

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

## The Influence of Ionic Strength on the Quenching of Fluorescence in Aqueous Solutions

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The quenching of fluorescence in solution has usually been discussed as a deactivation of the photoactivated molecule by a collision with the quenching ion in which some energy is transferred to the quencher. Such a process is analogous to the type assumed in the discussion of gaseous systems. A more specific picture to adopt for the mechanism of quenching would be to say that the activated molecule and the quencher react to form an intermediate, such as is assumed in the discussion of rates of reaction, and that when this intermediate decomposes into the original molecules, neither is in a state which can radiate. At first it may seem that there is no essential difference between the two views but there is a definite difference to be expected for the effect of ionic strength on the efficiency of a quencher. In this paper we shall show that the quenching process behaves exactly as a second order reaction.

According to the interionic attraction theory, the limiting law for the effect of ionic strength on the activity coefficient of an ion in water at 25° is

$$\log \gamma = -0.5 z^2 \mu^{1/2} \quad (1)$$

in which  $\gamma$  is the activity coefficient,  $z$  the charge on the ion and  $\mu$  the ionic strength. This shows that the activity of an ion at a given concentration will decrease as the ionic strength is increased. If the quenching action of ions in solution on fluorescence is considered as simply a collision of the second kind and it is assumed that the number of effective collisions is determined by the activities of the colliding molecules, it follows that raising the ionic strength will decrease the quenching efficiency of a given ion. On the other hand, if the quenching process is analogous to the rate of a bimolecular reaction involving ions in solution, the effect which will be observed will depend on the nature of the molecules involved. Thus it has been found that the logarithm of the rate constant for an ionic reaction is given by

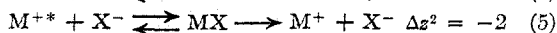
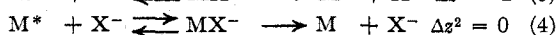
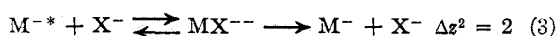
$$\log k = \log k_0 + 0.5 \Delta z^2 \mu^{1/2}$$

or

$$(2/\Delta z^2) \log k/k_0 = \mu^{1/2} \quad (2)$$

in which  $k$  is the rate constant at ionic strength  $\mu$ ,  $k_0$  is the constant at  $\mu = 0$ , and  $\Delta z^2$  is the dif-

ference between the square of the charge of the intermediate complex formed from the reactants and the sum of the squares of the charges of the reactant molecules. On the basis of this equation raising the ionic strength may increase, decrease, or have no effect on the quenching action depending on the sign of  $\Delta z^2$ . The three possibilities may be illustrated by writing the equations



The first step is written as reversible in accordance with the assumption usually made in deriving equation (2). It must be stressed, however, that the validity of that equation as a limiting law rests on an experimental basis and is independent of any assumptions which may be made in justifying it on theoretical grounds. Furthermore, the application of the equation to the quenching of fluorescence is independent of the nature of the products in (3), (4) and (5) except that the molecules of the fluorescent substance must be regenerated.

In the following paragraphs we present data on twelve systems in all of which the quenching changes in the way predicted for a bimolecular ionic reaction. The limiting law is not strictly valid at the concentrations used but the deviations observed are within the same range as found for electrolytes such as the alkali halides and halogen acids.

**Materials.**—The  $KNO_3$ ,  $NaClO_4$ ,  $KCl$ ,  $KBr$ ,  $KI$ ,  $KCNS$ ,  $AgNO_3$ , quinine sulfate and acridone used in these experiments were c. p. grade. The fluorescein was reprecipitated by dissolving it in an alkaline solution and acidifying. The anthracenesulfonic acid was prepared by a method outlined in the literature.<sup>1</sup> The acridone sulfonic acid was prepared by heating acridone and fuming sulfuric acid in the presence of glacial acetic acid to 100°, diluting, removing the excess sulfuric acid with barium hydroxide and evaporating to dryness.

**Apparatus.**—The apparatus is shown in Fig. 1. Light from the Heraeus type mercury arc S passed through the lens L and the filter F and excited the fluorescent solution in the cell C, which was placed in the water thermostat B. F was a no. 586 Corning filter and transmitted only the group of mercury lines around 3660 Å. The fluorescent light from C passed through the filter F', which absorbed

(1) *Chem. Zentr.*, **83**, 11, 1413 (1912).

all ultraviolet light, and onto the photocell  $P_1$ . In order to compensate for fluctuations in the arc, light from the same source was allowed to pass through the same type of filter  $F$  and illuminate a second photocell  $P_2$ . The currents of the two photocells were balanced with a potentiometer. With this arrangement the potentiometer readings were proportional to the intensity of light falling on  $P_1$  when adjustment was made so that no current flowed through the galvanometer  $G$ . The linearity of the potentiometer readings as the light intensity was varied was verified by placing calibrated screens between  $F'$  and  $P_1$ .

**Method.**—All the solutions used in these experiments were kept in a thermostat at  $25^\circ$ , and mixing was done immediately before the measurements were taken to prevent the possibility of photooxidation on standing. The thermostat in which the cell was placed was also kept at  $25^\circ$ .

The potentiometer reading obtained when the cell  $C$  was filled with a dye solution at a definite hydrogen ion concentration and at a definite ionic strength was recorded as  $I_0$ . Similar readings were taken at various concentrations of the quenching salt and recorded under the heading  $I$ . From these data values of the quenching constant  $k$  were calculated using the equation

$$I/I_0 = 1/(1 + kC_q)$$

where  $C_q$  is the concentration of the quenching ion.

In order to test the validity of the present explanation for the quenching of fluorescence, the charge on the fluorescent substance in solution had to be known. It was possible to fix the molecular species present by using the proper hydrogen ion concentration. Two different sets of data were recorded in the case of fluorescein: one in  $0.001 M$  perchloric acid ensuring the presence of only the un-ionized molecules and one in  $0.001 M$  potassium hydroxide to ensure having only the divalent negative ion in solution. All the measurements with quinine sulfate were taken in  $0.01 M$  nitric acid, so that the substance would be in the form of the divalent positive ion. No acid or base was added in the case of acridone, anthracenesulfonic acid or acridonesulfonic acid since acridone exists only as the neutral molecule and the other two are strong acids.

The values of  $k$  at a given ionic strength were determined for various concentrations of the quenching salts in the cases of fluorescein with potassium iodide in both acid and basic solutions and quinine sulfate with potassium chloride and bromide and silver nitrate. It was found that  $k$  depended only slightly on the concentration of

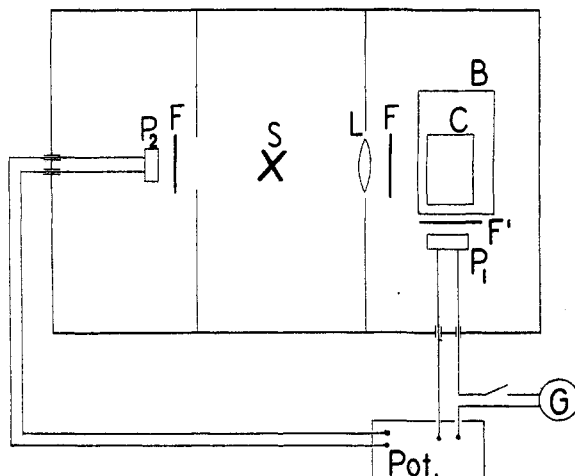


Fig. 1.—Diagram of apparatus.

the quencher. The variations that were observed showed a slight increase with increasing concentration with the negative ions but with  $Ag^+$  there was a decrease. In order to separate the effect of ionic strength from any variations of this type, values of  $k$  were determined in which the concentration of the quenching salt was kept constant and the ionic strength varied by adding sodium perchlorate in the case of fluorescein and potassium nitrate in all other cases. Small corrections were made for the quenching of quinine sulfate by potassium nitrate. No measurable quenching was observed by either potassium nitrate or sodium perchlorate with the other dyes. The results are given in the tables.

TABLE I  
QUENCHING OF QUININE SULFATE ( $c = 0.0004 M$ ) AT  $25^\circ$

$\mu$	$I/I_0$	$k$
By $0.025 M$ Silver Nitrate		
0.0367	0.882	5.3
.0617	.858	6.6
.1117	.840	7.6
.2117	.786	10.9
.4117	.720	15.5
By $0.005 M$ KBr		
0.0217	0.370	340
.0317	.393	308
.0417	.417	280
.0617	.438	256
.1117	.513	190
.2117	.531	176
.4117	.569	152
By $0.005 M$ KCl		
0.0217	0.438	256
.0617	.510	192
.1117	.562	156
.2117	.612	126
.4117	.668	100

TABLE II

QUENCHING OF FLUORESCHEIN ( $c = 1 \times 10^{-4} M$ ) BY KI AT 25°

$\mu$	$I/I_0$	$k$	$cKI$
In 0.001 M KOH			
0.031	0.745	11.4	0.03
.051	.732	12.2	.03
.101	.705	14.0	.03
.201	.550	16.4	.05
.301	.544	16.8	.05
In 0.001 M HClO <sub>4</sub>			
0.031	0.635	19.2	0.03
	.615	20.9	.03
.041	.558	19.8	.04
.051	.638	18.8	.03
	.621	20.3	.03
	.490	20.8	.05
	.500	20.0	.05
.091	.483	21.4	.05
	.508	19.4	.05
.101	.500	20.0	.05
	.333	20.0	.1
.201	.513	19.0	.05
	.330	20.5	.1
	.336	19.8	.1

TABLE III

QUENCHING OF ACRIDONE (SATD. SOLN.) AT 25°

$\mu$	$I/I_0$	$k$
By 0.01 M KI		
0.01	0.522	92
.05	.521	92
.11	.516	94
.21	.517	93.5
By 0.025 M AgNO <sub>3</sub>		
0.025	0.765	12.2
.125	.763	12.4
.425	.745	13.6

TABLE IV

QUENCHING OF ANTHRACENESULFONIC ACID ( $c = 1 \times 10^{-4} M$ ) AT 25°

$\mu$	$I/I_0$	$k$
By 0.05 M KI		
0.05	0.640	11.3
.10	.598	13.5
.25	.560	15.8
.45	.550	16.3
.55	.526	18.0
.85	.523	18.2
By 0.025 M AgNO <sub>3</sub>		
0.025	0.440	51.0
.050	.474	44.4
.125	.550	32.8
.425	.576	29.5
.525	.567	30.5
.905	.533	35.0

Fluorescein in Table II represents the first dye studied. Results in this case were not as

TABLE V

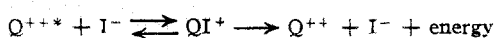
QUENCHING OF ACRIDONE SULFONIC ACID ( $c = 5 \times 10^{-5} M$ ) AT 25°

$\mu$	$I/I_0$	$k$
By 0.05 M AgNO <sub>3</sub>		
0.05	0.697	8.7
.15	.717	7.9
.25	.725	7.6
.45	.722	7.7
.65	.709	8.2
.85	.692	8.9
By 0.01 M KI		
0.0105	0.620	62.0
.03	.591	69.0
.05	.580	72.5
.11	.568	76.0
.21	.552	81.0
.31	.551	82.0
.41	.538	86.0
.51	.535	86.5
.81	.536	86.5
By 0.01 M KCNS		
0.01	0.698	43.3
.05	.663	51.0
.11	.648	54.5
.21	.634	57.5
.31	.629	59.0
.41	.624	60.0
.51	.626	60.0
.81	.620	61.0

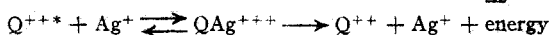
reproducible as they were in the later work due to slight improvements in the method and apparatus made after the studies on fluorescein. Also in Table II the values of  $k$  are given for more than one value of the concentration of the quencher.

### Discussion

From the data in Tables I to V, values of  $(2/\Delta z^2) \log k$  were determined and plotted against  $\mu^{1/2}$ . The values of  $\Delta z^2$  were obtained by subtracting the sum of the squares of the charges of the reacting ions from the square of the charge of the intermediate complex formed. The quenching ions in these reactions were the negative ions of the potassium halides and potassium thiocyanate used, and the silver ion in the case of silver nitrate. The charges on the dye ions were discussed before. Thus the  $\Delta z^2$  values for the reactions of the quenching of fluorescence of quinine sulfate by potassium iodide and silver nitrate would be as follows



$$\Delta z^2 = -4$$



$$\Delta z^2 = +4$$

$Q^{+++}$  and  $Q^{++}$  representing the activated and unactivated quinine ions, respectively.

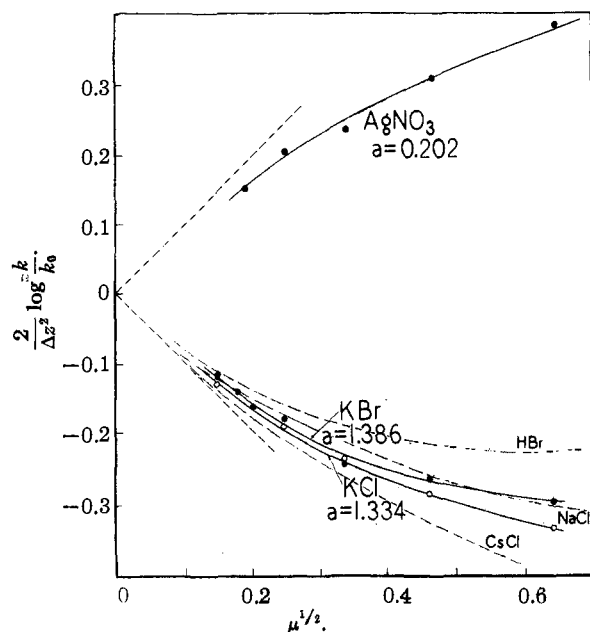


Fig. 2.—Quenching of fluorescence of quinine sulfate by  $\text{AgNO}_3$ ,  $\text{KBr}$  and  $\text{KCl}$ .

In order properly to compare the  $(2/\Delta z^2) \log k$  vs.  $\mu^{1/2}$  curves obtained with known activity coefficient curves, it was necessary to determine the value of  $\log k_0$ . This determination was accomplished by drawing in the limiting curve of slope  $\pm 1$  in the position which seemed most appropriate and extending it to the ordinate. The value of the ordinate at this intersection was taken as  $(2/\Delta z^2) \log k_0$ , and the entire curve was shifted by this amount toward the origin by moving it parallel to the  $(2/\Delta z^2) \log k$  axis. This method was equivalent to plotting  $(2/\Delta z^2) \log k/k_0$  vs.  $\mu^{1/2}$  and it brought all the curves into the

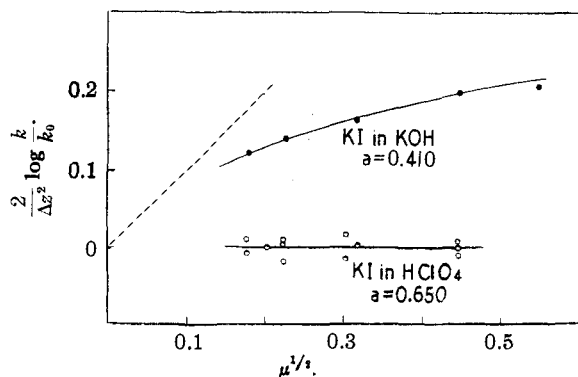


Fig. 3.—Quenching of fluorescence of fluorescein by potassium iodide in acid and basic solutions.

same region for comparison. The results are shown in Figs. 2, 3, 4, 5 and 6.

The values of  $a$  here represent  $(2/\Delta z^2) \log k_0$ . The dotted lines of slope  $+1$  and  $-1$  represent the limiting slope predicted by the Debye-Hückel theory. The activity coefficient curves for hydrobromic acid, sodium chloride, and cesium chloride are shown dotted in Fig. 2 for comparison.

The possibility of explaining the quenching of fluorescence as due to the tying up of part of the dye ions by complex formation with the quenching ions before activation of the dye was considered. Absorption measurements were made on solutions of quinine sulfate, fluorescein and acridone sulfonic acid in the presence and absence of the quenching ions used in each case. These measurements were taken with such concentrations of the dyes that about 50% of the incident light was absorbed. A negligible decrease in absorption in the presence of the quenching salts was noted in all cases except those involving  $\text{Ag}^+$ , where a small increase (less than 1%) was observed. Since it is very improbable that the complexes in all cases would have the same absorption coefficients as the dye molecules

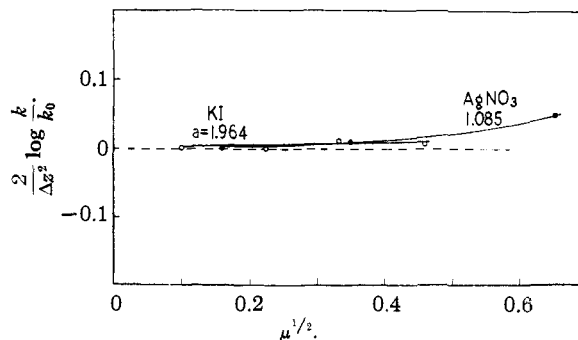


Fig. 4.—Quenching of fluorescence of acridone by silver nitrate and potassium iodide.

themselves, these results may be interpreted as eliminating the possibility of explaining the quenching by complex formation before activation of the dye.

It may be well to emphasize here the importance of fixing the molecular species in solution of those dyes which are weak electrolytes. For our purpose the charge on the ions had to be known, and it was pointed out by Desha, Sherrill and Harrison<sup>2</sup> and later by Eisenbrand<sup>3</sup> that the form of the molecules due to the value of  $\text{pH}$  has an enormous effect on the intensity of fluorescent

(2) Desha, Sherrill and Harrison, *THIS JOURNAL*, **48**, 1493 (1926)  
(3) Eisenbrand, *Z. physik. Chem.*, **A144**, 441 (1929).

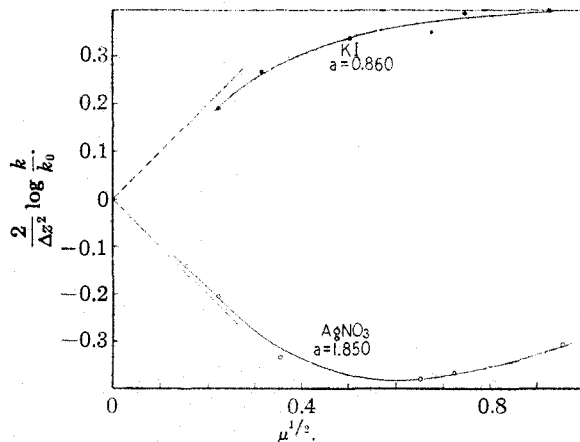


Fig. 5.—Quenching of fluorescence of anthracene sulfonic acid by silver nitrate and potassium iodide.

light in the region of hydrogen ion where dissociation takes place. This ensuring of a single definite molecular species in solution has been overlooked by many investigators of fluorescence quenching.

From the data presented it can be seen that the variations of values of  $(2/\Delta z^2) \log k/k_0$  with varying ionic strength are of the same type and magnitude as those known to occur in ordinary bimolecular reactions. In every case the curves are of the required form and in every case the increase or decrease of the values of the quenching constants at low values of  $\mu$  is in accordance with the sign of  $\Delta z^2$ . In view of these results and the considerations of the previous paragraphs, it follows that the quenching is due to an ordinary bimolecular reaction between the activated dye molecules and the quenching ions.

It must be pointed out here that in the present paper no attempt is made to attribute the decrease in fluorescence intensity with increasing dye concentration to the ionic strength effect on the quencher. Only the quenching due to known non-fluorescing quenching ions was explained in this way.

From the results of the present work the study of the quenching of fluorescence seems to offer a very convenient means for studying a simple bimolecular reaction. The first step of such a process may be determined directly without having to consider the products formed as is usually the case. A perhaps even more useful

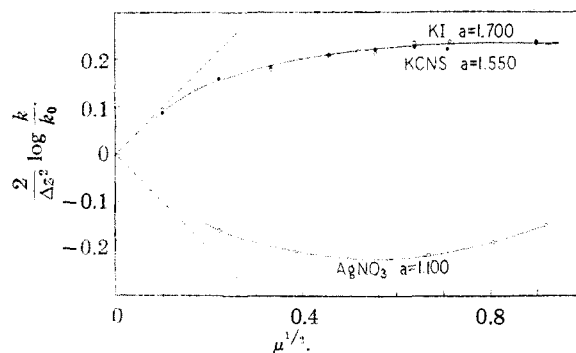


Fig. 6.—Quenching of fluorescence of acridone sulfonic acid by  $\text{AgNO}_3$ , KI and KCNS.

possibility is to reverse the process used here, that is, assume the reaction to be of the ordinary bimolecular type and then determine the molecular species involved by a study of the variations of  $\log k$  with varying ionic strength. Such an application should be of considerable importance in determining the form of the ions present in non-aqueous solutions.

#### Summary

The quenching constants at various values of ionic strength were determined for the quenching of fluorescence of quinine sulfate by potassium chloride and bromide and silver nitrate; of fluorescein by potassium iodide in acid and basic solutions; of acridone by potassium iodide and silver nitrate; of anthracene sulfonic acid by potassium iodide and silver nitrate and of acridone sulfonic acid by potassium iodide, potassium thiocyanate and silver nitrate.

The values of  $(2/\Delta z^2) \log k$  were plotted against  $\mu^{1/2}$  and in every case the curves obtained were of the same type as those expected for an ordinary bimolecular reaction.

Absorption measurements were made with the dye solutions in the presence and absence of the quenching ions. Only a negligible change of absorption was observed, thus eliminating the possibility of explaining the quenching as due to complex formation of the dye with the quencher before excitation of the former.

The quenching in all cases appeared to be due to an ordinary bimolecular reaction between the quenching ion and the activated dye molecule.