been shown that the action of a quencher is influenced by an increase of ionic strength in the same manner as a second-order reaction. In those papers the Stern and Volmer quenching law was assumed since it can be derived by considering that the fluorescence is a first-order reaction and the quenching a second-order reaction and that the intensity of the observed fluorescence is determined by a competition between these two processes. Since Perrin's equation cannot be derived on such a basis it seemed desirable to measure the quenching over a range of concentrations sufficient to establish the nature of the law and thus determine whether or not the process may be treated as a chemical reaction.

<sup>1</sup> J. Bouchard, *J. Chim. physique* **33**, 51, 127, 232, 325 (1936).

<sup>2</sup> F. Perrin, *Ann. de physique* **12**, 169 (1929).

<sup>3</sup> O. Stern and M. Volmer, *Zeits. f. wiss. Phot.* **19**, 275 (1920).

<sup>4</sup> I. M. Frank and S. I. Vavilov, *Zeits. f. Physik* **69**, 100 (1931).

<sup>5</sup> R. W. Stoughton and G. K. Rollefson, *J. Am. Chem. Soc.* **61**, 2634 (1939); *ibid.* **62**, 2264 (1940); *ibid.* **63**, 1517 (1941).

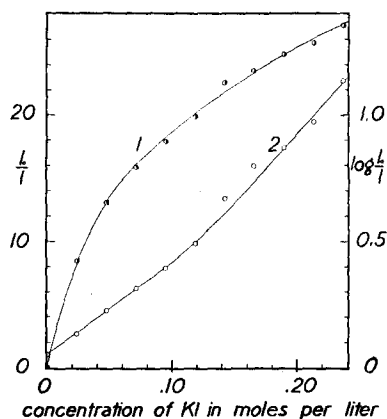


FIG. 1. Test of the quenching laws. Perrin's equation (curve 1) and Stern and Volmer's equation (curve 2).

The experiments which have been performed may be divided into two groups. The first experiments were carried out with a constant concentration of the fluorescing substance and various concentrations of the quencher. The second group was with various concentrations of the fluorescer but no quencher. The apparatus used was essentially the same as has been used in the previous work.<sup>5</sup> For excitation the light from a Hereaus type mercury arc was passed through a Corning 586 filter which transmitted only the group of lines around 3660Å. The photo-cells used in the measurements were shielded from the exciting light by glass filters which absorbed all wave-lengths less than 4000Å.

In order to secure the best test of the quenching equations it is desirable to have as few variables as possible in the system. Since the quenching of the fluorescence of an ion by an ion is markedly affected by changes in the ionic strength it was decided to use acridone as the fluorescing substance in one set of experiments since this is a neutral molecule in solution and it has been shown that the quenching of it by iodide is only slightly affected by large changes in the ionic strength.<sup>5</sup> Another set of experiments was carried out with solutions of fluorescein since the variations of the intensity of the fluorescence with the concentration of the fluorescent substance are quite large in these solutions.

In order to test the various quenching laws stock solutions were prepared of 1.183*M* potassium iodide saturated with acridone and of distilled water saturated with acridone. The solu-

tions needed for the measurements were made by mixing these two in the necessary proportions. The results obtained are represented graphically in Fig. 1. In order to test Eq. (2) we have plotted  $\log I_0/I$  vs. the concentration of the quencher. In this case it is immaterial whether we plot the ratio of the intensities as measured or convert them into specific intensities since the proportionality factor cancels out in taking the ratio. For Eq. (3) the ratio of the intensities is plotted against the concentration of the quencher. If the correct equation has been chosen the plot should be a straight line. It is apparent that the curve corresponding to Eq. (3) (2 in the figure) is a straight line up to a concentration of the quencher which quenches ninety percent of the fluorescence and the deviations are not great at larger concentrations. On the other hand, the curve for Eq. (2) shows a marked deviation from linearity at all concentrations. The deviations from linearity shown by the curve 2 may be due to an effect such as is represented by the exponential factor in Eq. (4) but the accuracy of the measurements when the quenching is large is not sufficient to warrant any decision concerning the validity of that equation. Since iodide is one of the most efficient quenchers for acridone it is to be expected that such a factor would be needed in this case if it is going to be needed in any. The fact that the Stern-Volmer equation fits as well as it does leads to the conclusion that the quenching process is correctly viewed as a bimolecular reaction competing with the monomolecular reaction of fluorescence.

Our second group of experiments was designed to study the factors influencing the intensity of fluorescence in the absence of a quencher. Under these conditions the intensity of the fluorescence will depend on (1) the amount of light absorbed by the solute, (2) the amount of the fluorescent light absorbed by the solution, and (3) the quenching of the fluorescence by the molecules of the fluorescent substance. In order to derive an equation for the intensity of fluorescence which will be observed at some point outside the solution let us consider an experimental arrangement such as is shown in Fig. 2. The solution to be studied is contained in a cell with plane parallel ends, the exciting light enters at

one end, and the fluorescence is observed through the other end. A suitable arrangement of filters removes any of the exciting light which is not absorbed by the solution.

If it is assumed that the exciting light is sufficiently monochromatic so that the absorption by the solution obeys Beer's law, the intensity at any point in the solution is given by

$$I' = I_0' \exp(-k_1 cs), \quad (5)$$

in which  $I_0'$  is the intensity of the light incident on the cell,  $k_1$  is the absorption coefficient,  $c$  the concentration of the solution, and  $s$  the distance indicated in the diagram. The amount of light absorbed by a layer  $ds$  in thickness is obtained by differentiating (5) which gives

$$dI' = -I_0' k_1 c \exp(-k_1 cs) ds.$$

The intensity of the fluorescence originating in any element of the solution  $ds$  in width will be proportional to this quantity  $dI'$ . If there is any quenching of the fluorescence by the absorbing molecule the intensity of the light emitted by any such element will be reduced by a factor  $1/(1+k_2c)$  in which  $k_2$  is the quenching constant as defined by Eq. (3). If the observer is far enough from the cell so that the light coming from all parts of the element  $ds$  can be considered as having passed through a layer of solution  $d-s$  in thickness, then according to Beer's and Lambert's laws the fraction of fluorescent light from any element which reaches the observer is given by  $\exp[k_3(l-s)c]$ . In this expression  $k_3$  is the absorption coefficient of the solution for the light involved. Since the fluorescent light obtained from solutions is never monochromatic and the absorption coefficient is a function of the wavelength of the light an exact solution of the problem would require that  $k_3$  be determined for each wave-length. However, since it is found experimentally that the absorption is small for all of the wave-lengths in the fluorescent light a reasonable approximation is to use an average value for the whole band. With this approximation we may write for the intensity of the light reaching the observer from the element  $ds$

$$dI = \frac{aI_0' k_1 c}{1+k_2 c} \exp[-k_1 s c - k_3(l-s)c] ds.$$

The factor  $a$  is the fraction of the absorbing molecules which would emit light toward the point of observation if there were no quenching. This factor can be considered constant for all positions of  $ds$  if the observer is relatively far from the cell. The total observed intensity is obtained by integrating the above expression between the limits 0 and  $l$ , the result is

$$I = \frac{aI_0' k_1 c \exp(-k_3 cl)}{1+k_2 c} \int_0^l \exp[-(k_1-k_3)cs] ds = \frac{A[\exp(-k_3 cl) - \exp(-k_1 cl)]}{1+k_2 c}. \quad (6)$$

In the final expression  $A$  is  $aI_0' k_1 / (k_1 - k_3)$  which is a constant. Similar equations can be derived for observation from the side or front of the solution.

Equation (6) was tested by diluting stock solutions of acridone and fluorescein and measuring the intensity of the fluorescence for each concentration. On account of the low solubility of acridone in water the experiments with that substance were carried out using 95 percent ethyl alcohol as the solvent. The stock solution was 0.00634M. The stock solution of fluorescein was 0.015M with water as the solvent. This solution was also 0.01M with respect to sodium hydroxide and 0.2M potassium nitrate. These two substances were added in order to keep the fluorescein in the form of the doubly charged ion and to keep the ionic strength constant. Their concentrations were kept constant in the dilution by adding a solution of the same composition except that fluorescein was omitted.

Before testing Eq. (6) as a whole the constants  $k_1$  and  $k_3$  can be determined by direct measurement. The absorption coefficient for the exciting light was determined by filling a cell, such as was used in the study of the fluorescence, with

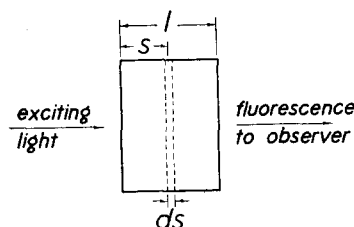


FIG. 2. Experimental arrangement corresponding to Eq. (6).

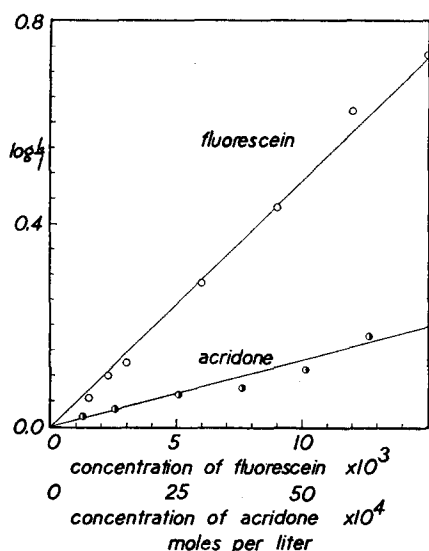


FIG. 3. Dependence of the absorption of the fluorescent light on the concentration of the fluorescer.

solutions of acridone or fluorescein at various concentrations and measuring the intensity of the transmitted light with a photronic cell. The slope of the curve obtained by plotting  $\log(I_0/I)$  against the concentration of the solution is  $k_1/2.3$ . Good straight lines were obtained for both substances. The absorption coefficient for the fluorescent light was determined in the same way except that the light used was the fluorescence from a thin cell which contained a relatively high concentration of the fluorescent substance. The results are shown in Fig. 3. The fact that the plots of  $\log(I_0/I)$  vs. concentration of the absorbing solution are straight lines justifies the procedure of using a single value for  $k_3$  for each substance.

The self-quenching constant  $k_2$  is obtained from the data obtained by the arrangement shown in Fig. 2. For this purpose Eq. (6) may be put into the form

$$(1 + k_2 c)/A = [\exp(-k_3 cl) - \exp(-k_1 cl)]/I.$$

The right-hand member of this equation may be evaluated by means of the previously determined values for  $k_1$  and  $k_3$  and the measurements of the intensity of the fluorescence leaving the cell. If these values are plotted against  $c$  the intercept is the value of  $1/A$  and the slope is  $k_2/A$ . Hence both  $k_2$  and  $A$  may be evaluated. The plots of the data are shown in Fig. 4. It is apparent from

an inspection of the figure that  $k_2$  is quite small, i.e., there is little self-quenching, for acridone but with fluorescein this effect is of considerable importance. The final values of the constants in Eq. (6) are tabulated for each substance in Table I. Since the intensities of the fluorescence are expressed in arbitrary units, the value of  $A$  is in arbitrary units, the other constants are in the units indicated.

The points corresponding to the first two values listed in Table II are outside the range covered by the plot in Fig. 4. Both points fall well above the line shown. This fact may be due to an effect such as is accounted for by the exponential factor in Eq. (4) (the quenching constant is about four times as large as for iodide on acridone and the fluorescein ion is much larger than the iodide ion) but the data at these low intensities are not sufficiently accurate (25 percent as compared to 1 percent for the higher intensities) to warrant any such conclusion. If the data are plotted on the assumption that the self-quenching follows Eq. (1), a curve similar to 1 in Fig. 1 is obtained. In such a plot the data cannot be fitted by any straight line passing

TABLE I. Constants for Eq. (6).

	Fluorescein	Acridone
$A$ (arbitrary units)	360	874
$k_1$ (mole <sup>-1</sup> cm <sup>-1</sup> liter)	1580	30250
$k_2$ (mole <sup>-1</sup> liter)	310	5.84
$k_3$ (mole <sup>-1</sup> cm <sup>-1</sup> liter)	29.5	59.8

TABLE II. Comparison of the observed intensities of fluorescence with those calculated by Eq. (6). Length of the cell used 3.70 cm.

Fluorescein			Acridone		
Molality $\times 10^3$	Obs.	Calc.	Molality $\times 10^4$	Obs.	Calc.
15.0	7	12	63.4	575	576
12.0	15	21	50.7	628	625
9.0	33	35	38.0	685	680
6.0	70	65	25.4	740	739
3.0	140	134	12.7	800	803
2.25	172	166	10.8	810	814
1.5	211	208	8.2	827	827
1.2	230	230	6.34	835	837
0.9	251	254	5.07	843	844
.6	277	275	3.80	855	851
.3	273	262	2.54	855	858
.15	209	195	1.27	850	848
.12	187	171	.634	775	740
.09	150	139	.507	700	681
.06	110	102	.380	610	595
.03	60	56	.254	493	465
			.127	320	277

through the origin as required by the equation. These data therefore furnish additional support for Eq. (3).

The accuracy with which the data are fitted by Eq. (6) is shown by substituting the values for the constants as given in Table I and comparing the calculated and observed intensities. Such a comparison is given in Table II. It is to be noted that  $A$  is merely a proportionality factor and does not affect the form of the curve in any way. Since  $k_2$  is the only one of the other constants determined from the intensity measurements it follows that this table shows how well the data can be fitted with what is essentially a one constant equation.

The fact that the calculated intensities fall below the observed ones at low concentrations may be accounted for by a failure to fulfill the condition that the photo-cell used for the observations should be so far from the cell that the factor  $A$  is constant. As the concentration of the fluorescent substance decreases the fluorescence spreads more uniformly throughout the cell and the average distance of the source of fluorescence from the photo-cell decreases with a resultant increase in  $A$ . The completeness with which the intensity *vs.* concentration curve is fitted on the

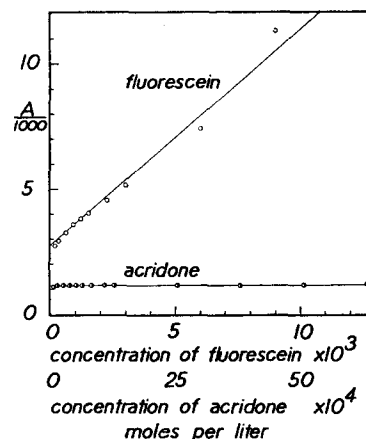


FIG. 4. Plot for determination of  $A$  and  $k_2$  in Eq. (6). (The values for acridone were actually determined from a plot on a much larger scale.)

basis proposed in this paper shows that for the two substances considered no other assumptions are needed. It is possible that with other substances, or even possibly with fluorescein at higher concentrations, it may be necessary to consider other effects such as the formation of more complex molecular species. However, such assumptions should not be introduced until correction has been made for the effects described in this paper which obviously must apply to all systems.

## Erratum: Frequency Spectrum of Crystalline Solids. II. General Theory and Applications to Simple Cubic Lattices

[J. Chem. Phys. 11, 481-495 (1943)]

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**P**AGE 494, Table IV: Headings should be

instead of  $p_0, p_1 \times 10^{-1} \dots p_7 \times 10^{-7}$   
 $p_1, p_2 \times 10^{-1} \dots p_8 \times 10^{-7}.$

Page 495, Eq. (54): Should be a power series in  $(h\nu_L/KT)$  instead of in  $(kT/h\nu_L)$ .

The denominator in the first equation of the second column of page 495 should be

instead of  $/2\pi^2\tau$   
 $/2\pi^2.$

Page 495, Eq. (55): The integral above this equation should be

instead of  $4\pi^4/15$   
 $4\pi^2/15.$