From the experiments carried out in the absence of an organic solvent it follows conclusively that suspended BP reacts with ferrous iron in the detergent solutions. Other conditions being the same the rate of reaction increases with increasing detergent concentrations and also with the solubilizing power of the detergents. However, a definite reaction also is found below the critical concentration where the detergent acts only as an emulsifier and not as a solubilizer. This result is not surprising since the rate of reaction would be expected to increase with an increase in the surface exposed by the BP. In agreement with this conclusion are the results of experiments carried out in the presence of benzene. Potassium myristate in 0.005 molar solution and potassium caprate in 0.05 molar solution are below the respective critical concentrations, but act as good emulsifiers at those concentrations. A measurable rate of reaction of ferrous iron and BP which is considerably greater than the blank is found at these concentrations. It is seen that the rate of reaction in 0.10 molar myristate is greater than in 0.005 molar myristate. This is attributed to the solubilized BP. When the pyrophosphate concentration in a 0.10 molar myristate solution is increased from 0.05 to 0.15 molar, the rate of reaction decreases somewhat. This effect of pyrophosphate deserves further systematic study.

The rate of reaction found in 0.005 molar oleate is somewhat greater than in 0.005 molar myristate. This is not surprising since the critical concentration of sodium oleate is of the order of and less than 0.001*M*. If the reaction between ferrous iron and BP would take place in the benzene layer, a considerably greater rate of reaction would have been expected with oleate since ferrous oleate is of the order of 300 times more soluble in benzene than is ferrous myristate. Thus, these results substantiate again the conclusion that the reaction takes place in the aqueous layer in the presence of pyrophosphate.

Acknowledgment.—The authors are indebted to Dr. A. I. Medalia for his interest and constructive discussions and to Mrs. Alma Blazic for carrying out polymerization experiments.

Summary

It has been shown that the reaction between ferrous iron dissolved in aqueous pyrophosphate solution and BP dissolved in organic solvents occurs in the aqueous layer. Evidence has been given that both solubilized and emulsified BP reacts with ferrous iron.

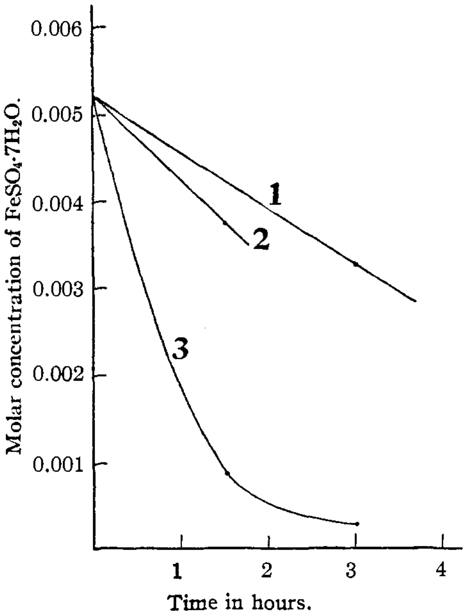

Fig. 2.—Reaction between ferrous iron in 0.10 *M* aqueous solution of sodium dodecane sulfate and BP in benzene solution. Mixtures composed of 30 ml. of aqueous solution 0.0051 *M* in $FeSO_4 \cdot 7H_2O$ and 25 ml. of benzene solution 0.05 *M* in BP. Reaction studied at 30° in the absence of air: 1, mixture contained 0.05 *M* acetic acid in aqueous layer; 2, mixture as described; 3, mixture contained 0.036 *M* $Na_4P_2O_7 \cdot 10H_2O$ in aqueous layer.

The most conclusive evidence that the aqueous layer is the locus of the reaction of BP and ferrous iron in an emulsion polymerization recipe is derived from experiments with the cationic detergent, CBAC. With this detergent no organic soluble iron can be formed but the polymerization reaction occurs with a reasonable rate.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 9, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

The Quenching of Fluorescence. Deviations from the Stern–Volmer Law

BY HAROLD BOAZ AND G. K. ROLLEFSON

The fluorescence of a gas or solution is said to be quenched if the intensity of that fluorescence is diminished by the addition of another substance which does not undergo any net reaction with the fluorescent substance. It is of course possible that the quenching process may involve some reaction between the photoactivated molecule and the quencher but both molecules must ultimately return to their original states. Some possible mechanisms for the quenching process have been discussed in a previous paper.[1] In this paper we wish to present data dealing with systems which deviate from the law derived by Stern and Volmer in 1920 and to show how these deviations can be accounted for in a simple manner.

Stern and Volmer considered the quenching process to be a bimolecular reaction which competes with the radiative process and all other molecular processes. On this basis they derived an equation which can be put in the form

$$I_0/I = 1 + k_q(Q) \qquad (1)$$

in which I_0 is the intensity of fluoresence in the

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(1) G K. Rollefson and H. Boaz, *J. Phys. Colloid Chem.*, **52**, 518 (1948).

absence of a quencher, I the intensity in the presence of a quencher at a concentration (Q) and k_q is called the quenching constant. This constant can be expressed as $k_2\tau_0$ in which k_2 is the rate constant for the bimolecular quenching process and τ_0, the mean life of the photoexcited state, is the reciprocal of the sum of the rate constants for all the first order reactions of the excited molecule. Apparent deviations from this law may arise from a number of causes. For example, if the fluorescence is produced by more than one excited state the quenching constants for the various states will differ and as a result a more complicated function will be needed to express the intensity of the total fluorescence as a function of the concentration of the quencher added although the simple law may apply to each of the excited states separately. Other deviations may arise from the effect of ionic strength on the magnitude of the quenching constant. Such effects have been discussed by Stoughton and Rollefson.[2] Even in systems in which these sources of deviations do not exist it has been found that there are significant departures from the behavior predicted by equation (1). In order to account for the positive deviations calculated from the data of Jette and West[3] Frank and Vavilov[4] postulated a sphere of action about the excited molecule. They assumed that the probability of deactivation of the excited molecule by a collision of the second kind with the quencher is unity inside this sphere and zero outside it. The probability that no quencher molecule be within the sphere at the time of excitation is given by the expression $\exp(-(Q)Nv/1000)$ in which v is the volume within the sphere actually available to the point-center of the quencher molecule and $(Q)N/1000$ is the number of quencher molecules per unit volume of the solution. This probability expresses the fraction of the excited molecules which is available for the competition between fluorescence and collisions of the second kind requiring diffusion. Hence Frank and Vavilov modified the Stern and Volmer equation to

$$I_0/I = (1 + k_q\,(Q))\exp((Q)Nv/1000) \qquad (2)$$

By means of this equation they calculated the sphere of action for the quenching of quinine ion (+2 charge) by iodide ion in water solution to be 2.0×10^{-20} cm.^{3}. This value corresponds to a distance of over 17 Å. between the centers of the ions at the time of deactivation. For the ionic radii assumed in their calculation this distance allows three water molecules to be placed on a line between the two ions. It is hard to justify the assumption of a resonance transfer of a large amount of energy across such a distance unless it can be shown that the difference between electronic states of the fluorescer and quencher are nearly equal.

Experimental Methods

The apparatus used to measure the fluorescence quenching was similar to the one described by Stoughton and Rollefson.[2] The principal difference was that the compensating beam instead of being taken from the side of the exciting arc opposite the fluorescent solution was reflected from the filter which was set at an angle of 45° to the main beam between the lens and the fluorescent solution. This arrangement eliminated some fluctuations which were found to be due to a wavering of the discharge current in the arc. The potentiometer consisted of two plug type resistance boxes which satisfactorily eliminated contact potentials even at very low values of the photo-current. The photo-currents came from two Visitron F-2A barrier layer type photocells (made by G. M. Laboratories, Inc.).

Transmission screens and photocells were calibrated by extrapolation to zero light intensity. For most experiments the response was linear within experimental error. For high intensity fluorescence, corrections were applied for non-linearity. Corrections seldom exceeded 2%.

The exciting light for all the experiments consisted of the mercury lines at 3650 and 3341 Å. or of the lines at 3650 Å. alone. A square of lead glass was placed between the fluorescent solution and any other filters in line to the photocell. The lead glass decreases the blank by absorbing most of the scattered and reflected exciting light without emitting a fluorescence of its own.

Two types of Pyrex cells were used for the fluorescent solutions: one, the standard 5.00 cm. cylindrical type manufactured by Central Scientific Co. for use in the "Photelometer;" the other, $^7/_8$ inch thin-walled test-tubes. The cylindrical cells were illuminated through the plane face. Fluorescence was observed at right angles. This arrangement gives a very low blank with distilled water. The test-tubes were clamped vertically in the beam. Fluorescence was observed at right angles radially. Although the test-tubes give a higher blank than the horizontal cylindrical cells, the blank is reproducible. Test-tubes were used in all the measurements requiring temperature control and in all experiments done in an atmosphere of nitrogen.

The absorption spectra in Figs. 4, 5 and 6 were obtained with a Cary recording spectrophotometer, model 11, serial 4. Several of the spectra were traced both with and without filters that remove the fluorescence. No difference could be detected.

For the fluorescence spectra the Cary instrument was used with the fluorescent solution in a test-tube in the normal position of the light source. The solution was illuminated from the side with a d. c. mercury arc. The instrument records the logarithm of a quantity proportional to the photo-current (dotted line in Figs. 7 and 8). The sharp peaks at 3650 and 4047 Å. are mercury lines transmitted by the filter.

In a typical experiment a distilled water blank was obtained for each of eight numbered test-tubes. The first seven tubes were then filled with the solution containing no quencher. These were read in order until two or more sets of readings gave satisfactory agreement—better than 0.5% in most cases. The solutions in five of the seven tubes were exchanged for solutions containing the different concentrations of quencher. All eight tubes were read in order until agreement was satisfactory. From these data values for I_0/I were calculated.

Reagents.—The methyl alcohol was distilled once. The carbon tetrachloride was purified according to the procedure in Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1941, p. 365. The acridone was recrystallized by Dr. Harold Dodgen (Rollefson and Dodgen, *J. Chem. Phys.*, 12, 107 (1944)). Merck U. S. P. quinine sulfate was used without further purification. α and β-naphthylamine were carried in a stream of nitrogen from the molten compound at 120° and collected

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[2] R. W. Stoughton and G. K. Rollefson, THIS JOURNAL, **61**, 2634 (1939).

[3] E. R. Jette and W. West, *Proc. Roy. Soc.* (*London*), **A121**, 294, 299 (1928).

[4] I. M. Frank and S. I. Vavilov, *Z. Physik*, **69**, 100 (1931).

in an air condenser. α-Naphthylamine was distilled twice in this manner; β-naphthylamine once. The purified α-naphthylamine melted 47.7–48.8°; β-naphthylamine, 110.2–110.5°. Eastman Kodak Co. reagent grade naphthionic acid was recrystallized under nitrogen twice from hot water. The equivalent weight was determined in duplicate by titration: 224.0, 224.6. Reagent grade inorganic salts were used without further purification. Some of the solutions, however, were filtered to minimize light scattering.

Positive Deviations from the Stern–Volmer Law.—The quenching of the fluorescence of acridone by potassium iodide was chosen for a detailed study of the positive deviations from the Stern–Volmer law. Acridone is a neutral molecule in aqueous solutions of *p*H 7, hence the ionic strength effects are much smaller than those found for reactions between ions. Such effects are, however, not completely absent since we found that at 24° the quenching constant in a solution which is 0.0400 *M* potassium iodide is 107.3 whereas in a solution at the same temperature and iodide concentration but in the presence of 1.06 *M* potassium nitrate it is 89.5. Therefore, to avoid variations due to this cause the quenching experiments were performed in solutions in which the total salt concentration was maintained at 1.100 moles per liter. Such a composition was maintained by using mixtures of potassium iodide and potassium nitrate. Iodide ion quenches the fluorescence of acridone but potassium and nitrate ions do not. The results obtained are given in Table I and analyzed graphically in Figs. 1 and 2.

Table I

The Quenching of Acridone by Iodide

Temperature 26°; acridone 0.85 saturation concentration; filters used: Corning 5860, lead glass, Corning 306.

M KI	*M* KNO_3	I_0/I Obsd.	I_0/I Calcd.	"k_q"
0.0000	1.10	....	....	...
.0400	1.06	4.64	4.64	91.0
.1000	1.00	10.59	10.73	95.9
.2000	0.90	23.0	22.52	110
.3000	.80	37.2	36.4	121
.5000	.60	68.6	70.3	135
.8000	.30	137	136.5	170

The fourth column was calculated by means of equation (3) with the values of k_q and K as 85.8 and 1.20, respectively. It has been pointed out by Umberger and LaMer (This Journal, **67,** 1099 (1945)) that the quenching constant for the quenching of the fluorescence of the doubly negative ion of fluorescein by iodide is dependent on the concentration of the fluorescein ion. We have also observed this effect when working with fluorescein but such behavior seems to be exceptional since we have not found such a dependence in any of the systems reported in this paper.

The broken line in Fig. 1 corresponds to the requirements of the Stern–Volmer law. It is evident that the data deviate greatly at high quenching. If we calculate apparent values for the quenching constant, "k_q," and plot these values (last column of Table I) against the concentration of the quencher the straight line shown in Fig. 2 is obtained. This result shows that the data can be represented by the addition of another term to the Stern–Volmer equation to give

$$I_0/I = 1 + k_1(Q) + k_2(Q)^2$$

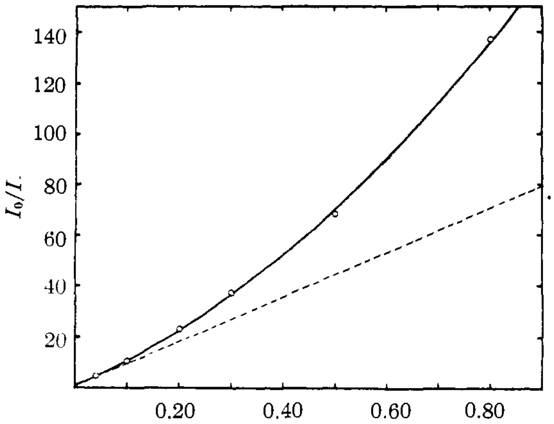

Fig. 1.—Quenching of the fluorescence of neutral acridone by iodide ion in water.

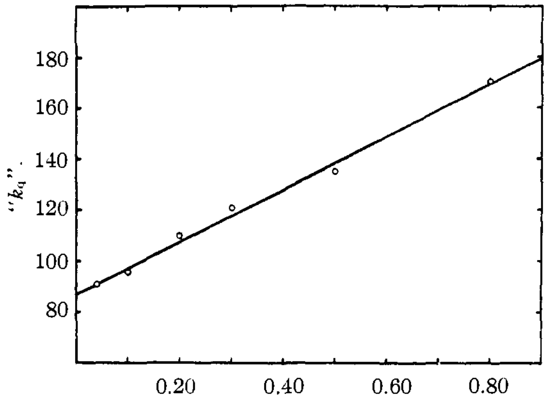

Fig. 2.—The function $(I_0/I - 1)/(Q)$ or "k_q" for neutral acridone and iodide ion in water.

Such an equation is not sufficient for all cases of positive deviations from the simple law. The quenching of the fluorescence of quinine by chloride ion requires the addition of still another term involving the third power of the quencher concentration.

Table II

The Quenching of Quinine by Chloride Ion

Temperature 22–23°. Filters same as for Table I. Total salt concentration maintained at 0.500 *M* with KNO_3. +2 quinine ion 3 × 10^{-5} *M;* nitric acid 0.010 *M*. The last column is calculated by equation (4) with values of k_q, K_1, K_2 of 86.7, 1.69 and 1.15, respectively.

M Cl^-	I_0/I Obsd.	I_0/I Calcd.
0.0050	1.446	1.446
.0100	1.898	1.899
.0500	5.82	5.81
.1000	11.46	11.49
.2000	25.8	25.94
.5000	103	103.2

An equation of the type indicated above can be deduced in several ways from theoretical

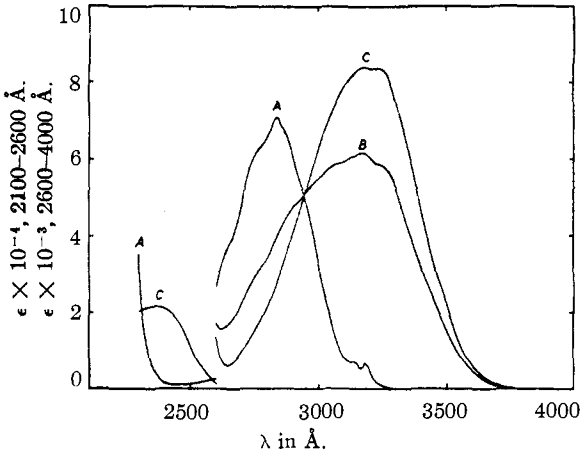

Fig. 3.—Absorption of naphthionic acid and naphthionate ion in water solutions: A, 0.98 M HCl; B, 1.02 $\times$ 10^{-3} M H^+; C, 1.00 $\times$ 10^{-4} M NaOH with 0.500 M NaCl, 0.100 M NaOH with 0.400 M NaCl, 0.500 M NaOH with no NaCl: $\epsilon_{\max.}$ = 8392 at 3180 Å. in curve C.

considerations. Thus if the exponential part of equation (2) is expanded into a power series and multiplied by $1 + k_q(Q)$ a power series is obtained which is convergent for values of (Q) less than unity. In such a series there is a definite relationship between the coefficients of the successive terms which in general will determine the number of terms of the series which must be considered in any given case. Application of this equation to the quenching of the fluorescence of acridone by iodide shows that the cubic term should be of such magnitude that the data should deviate markedly from the straight line in Fig. 2 if such an equation were applicable. It is obvious that such is not the case. On the other hand, the quenching of the fluorescence of quinine by chloride ion is fitted equally well by equation (2) or by a cubic equation such as mentioned above, *i. e.*, the fourth power and higher terms resulting from the expansion of equation (2) are negligible within the limits of experimental error. The variability in the applicability of equation (2) is probably due to the failure of the systems to conform to the condition of a random distribution of quencher molecules assumed in the derivation of that equation. A random distribution requires that there be no interaction between quencher molecules for any distance between centers.

In order to explain the equations which fit the experimental data we have used a model which embodies the same principle as that used by Frank and Vavilov but is more specific in its definition of the sphere of action and provides a basis for the deviations from the idealistic conditions indicated above. Consider that molecules of the fluorescer and quencher are continually coming together and separating in a way that can be represented by

$$M + Q \rightleftarrows M..Q;\ K = (M..Q)/(M)(Q)$$

M..Q is a fluorescer–quencher pair within a solvent cage and K is a constant, characteristic of the steady state, which is determined by the rates of formation and separation of these pairs. In the absence of excitation of M the effect of Q on M is not significantly different from that of a solvent molecule. This assumption is in accord with the experimental fact that the activity of a substance like acridone is not affected by the addition of a large concentration of a quencher such as potassium iodide.[5] If a molecule of M absorbs a photon while it is in the same cage with a molecule of Q the probability of quenching is high; for the present we shall consider it unity. The existence of such pairs decreases the number of M* molecules that can emit light or be quenched by a molecule of Q diffusing into the necessary sphere of action during the lifetime of the excited state. Assuming that the Stern–Volmer equation applies rigorously to the competition between the fluorescence and diffusional quenching processes the allowance for the type of static quenching described is made by multiplying $1 + k_q(Q)$ by M_0/M. M is the number of fluorescer molecules not in the same cage with a quencher molecule and M_0 is the total number of fluorescer molecules. This ratio is

$$((M) + (M..Q))/(M) = 1 + K(Q)$$

The result obtained is

$$I_0/I = 1 + (k_q + K)(Q) + k_qK(Q)^2 \qquad (3)$$

It follows that the intercept at (Q) = 0 in Fig. 2 is the value of $k_q + K$ and the slope of the line k_qK. These values are 87.0 and 103 in this case from which we calculate $k_q = 85.8$ and $K = 1.20$. It is to be noted that the limiting value of the quenching constant obtained by extrapolating to zero concentration is not the constant of the simple Stern–Volmer formulation as postulated by Williamson and LaMer.[6] (This is also true for the expanded form of equation (2).)

An equation of the type applied to the quenching of the fluorescence of quinine by chloride (Table II) can be derived in a similar manner if it is assumed that molecules of the fluorescer may be in the same solvent cage as either one or two molecules of the quencher. The result obtained is

$$I_0/I = 1 + (k_q + K_1)(Q) + K_1(k_q + K_2)(Q)^2 + k_qK_1K_2(Q)^3 \qquad (4)$$

in which $K_1 = (M..Q)/(M)(Q)$ and $K_2 = (Q..M..Q)/(M..Q)(Q)$. Equations with still higher powers of (Q) can be obtained by assuming the possibility of more molecules of quencher being in the same cage with the fluorescer but we have not studied any case in which that has been necessary. In all of these derivations it is assumed that there is no fluorescence from a

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[5] G. K. Rollefson and R. W. Stoughton, This Journal, **63**, 1517 (1941).

[6] B. Williamson and V. K. LaMer, *ibid.*, **70**, 717 (1948).

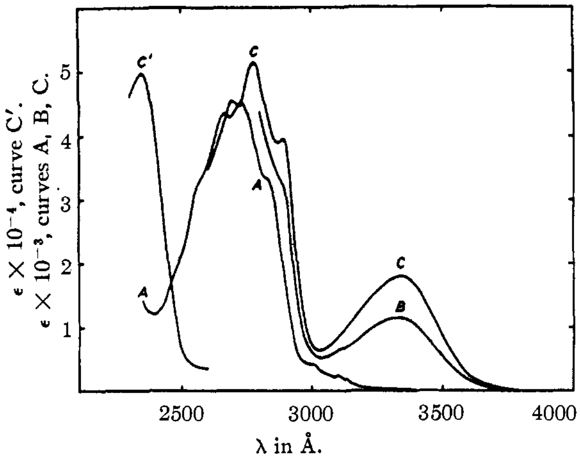

Fig. 4.—Absorption of β-naphthylamine and its positive ion in water solutions: A, 0.98 M HCl, B, 6.2 × 10^{-5} M H^+; C, same NaOH and NaCl concentrations as in Fig. 4; C', 1.00 × 10^{-4} M NaOH with 0.500 M NaCl; $\epsilon_{\text{max.}}$ = 1797 at 3340 Å. in curve C.

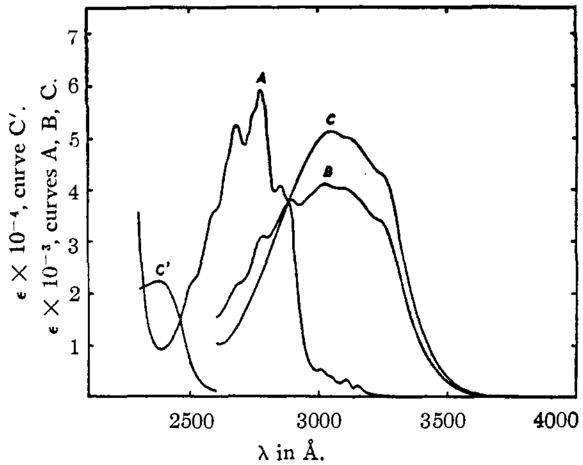

Fig. 5.—Absorption of α-naphthylamine and its positive ion in water solutions: A, 1.00 M HCl; B, 8.2 × 10^{-5} M H^+; C and C', same NaOH and NaCl concentrations as in Fig. 5; $\epsilon_{\text{max.}}$ = 5115 at 3040 Å. in curve C.

molecule which is excited while it is in the same solvent cage as one or more quencher molecules.

Negative Deviations.—Relatively few systems have been studied in which the apparent quenching constant, k_q, decreases as the concentration of the quencher is increased. Stoughton and Rollefson found such an effect for quinine quenched by silver ion and Sveshnikov[7] found the same behavior for the quenching of the plus one ion of rhodamine B by iodide. In the latter case it was reported that both the absorption and fluorescence spectra of the dye were changed by the addition of the quencher so it was believed that there was a reaction of the dye and quencher before activation. In this paper we wish to report results on the quenching of naphthionate ion, α-naphthylamine and β-naphthylamine, by hydroxide ion, all systems which show negative deviations from the Stern–Volmer law.

The absorption spectra of these substances are shown in Figs. 4, 5 and 6. It is apparent that there is a dependence on the pH of the solution but the absorption is not affected by the hydroxide concentration in the range which is effective for quenching. The latter fact may be taken as an indication that there is no change in the nature of absorbing species in the quenching experiments. In order to obtain additional confirmation of this fact the distribution of α- and β-naphthylamines between water and carbon tetrachloride was studied. The results are shown in Table III. There is no significant effect with β-naphthylamine as the ionic solute is changed from 0.333 M sodium chloride to sodium hydroxide of the same concentration. The effect with α-naphthylamine is beyond the limits of experimental error but is the opposite of what might be expected if the hydroxide acted to convert the

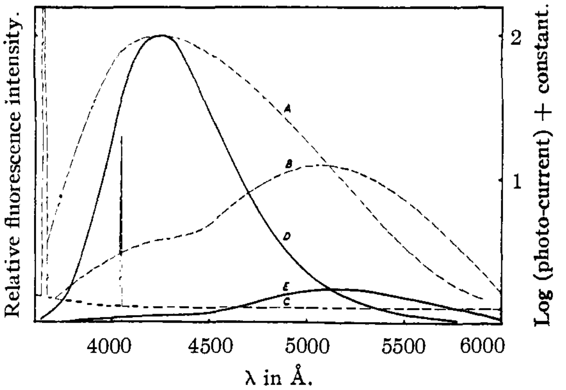

Fig. 6.—Fluorescence of naphthionate ion in water: A, B, C, smooth curves through the logarithmic tracings by the Cary recording spectrophotometer for 2.0 × 10^{-4} M NaOH and 0.400 M NaOH solutions and for a distilled water blank; D, E, curves obtained from the net antilogarithms of A and B corrected for quartz dispersion and the quantum response of a photo-multiplier tube 1-P28—the latter as obtained from the RCA tube handbook with conversion from energy units to quantum units. The dotted curves A, B, C, in this figure and in Fig. 8 contained the same constant.

TABLE III

THE DISTRIBUTION OF α- AND β-NAPHTHYLAMINES BETWEEN WATER SOLUTION AND CARBON TETRACHLORIDE

	Composition of H_2O phase	C_{H_2O}/C_{CCl_4}
α-NH_3	0.333 M NaOH	0.00957
		.00957
	.333 M NaCl	.01054
		.01060
β-NH_2	.333 M NaOH	.01237
		.01231
	.333 M NaCl	.01237
		.01244

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(7) Sveshnikov, *Acta Physicochim. U. R. S. S.*, **4**, 453 (1936).

amine into some ionic form. Furthermore the effect is not great enough to account for deviations from the Stern–Volmer law of the magnitude observed.

The solid curves in Fig. 7 show the fluorescence spectrum of naphthionate ion at two different concentrations of hydroxide ion. At *p*H 10 the fluoresence is blue-violet in appearance with a maximum at 4250 Å. In 0.40 *M* hydroxide it is yellow-green. Since the absorption spectrum is the same for both of these solutions it is believed that the change in the fluorescence is due to a reaction of the photoactivated molecule with hydroxide ion. The data for the quenching are collected in Table IV. The last column is calculated from a consideration of the following mechanism in which BH^{*-} represents an excited naphthionate ion

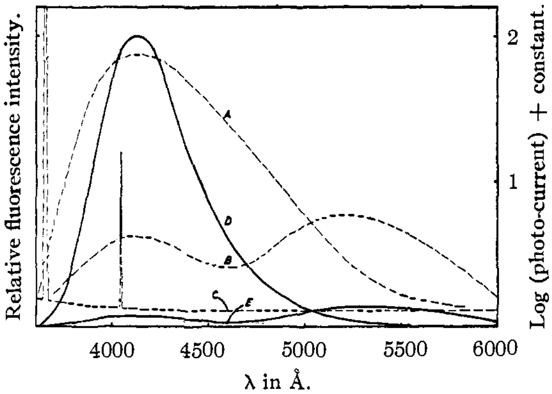

Fig. 7.—Fluorescence of β-naphthylamine in water solutions. The description of this figure is the same as for Fig. 7.

$$\begin{array}{ll}
BH^{*-} \longrightarrow BH^- + h\nu_1 & k_1 \\
BH^{*-} + OH^- \longrightarrow B^{*--} + H_2O & k_2 \\
B^{*--} \longrightarrow B^{--} & k_3 \\
B^{*--} \longrightarrow B^{--} + h\nu_2 & k_4 \\
B^{--} + H_2O \longrightarrow BH^- + OH^- &
\end{array}$$

from which

$$I_0/I = (1 + c_1(OH^-))/(1 + c_2c_1(OH^-)) \quad (5)$$
$$c_1 = k_2/k_1$$
$$c_2 = Rk_4/(k_3 + k_4)$$

R is a factor which corrects for the fact that the response of the photocell is not the same for equal numbers of quanta of different frequency distributions. In this case it is the ratio of the response to the frequency distribution ν_2 to the response to the distribution ν_1 with both distributions modified by passing through the filters used to absorb the exciting light from the mercury arc.

The values of c_1 listed in Table IV were calculated by applying equation 5 to two successive values of I_0/I and eliminating c_2 from the equations thus obtained. The average of the values obtained for c_1 was put into equation 5 to calculate a value of c_2 for each hydroxide concentration. Finally the average values of c_1 and c_2 were inserted in equation 5 to calculate the values of I_0/I listed in the last column of the table. It is apparent that the agreement between the calculated and observed values is quite good although the hydroxide concentration is varied twenty-fold.

TABLE IV

THE QUENCHING OF NAPHTHIONATE ION BY HYDROXIDE ION

M OH⁻	I_0/I, obsd.	c_1	c_2	I_0/I, calcd.
		0°		
0.00996	1.370	...	0.253	1.375
.01992	1.672	52.5	.243	1.669
.04992	2.277	56.3	.241	2.263
.0999	2.812	57.3	.242	2.792
.1998	3.258	61.2	.246	3.266
	Av.	56.8	.245	
		19.7°		
0.00996	1.501	...	0.248	1.507
.01992	1.872	76.4	.242	1.872
.04992	2.557	78.2	.239	2.540
.0999	3.089	80.6	.239	3.066
.1998	3.497	84.6	.241	3.491
	Av.	80.0	.242	
		50°		
0.00996	1.594	...	0.261	1.600
.01992	1.992	99.0	.257	1.995
.04992	2.658	99.4	.254	2.645
.0999	3.118	102.6	.254	3.101
.1998	3.444	107.0	.256	3.439
	Av.	102.0	.256	

The results obtained with β-naphthylamine were similar to those with naphthionate ion. They are summarized in Table V and Fig. 8. It is to be noted that in obtaining the second set of data at 24.5° the fluorescence was passed through a different set of filters from that used for the other data. The results at different temperatures both with the β-naphthylamine and naphthionate show the dependence of c_1 and comparative independence of c_2 on the temperature. This fact is in accordance with what should be expected on the basis of the significance assigned to these constants by the assumed mechanism. Furthermore the dependence of c_2 and independence of c_1 on the filters used is in accord with these views.

The case of α-naphthylamine is of special interest because in addition to showing an effect similar to that found with naphthionate and β-naphthylamine there is evidence for an effect of the type discussed in connection with the positive deviations from the Stern–Volmer law. As a result a three-constant equation is required to fit the data. The following equation which is derived in an appendix has been found to be suitable.

$$I_0/I = [1 + c_1((OH^-) + c_3(OH^-)^2)]/[1 + c_1c_2((OH^-) + c_3(OH^-)^2)] \quad (6)$$

TABLE V

THE QUENCHING OF β-NAPHTHYLAMINE BY HYDROXIDE ION

T, °C.	M OH$^-$	I_0/I	c_1	c_2
14	0.01064	1.480		0.220
	.02128	1.850	65.6	.218
	.0532	2.59	65.1	.214
	.1064	3.19	69.9	.217
		Av.	66.9	.217
24.5	0.01064	1.530		0.214
	.02128	1.932	73.1	.212
	.0532	2.70	73.5	.211
	.1064	3.32	75.8	.212
		Av.	74.1	.212
24.5	0.01064	1.586		0.153
	.02128	2.062	71.9	.152
	.0532	3.05	71.8	.153
	.1064	3.97	74.5	.156
		Av.	72.7	.153
44	0.01064	1.595		0.221
	.02128	2.035	82.7	.214
	.0532	2.83	85.4	.213
	.1064	3.41	90.6	.216
		Av.	86.2	.216

The constants have the significance assigned to them in the derivation. It is apparent that if $c_3 = 0$ the equation reduces to the same form as (5). The data which have been obtained are listed in Table VI along with the values of I_0/I calculated by means of the above equation using the indicated values for the constants. Again we call attention to the fact that c_1 and c_3 are independent of the nature of the filters used but c_2 depends on the filters, as is to be expected from the assigned significance. The agreement between the calculated and observed values of I_0/I is quite satisfactory except for the last point observed with the blue filter. In this case the discrepancy is probably due to experimental error since the fluorescence is quenched more than 99% in that particular experiment. The value of K computed from the values for c_1 and c_3 is essentially 3.0, which is of the same order of magnitude as the values found for the systems which show marked positive deviations from the Stern–Volmer law.

TABLE VI

THE QUENCHING OF α-NAPHTHYLAMINE BY HYDROXIDE ION

Fluorescence viewed through yellow filter number 306: $c_1 = 145$, $c_2 = 0.011$, $c_3 = 3.0$

M OH$^-$	I_0/I, obsd.	"k_q"	I_0/I, calcd.
0.00998	2.449	145.2	2.45
.01998	3.964	148.4	3.94
.04992	8.55	151.3	8.54
.0998	15.95	149.8	16.4
.1998	30.7	148.5	31.4
.5006	63.9	126	61

Fluorescence viewed through blue filter number 5543: $c_1 = 145$, $c_2 = 0.0050$, $c_3 = 3.0$

M OH$^-$	I_0/I obsd.	"k_q"	I_0/I, calcd.
0.00998	2.462	146.5	2.47
.01998	4.012	150.7	4.01
.04992	8.91	158.5	8.95
.0998	17.8	168	18.1
.1998	38.6	188	38.4
.5006	112	223	96

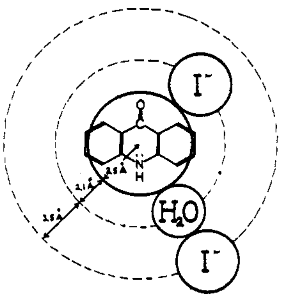

Fig. 8.—A model for approximating the fraction of acridone molecules that are nearest neighbors to an iodide ion in water solution.

Discussion.—The fact that some photoactivated molecules can undergo reactions with quenchers so as to give the observed negative deviations from the Stern–Volmer law whereas other molecules do not react thus is not surprising since it is quite common to observe a high degree of specificity with respect to chemical reactivity and a considerable variation in fluorescence yield even among compounds that are structurally very similar. The yield of fluoresence from the excited negative ion of α-naphthylamine is so small that it was not detected from the fluorescence spectrum nor would it have been detected with certainty even with the yellow filter except at the higher values of I_0/I (see Table VI). Some examples of systems in which a change in the combining power of atoms or molecules on excitation has been reported are as follows: Glockler[8] reported banded fluorescence from a molecule formed from excited mercury atoms and methane; Rabinowitch[9] found visible fluorescence bands on excitation of mercury in the presence of 20 mm. pressure of ammonia; Melville[10] explained his observations of the mercury sensitized polymerization of acetylene by the formation of a complex with the excited mercury atoms; anthracene forms an unstable dimer upon illumination[11] and also forms a peroxide when illuminated in the presence of oxygen.[12] More data must be obtained before any general rules with respect to this type of behavior can be set up. On the other hand, some interesting information can be obtained from a more detailed consideration of the model used to derive equations (3) and (4).

[8] G. Glockler, *J. Chem. Phys.*, **2**, 46 (1934).

[9] E. Rabinowitch, *Ann. N. Y. Acad. Sci.*, **41**, 203 (1941).

[10] H. W. Melville, *Trans. Faraday Soc.*, **32**, 258 (1936).

[11] R. Luther and F. Weigert, *Z. physik. Chem.*, **51**, 297 (1905); **53**, 385 (1905).

[12] C. Dufraisse and M. Gerard, *Compt. rend.*, **201**, 428 (1935).

Consider a model, Fig. 8, in which the fluorescer, quencher and solvent molecules are represented as spheres, the dimensions of which can be calculated from the physical properties of these substances. Let us assume that two molecules can be said to be in the same solvent cage if their separation is not sufficient for a molecule of solvent to come between them. The figure shows a reasonable set of dimensions to use for the discussion of the quenching of acridone by iodide. The probability of finding m ions in the spherical shell if that shell on the average contains a ions is given by Poisson's formula as $W_m = a^m.(\exp(-a))/m$. For the dimensions indicated in Fig. 8, if the concentration of iodide is one mole per liter the value of a is 1.46. The various values of W are $W_0 = 0.232$, $W_1 = 0.339$, $W_2 = 0.247$, $W_3 = 0.124$, etc. The constants K_1 and K_2, etc., of an equation such as (4) may be expressed in terms of these probabilities as $K_1 = W_1/W_0$, $K_2 = W_2/W_1$, etc., if we assume that these constants are determined solely by the statistical distribution of the quencher molecules throughout the solvent. The values of K_1 and K_2 are 1.46 and 0.73, respectively. It is apparent that the value for K_1 agrees fairly well with the value calculated for K in equation (3) for the quenching of acridone by iodide but there is no evidence for a term involving K_2 of a magnitude required by this type of calculation. This fact is not too surprising since it could be expected that the presence of the first iodide ion in the solvent cage would tend to prevent the entry of a second by electrostatic repulsion. In the quenching of quinine fluorescence by chloride ion the first chloride ion neutralizes only half the charge on the quinine so that an electrostatic attraction still exists between the fluorescer and quencher with the result that the term with K_2 is of significance. Electrostatic effects may further modify the values of K_1, K_2, etc., as calculated above in that if the forces are attractive a quencher molecule which is originally slightly outside the cage may be drawn into the cage in a time much less than the lifetime, and therefore will have the same effect as a molecule originally in the cage. Similarly repulsive forces would tend to keep quencher molecules out of the cage and thus yield low values for the K's. That such electrical effects are not the sole source of complications is apparent from the fact that in the quenching of the fluorescence of the naphthylamines by hydroxide ion the electrostatic effects should be identical but we find that with the alpha compound there is a definite static quenching but with the beta compound there is none. Actually a close examination of our data for the β-naphthylamine and naphthionate cases shows a slight systematic variation which might be due to a value for K of 0.1 or less. Even if this effect is real the large difference in behavior between the alpha and beta compounds requires some explanation. If it is assumed that the mere presence of a quencher molecule within a given sphere of action is not sufficient to cause quenching but that some specific orientation of the two molecules is also required the calculations given above must be modified. Under such conditions some of the fluorescer–quencher pairs will fluoresce since the right orientation will not occur within the lifetime of the excited state, or the molecules will separate before the right orientation occurs. Let c be the fraction of the pairs which do fluoresce when excited. Then equation (3) or (4) will be modified by a factor of $1/(1 + cK(\mathrm{Q}))$ to give

$$I_0/I = (1 + (k_q + K)(\mathrm{Q}) + k_qK(\mathrm{Q})^2)/(1 + cK(\mathrm{Q})) \qquad (7)$$

It is apparent that for $c = 1$ this equation reduces to (1) and for $c = 0$ it becomes identical with (3). For intermediate values of c if the apparent quenching constant is plotted against the concentration of quencher as in Fig. 2 a curve is obtained which is approximately a straight line but is slightly concave toward the concentration axis. The principal effect of assuming that some pairs fluoresce is to decrease the value calculated for K if one attempts to fit the data with a straight line. In view of this fact and the fact that at high percentage quenching where the effect is large the experimental errors are also apt to be large enough to mask any curvature it is difficult to get a quantitative check of the above equation. Therefore at least some of the variations in the values obtained for K are probably due to fluorescence occurring from some of the pairs.

If the variations in K are in part due to the cause just mentioned it might be expected that a correlation would exist between the magnitude of K and the magnitude of the constant for the bimolecular quenching process since both effects are concerned with the probability of quenching occurring when a quencher molecule is within a certain distance of a fluorescer molecule. Unfortunately data are not available for testing this hypothesis. The experimentally determined k_q is the product of the bimolecular rate constant and the lifetime of the excited state. At the present time experiments are under way in this Laboratory to determine these lifetimes and thus permit the determination of the desired rate constants. If our theory is correct it is to be expected that low values for K will be associated with low values for the second order rate constants.

Appendix. Derivation of Equation (6).—The following scheme is used to describe the reaction of α-naphthylamine with hydroxide ion

$$\begin{array}{lr}
\mathrm{BH} + \mathrm{OH}^- \rightleftarrows \mathrm{BH..OH}^- & K \\
\mathrm{BH}^*..\mathrm{OH}^- \longrightarrow \mathrm{B}^{*-} + \mathrm{H_2O} & \text{very rapid} \\
\mathrm{BH}^* \longrightarrow \mathrm{BH} + h\nu_1 & k_1 \\
\mathrm{BH}^* + \mathrm{OH}^- \longrightarrow \mathrm{B}^{*-} + \mathrm{H_2O} & k_2 \\
\mathrm{B}^{*-} \longrightarrow \mathrm{B}^- & k_3 \\
\mathrm{B}^{*-} \longrightarrow \mathrm{B}^- + h\nu_2 & k_4 \\
\mathrm{B}^- + \mathrm{H_2O} \longrightarrow \mathrm{BH} + \mathrm{OH}^- &
\end{array}$$

The fraction of excited molecules that reacts with hydroxide ion at the time of excitation is

$$K(\mathrm{OH}^-)/(1 + K(\mathrm{OH}^-)) \qquad (1)$$

the fraction that does not is

$$1/(1 + K(\mathrm{OH}^-)) \qquad (2)$$

Of this last fraction the fraction

$$1/(1 + k_q(\mathrm{OH}^-)) \qquad (3)$$

emits the fluorescence of the neutral excited α-naphthylamine molecule and the fraction

$$k_q(\mathrm{OH}^-)/(1 + k\ (\mathrm{OH}^-)) \qquad (4)$$

reacts with hydroxide ion; $k_q = k_2/k_1$.

The molecules that react with hydroxide ion fluoresce with the apparent efficiency c_2.

In the solution containing hydroxide ion the intensity of fluorescence from neutral excited α-naphthylamine molecules is the product of *2*, *3* and I_0

$$I_0 \left[\frac{1}{1 + K(\mathrm{OH}^-)}\right] + \left[\frac{1}{1 + k_q(\mathrm{OH}^-)}\right] \qquad (5)$$

The measured intensity of fluorescence from the molecules that react with hydroxide ion is the product of c_2, *1* and I_0 plus the product of c_2, *2*, *4* and I_0

$$I_0c_2 \left\{\left[\frac{K(\mathrm{OH}^-)}{1 + K(\mathrm{OH}^-)}\right] + \left[\frac{1}{1 + K(\mathrm{OH}^-)}\right]\left[\frac{k\ (\mathrm{OH}^-)}{1 + k_q(\mathrm{OH}^-)}\right]\right\} \qquad (6)$$

Adding *5* and *6* and inverting we get

$$I_0/I = \frac{1 + c_1((\mathrm{OH}^-) + c_3(\mathrm{OH}^-)^2)}{1 + c_1c_2((\mathrm{OH}^-) + c_3(\mathrm{OH}^-)^2)}$$

in which

$$c_1 = k_q + K$$
$$c_3 = k_qK/(k_q + K)$$

Summary

A study has been made of the deviations from the Stern–Volmer law in the quenching of the fluorescence of (1) acridone by iodide, (2) quinine by chloride, (3) naphthionate, α-naphthylamine and β-naphthylamine by hydroxide. The first two systems show marked positive deviations and the last three negative deviations. The case of α-naphthylamine is of particular interest since in addition to the negative deviation it shows an effect explainable in the same way as the positive deviations for the first two systems.

The positive deviations are explained as due to the presence of a quencher molecule in the same solvent cage as the fluorescer molecule at the moment of excitation. On the basis of this model an equation is derived which takes into account the fact that some of the activated fluorescer–quencher pairs may fluoresce.

Negative deviations from the Stern–Volmer law are explained by the assumption that the quencher molecules react with the photoactivated fluorescer molecules to form a new activated species which emits light of a different energy distribution from that emitted by the original fluorescer molecule. The distribution of the emitted energy with respect to frequency has been determined for the fluorescence both in the absence and presence of the quencher. The results obtained support the theory.

BERKELEY 4, CALIF. RECEIVED JULY 15, 1949

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Microwave Absorption and Molecular Structure in Liquids. V. Measurement of the Dielectric Constant and Loss of Low-loss Solutions[1]

BY W. M. HESTON, JR., A. D. FRANKLIN, E. J. HENNELLY AND C. P. SMYTH

The investigations of the twenty-seven liquid organic halides reported and discussed in the first three papers[2,3,4] of this series made apparent the desirability of measuring selected molecules in dilute solution in a variety of different solvents. The present paper describes the experimental method employed in these measurements and gives examples to illustrate the data obtained.

Experimental Method

The high dielectric loss method presented in the first two papers[2,3] made use of the large variation in the reflection coefficient as the sample length was changed. The absolute magnitude of the reflection coefficient for low-loss dielectrics, however, is very close to unity since power absorption by the dielectric-filled waveguide section is relatively small. Because the variation in reflection coefficient with sample length is very small and thus difficult to measure with any degree of reliability, an alternative approach has been adopted. The voltage standing wave ratio, *SWR*, is quite sensitive to small changes in dielectric loss since for low loss dielectric the *SWR* is large. The *SWR* is represented in equation (1) as

$$\rho_n = SWR = \left|\frac{E_{\mathrm{max.}}}{E_{\mathrm{min.}}}\right|_n = \frac{1 + |G|_n}{1 - |G|_n} \qquad (1)$$

where G_n is the reflection coefficient and $E_{\mathrm{max.}}$ and $E_{\mathrm{min.}}$ are the voltages of the maximum and minimum points, respectively, at the n^{th} minimum along the line. Calculation of ρ_n for various values of G_n in the neighborhood of $G_n = 1.0$ indicates that ρ_n is very sensitive to small changes in G_n. This sensitivity of ρ_n to small changes in the reflection coefficient when near unity is used to measure the dielectric absorption of low loss systems presented in this paper. Figure 1 illustrates the variation in ρ with $|G|$.

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[1] This research was carried out with the support of the Office of Naval Research. Reproduction in whole or in part permitted for any purpose of the United States Government.

[2] Heston, Hennelly and Smyth, THIS JOURNAL, **70**, 4093 (1948).

[3] Laquer and Smyth, *ibid.*, **70**, 4097 (1948).

[4] Hennelly, Heston and Smyth, *ibid.*, **70**, 4102 (1948).