

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

The Kinetics of Diffusion Controlled Molecular and Ionic Reactions in Solution as Determined by Measurements of the Quenching of Fluorescence^{1,2}

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Introduction

The prevailing theory of chemical kinetics asserts that the absolute rate of reaction is determined by the concentration of activated complexes, in equilibrium with the reactants and products, multiplied by the frequency with which these critical complexes decompose to form the end products. However, in exceedingly rapid reactions, such as the quenching of fluorescence by iodide ions, the rate controlling process is the diffusion of the reactants toward one another, as proved by the inverse dependence of the rate on the viscosity of the medium. In such cases the concept of a critical complex intermediate between the reactants and products is still real but is not important in calculating the reaction rate.

Since Brönsted⁴ postulated his theory of the primary kinetic salt effect upon the existence of critical complexes, the dependence of the rate of quenching of fluorescence on the square root of the ionic strength of the solution has been taken^{5,6} as *prima facie* evidence that the quenching process involves more than mere collision, *i. e.*, the rate is controlled by the formation of an activated complex. This view, however, is erroneous, because the Brönsted kinetic salt effect can be explained equally well from the viewpoint of a rate controlled by collision processes.^{7,8,9,10}

Recently, Debye¹⁰ has revived the classical Smoluchowski¹¹ diffusion treatment of collision processes as a means of evaluating the frequency factor of the absolute rate of reactions in solution. He extends the Smoluchowski treatment to include ionic reactions by considering the electrostatic interactions arising from the net charges of the ions of the reacting species as well as the

ions of the medium. This calculation yields the same salt effect of the ions of the medium as the equilibrium (activation-controlled) theory, but the dependence upon the self-potential of the reactants takes a form which approaches the familiar Christiansen-Scatchard term only as a limiting case.

Previous to Debye, Sveshnikoff and Wawilow¹² applied Smoluchowski's theory to fluorescence quenching and included transient state (*i. e.*, time dependent) diffusion but not charge effects. Debye, on the other hand, includes charge effects but not transient state diffusion. Hence we present our own development of the general case *including charge and transient effects* and apply it to our new quenching data, which were obtained with a specially designed fluorimeter.

Theory

I. The Model for Calculating Diffusion-Controlled Reaction Rates in Solution.—Because of the condensed nature of liquids, collisions in solutions are not as simple as in gases. The reactants A and B diffuse through the solution, as a result of thermal motion, and finally collide. The pair is surrounded by a cage of solvent molecules and in this manner is held in coordination so that A and B execute motion or vibration relative to each other. This "sticky" collision is termed an *encounter* by Fowler.¹³ In ideal solutions, the product of the encounter frequency by the number of vibrations per encounter very roughly equals the gaseous collision frequency,¹⁴ but the distribution of collisions is different (collisions occur in sets). In activation controlled reactions this product, the number of encounters per second times the number of vibrations per encounter, is the "frequency factor." However, in diffusion controlled reactions the reaction rate equals the encounter rate or the number of times A and B diffuse together per second, because every encounter results in reaction, which is not true for reactions of the slow activation type. Therefore the problem is to calculate the encounter rate of ions A^a and B^b in solution.

The macroscopic diffusion model which represents the microscopic Brownian motion under discussion derives from Smoluchowski,¹¹ who obtained an expression for the rate of coagulation of homogeneous sols. According to his model, the particles in a coagulating sol collide owing to thermal motion and adhere to each other. Tu-

(1) Presented at American Chemical Society meetings in May, 1943 (New York Section), September, 1943 (Pittsburgh), and September, 1944 (New York).

(2) Dissertation submitted by Jacob Quentin Umberger in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

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(4) Brönsted did not assume equilibrium between the complex and the reactants and products. Bjerrum, however, introduced the concept of equilibrium to kinetics in his derivation of the Brönsted equation.

(5) R. W. Stoughton and G. K. Rollefson, *THIS JOURNAL*, **61**, 2634 (1939); **62**, 2264 (1940).

(6) Glasstone, Laidler and Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 337.

(7) V. K. LaMer and M. E. Kammer, *THIS JOURNAL*, **57**, 2662 (see p. 2668) (1935).

(8) H. G. Davis and V. K. LaMer, *J. Chem. Phys.*, **10**, 585 (see p. 594) (1942).

(9) O. K. Rice, *Am. N. Y. Acad. Sci.*, Vol. XLI, Art. 3, 227 (1941).

(10) P. Debye, *Trans. Electrochem. Soc.*, **82**, 265 (1942).

(11) M. v. Smoluchowski, *Z. physik. Chem.*, **92**, 129 (1917).

(12) Sveshnikoff, *Acta Physicochim.*, U.R.S.S., **3**, 257 (1935).

(13) R. H. Fowler and E. A. Guggenheim, "Statistical Thermodynamics," Cambridge University Press, 1939.

(14) *Ibid.*, p. 530.

mila¹⁵ found close agreement between experiments with coagulating sols and the equation of Smoluchowski.

II. The Calculation.—Consider a solution of bulk concentrations n_a^0 and n_b^0 of particles A and B per cc. Hold A fixed, and let B approach A by spherically symmetrical thermal motion. At distance R (near the surface of A, see Fig. 1) the concentration of B is kept at zero by the reaction, and at large distances the concentration is n_b^0 .

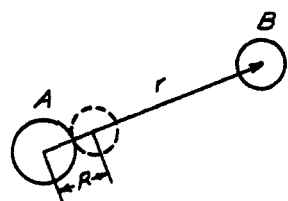


Fig. 1.— $R = r_a + r_b$ is the distance between A and B at which reaction occurs.

At intermediate distances from A, the concentration of B is n_b , except when time is zero the concentration is uniformly n_b^0 . We want to calculate the flow of B particles diffusing, under this concentration gradient, toward A, and to this end we consider the forces acting between A and B and set up the differential equation of motion of B toward A:

(1) Diffusion or thermal force = $-kT/n_b \cdot (\partial n_b / \partial r)_{i,T}$, *i. e.*, a force proportional to the concentration gradient. k is Boltzman's constant, T is the absolute temperature, and t is the time.

(2) Friction force = ρ_b (velocity of B toward A) = $-\rho_b dr/dt$. This force represents the viscous drag of the solvent; ρ_b is the friction coefficient of B in the given solvent and equals $6\pi\eta r_b$ if Stokes' law is applicable.

(3) Electrical force = $-(\partial U / \partial r)_{i,T}$, where U is the mutual electrical potential energy between A and B.

The sum of these forces (dynamical reaction, $-ma$, is neglected since the mass is only 10^{-22} g.) is zero, therefore

$$-\frac{kT}{n_b} \left(\frac{\partial n_b}{\partial r} \right)_{i,T} - \rho_b \frac{dr}{dt} - \left(\frac{\partial U}{\partial r} \right)_{i,T} = ma \simeq 0 \quad (1)$$

Solving for the velocity and multiplying by the concentration, at the point in question, yields the flow, *i. e.*, the negative of the number of B particles flowing per second toward A through an area of one square centimeter at right angles to the direction between A and B.

$$\text{Flow} = n_b \frac{dr}{dt} = - \left[\frac{kT}{\rho_b} \left(\frac{\partial n_b}{\partial r} \right)_{i,T} + \frac{n_b}{\rho_b} \left(\frac{\partial U}{\partial r} \right)_{i,T} \right] \quad (2)$$

This expression for the flow is incomplete for the reason that A was held fixed. Correcting for the motion of the central molecule-ion A, we have

$$n_b \frac{dr}{dt} = - \left[kT \left(\frac{1}{\rho_a} + \frac{1}{\rho_b} \right) \left(\frac{\partial n_b}{\partial r} \right)_{i,T} + \left(\frac{1}{\rho_a} + \frac{1}{\rho_b} \right) n_b \left(\frac{\partial U}{\partial r} \right)_{i,T} \right] \quad (3)$$

Introduce the new dependent variable $a = n_b e^{U/kT}$, where a is the thermodynamic activity at position r at time t (a is normalized to equal

n_b when U is zero). The expression for the flow now simplifies to

$$n_b \frac{dr}{dt} = -(D_a + D_b) \frac{n_b}{a} \left(\frac{\partial a}{\partial r} \right)_{i,T} \quad (4)$$

where $D_a = kT/\rho_a$ and $D_b = kT/\rho_b$.

The divergence of the flow is the source strength of ions B, at position r at time t , and is equal to the negative of the rate of change of B concentration with time

$$\left(\frac{\partial n_b}{\partial t} \right)_{r,T} = D \nabla \cdot \left[\frac{n_b}{a} \left(\frac{\partial a}{\partial r} \right)_{i,T} \right] \quad (5)$$

which simplifies to

$$\frac{\partial a}{\partial t} = D \left[\frac{\partial^2 a}{\partial r^2} + \frac{\partial a}{\partial r} \left(\frac{2}{r} - \frac{1}{kT} \frac{\partial U}{\partial r} \right) \right] \quad (6)$$

This is Fick's equation corresponding to the spherical interdiffusion of ions with an interaction energy U . The expression $-\partial U/kT\partial r$ equals L/r^2 , where L is the distance between A and B at which the thermal energy, kT , equals the coulombic energy of interaction of the net charges, $z_a e$ and $z_b e$, of A and B. In terms of the new independent variables $x = r/L$ and $\tau = t/L^2$, equation (6) takes the form

$$\frac{\partial a}{\partial \tau} = D \left[\frac{\partial^2 a}{\partial x^2} + \frac{\partial a}{\partial x} \left(\frac{2}{x} + \frac{1}{x^2} \right) \right] \quad (7)$$

Debye solved only the special case corresponding to the stationary state, *i. e.*, $\partial a / \partial t = 0 = \partial n_b / \partial t$. His solution is

$$a = n_b^0 \left[1 - \int_r^\infty e^{U/kT} \frac{dr}{r^2} / \int_R^\infty e^{U/kT} \frac{dr}{r^2} \right] \quad (8)$$

We have not yet succeeded in solving the general equation (7), but for the case of $2/x \gg 1/x^2$ the solution is

$$a = n_b^0 \left[1 - \frac{R}{r} + \frac{R}{r} \frac{2}{\sqrt{\pi}} \int_0^{r-R} \frac{e^{-z^2}}{2\sqrt{Dt}} e^{-z^2} dz \right] \quad (9)$$

or

$$n_b = n_b^0 e^{-U/kT} \left[1 - \frac{R}{r} + \frac{R}{r} \frac{2}{\sqrt{\pi}} \text{Erf} \left(\frac{r-R}{2\sqrt{Dt}} \right) \right] \quad (10)$$

Our expression (10) is the final approximate form of the dependence on position and time of the average concentration of species B around A, as A moves about in solution.

The number of encounters between one central body A and all bodies B per second is I , the flow toward A through a spherical area of $4\pi R^2$ square cm.

$$I = 4\pi R^2 D e^{-U/kT} \left. \frac{\partial a}{\partial r} \right|_{r=R} = 4\pi D R n_b^0 \left[1 + \frac{R}{\sqrt{\pi Dt}} \right] / e^{U/kT} \quad (11)$$

In the case of the stationary state (without the approximation that $2/x \gg 1/x^2$)

$$I = 4\pi D R n_b^0 / R \int_R^\infty e^{U/kT} \frac{dr}{r^2} \quad (12)$$

It is seen that, after a short time, equations (11) and (12) become identical except for the effect

of the interaction energy, U . This difference, which is due to the approximate nature of (11), is seen when the respective U expressions are expanded: $e^{U/kT} = e^{-L^2/LR}$, and

$$R \int_R^\infty e^{U/kT} \frac{dr}{r^2} = e^{-L^2} (e^{L/R} - 1)/L/R$$

Further calculations indicate that equation (11) is improved by substituting

$$f = R \int_R^\infty e^{U/kT} \frac{dr}{r^2} \text{ for } e^{U/kT}$$

Assume the stationary state solution $4\pi DRn_b^0/f$, where f is the factor expressing the effect of interionic forces on the rate of diffusion. This calculation is for just one central body A. However, there are n_a^0 bodies of species A per cc. and, accordingly, the number of encounters per second per cc. between species A and species B is

$$4\pi DRn_a^0n_b^0/f = \text{encounter frequency} \quad (13)$$

For reactions involving little chemical activation energy, every encounter with the proper orientation (insert a probability factor P) is effective in reaction. Hence the reaction rate is $P4\pi DRn_a^0n_b^0/f$ molecules per second per cc., and the molar rate constant is

$$P4\pi DRN_{Av}/1000f \text{ liters per second per gram mole} \quad (14)$$

If, due to an energy of activation, the concentration of species B is not zero on the surface of species A, but instead is some fraction, α , of the bulk concentration, n_b^0 , we must then multiply the rate expression (14) by $(1 - \alpha)$.

Assuming that Stokes' law for the viscous drag of the solvent on the diffusing species can be employed¹⁶ for molecules in solution, D becomes

equal to $\frac{kT}{6\pi\eta} \left(\frac{1}{r_a} + \frac{1}{r_b} \right)$, and the molar rate constant

$$k_r = \frac{P(1 - \alpha)}{f} \frac{8N_{Av}kT}{3000\eta} \left(\frac{2 + r_a/r_b + r_b/r_a}{4} \right) \quad (15)$$

It is apparent that kT/η is important in calculating encounter frequencies in condensed media in which Stokes' law is applicable.

III. The Significance of kT/η in Calculating Collision Frequencies in Gaseous Media.—In gases, the mean free path is larger than the particle size and, therefore, equation (15) is not applicable. However, one can show the significance of kT/η by the following inductive method, in which the gaseous collision frequency is expressed in terms of the gaseous viscosity. Gas kinetic theory obtains for the self-collision frequency of molecules A

$$\nu_a = 8r_a^2 \sqrt{\frac{\pi kT}{m_a}} n_a^2$$

The best value for the gaseous viscosity¹⁷ is

(16) H. Falkenhagen, "Electrolytes," Oxford University Press, New York, N. Y., 1934, p. 179.

(17) J. H. Jeans, "The Dynamical Theory of Gases," Cambridge University Press, 1925.

$$\eta_a = 0.084 \left(\frac{2}{\pi} \right)^{1/2} \frac{\sqrt{m_a kT}}{r_a^2}$$

Therefore in terms of the viscosity, the gaseous collision frequency becomes $\sim 2kTn_a^2/3\eta_a$. This result indicates that kT/η , the ratio of the driving energy of heat motion to the viscosity resisting motion, is a fundamental frequency factor for calculating encounter frequencies in liquids and gases.

IV. The Application of Smoluchowski's Theory to the Quenching of Fluorescence.—It is important to consider the effect of the time dependent, or transient state, part of the general solution, equation (11), for the encounter frequency as discussed by Sveshnikoff¹² and Wawilow, "It is not hard to see, by calculating the diffusion for the time of the mean life of the excited molecule, that the second term (time dependent) is comparable to the first." Their calculation yields the ratio of unquenched to quenched fluorescence intensity to be

$$I_0/I = [1 + \beta\tau_0]\delta \quad (16)$$

where β is the stationary state encounter frequency between one excited molecule and all quenching molecules, τ_0 is the average life of the excited molecules with no quencher present, and δ is a factor (> 1) expressing the effect of the transient state diffusion. The factor δ is complicated, but we find that if the quenching is not too large ($< 50\%$), equation (16) becomes

$$\frac{I_0}{I} - 1 = \beta\tau_0 \left[1 + \frac{R}{\sqrt{\tau_0 D}} \right] \quad (17)$$

Sveshnikoff¹⁸ uses δ to explain the experimental fact that the molar rate of quenching of the fluorescence of fluorescein ion by iodide ion increases as the concentration of potassium iodide is increased. However, the data of Stoughton and Rollefson and of the present authors prove conclusively, that this increase in rate is due mainly to the reduction of the repulsion between the fluorescein and iodide ions caused by the screening effect of the potassium ion atmospheres about these two negative ions. The effect of charges can be approximately expressed by dividing the right side of equation (17) by the factor f , as shown by equation (11) in conjunction with the derivation of Sveshnikoff and Wawilow. For high concentrations of potassium iodide, however, the salt effects are less important than the factor δ in explaining abnormally high quenching.

In their derivation, Sveshnikoff and Wawilow assume that the concentration of the quencher is zero on the surface of the excited fluorescent species, in spite of the fact that the quencher is regenerated by the reaction. To test this assumption, we have repeated the calculation in a different way, namely, the quencher is now made the center of diffusion, and the concentration of

(18) B. Sveshnikoff, *Acta Physicochim. U.R.S.S.*, **4**, 462 (1936).

TABLE I
 IODIDE QUENCHING OF H₂FI

Observer	KI, M	Ionic strength, M	H ₂ FI, M	HClO ₄ , M	k _Q , a.d.	Temp. °C.
Stoughton and Rollefson ⁴	0.03 to 0.1	0.031 to 0.201	10 ⁻⁴	0.001	20.0 ± 0.6	25
Umberger	.005 to .025	.007 to .027	10 ⁻⁵	.002	21.4 ± .2	24
Hodges	.005 to .025	.007 to .027	10 ⁻⁵	.002	21.5 ± .7	24
Hodges (unpublished)	.005 to .025	.007 to .027	2 × 10 ⁻⁵	.002	20.9 ± .4	24

excited fluorescent molecules is taken to be zero on the surface of the quencher, due to the quenching. Certain simplifying approximations are made in the calculation, but the important result is that the final form contains the factor $[1 + (R/\sqrt{\tau_0 D})]$ as in equation (17). This is evidence for the almost obvious conclusion that the diffusion calculation is the same whether the fluorescent molecule or the quencher is made the center of diffusion.

Experimental Results and Discussion

I. The Kinetics of Quenching.—The quench-

ing process, $FI^{*-} + I^- \xrightarrow{k} FI^- + I^- + \text{heat}$, involves an encounter between a dye molecule excited by light absorption (fluorescein ion) and a quencher (iodide ion). Following the encounter, the energy of excitation is converted into thermal motion of the surrounding solvent molecules. The detailed mechanism and the forces involved in the degradation of the excitation energy to thermal energy will be treated in a later paper.

A direct measurement of the time decay of fluorescence, although the most obvious means of investigating quenching, is too difficult for precision studies because of the extremely short half-life ($\sim 10^{-8}$ sec.). Recourse must be taken in measurement of the photo-stationary state. In this state, the intensity of fluorescence is constant, indicating that the concentration of excited molecules remains constant, *i. e.*, $d[FI^{*-}]/dt = 0$, and the rate of production of excited molecules by absorption of incident light, $CI[FI^-]$, equals the rate of destruction of excited molecules by fluorescence and by quenching encounters. For the quenched solution

$$CI[FI^-] = \frac{1}{\tau_0} [FI^{*-}] + k[FI^{*-}][I^-] \quad (18)$$

and for the unquenched solution

$$CI[FI^-] = \frac{1}{\tau_0} [FI^{*-}]_0 \quad (19)$$

C is the molar absorption parameter for the fluorescent dye, I is the intensity of the exciting light, and k is the molar rate constant for the quenching process. The quenching constant, k_Q , is defined as the ratio of the molar quenching and fluorescence rates and equals $\tau_0 k$. Solving (18) and (19) for the respective concentrations of the excited molecules yields

$$[FI^{*-}] = CI[FI^-] \frac{1}{\tau_0 + k[I^-]} = CI[FI^-] \tau_0 \quad (20)$$

$$[FI^{*-}]_0 = CI[FI^-] \tau_0 \quad (21)$$

TABLE II

IODIDE QUENCHING OF H ₂ FI	
10 ⁻⁵ M fluorescein; 0.002 M HClO ₄ ; T = 24°	
KI, M	k _Q
0.005	21.8
.010	21.1
.015	21.7
.025	21.5
.100	25.3

τ is the average life of the excited molecules in the quenched solution and is always less than τ_0 , *i. e.*, $1/\tau = 1/\tau_0 + 1/t$ where t is the average time between quenching encounters.

The quenched and unquenched solutions are illuminated equally, and since the fluorescence intensities, I and I_0 , are proportional to the stationary state concentrations of the excited molecules

$$[FI^{*-}]_0/[FI^{*-}] = I_0/I = \tau_0/\tau = 1 + \tau_0 k [I^-] \quad (22)$$

This reduces to

$$\frac{I_0}{I} - 1/[I^-] = k_Q \quad (23)$$

Equation (23) is the classical equation first developed by Stern and Volmer²⁹ and is equivalent to equation (17). Since equation (17) is a limiting form of equation (16), it is clear that the Stern-Volmer equation is applicable only when the per cent. quenching is not too large. However, the experimental results now to be described are in the range where the Stern-Volmer expression is valid.

With the photoelectric apparatus described in the appendix, the quenched and unquenched fluorescence intensities of fluorescein, in the *basic* (FI⁻) and the *acid* (H₂FI) forms, have been measured using the 0.365 μ line of Hg as the exciting light. Table I summarizes the quenching constants of the acid form of fluorescein. In Table II are the data of a typical run.

The high value of k_Q at 0.1 M KI is probably explained by the failure of the Stern-Volmer equation for large quenching.

The quenching reactions of the acid form of fluorescein exhibit no salt effect (because H₂FI is a neutral molecule) and little dependence on the concentration of fluorescein. The molar quenching rate is about twenty-one times faster than the molar fluorescence rate. Since τ_0 for the acid form of fluorescein has not been measured, we cannot compare the experimental quenching rate with theory.

Considering next the quenching of the basic

form of fluorescein, the results of Stoughton and Rollefson⁵ for 10^{-4} *M* fluorescein have been verified, but in addition it is found that as the concentration of fluorescein is decreased, the quenching constant decreases also. This phenomenon can be explained as a result of the partial absorption of the fluorescence light, by the fluorescein solution, in its transit from the body of the solution to the photocell. Qualitatively, one can understand that if a photon of fluorescence light is absorbed, the probability of its being converted into heat by an iodide ion encounter increases, because the time spent by the photon in solution is increased. This absorption effect depends upon the geometry of the apparatus as well as on the concentration of fluorescein and probably explains the discordance between the quenching measurements of various experimenters.

This same dependence on the concentration of fluorescein ion was observed when the solvent was methanol or 2 *M* aqueous sodium perchlorate. However, in the case of the acid form of fluorescein the dependence of the quenching constant on the fluorescein concentration is small, because the absorption coefficient and the fluorescence efficiency are less than for fluorescein ion.

II. The Calculation of Fluorescence Absorption.—The experimental quenching data were obtained with the arrangement of Fig. 2, and it can be seen that for dilute solutions of dye the fluorescence light must be transmitted through an average thickness of about 1 cm. of solution. However, for concentrated solutions the fluorescence reflected by the mirror is less important, and the average transit distance is less than 1 cm. and decreases with the concentration.

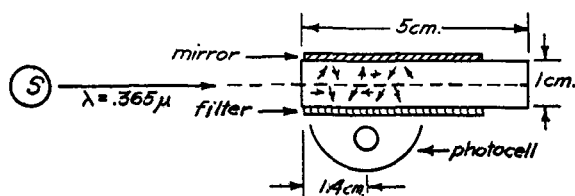


Fig. 2.—Arrangement of light source, fluorescent solution, and photocell.

It has been noted that when observed through even a 1 mm. layer of solution, the fluorescence of 10^{-4} *M* fluorescein ion is yellow, while for 10^{-6} *M* fluorescein ion the fluorescence is green. This is shown in Fig. 3, which displays the overlap and the mirror symmetry of the absorption and fluorescence spectra of fluorescein ion in dilute aqueous solution. The fluorescence peak is calculated to be lowered and shifted from 0.521 to 0.533 μ by being viewed through a 1 cm. layer of 10^{-4} *M* fluorescein, as shown by the dotted curve.

The intensity of fluorescence of a given molecular species varies from solvent to solvent

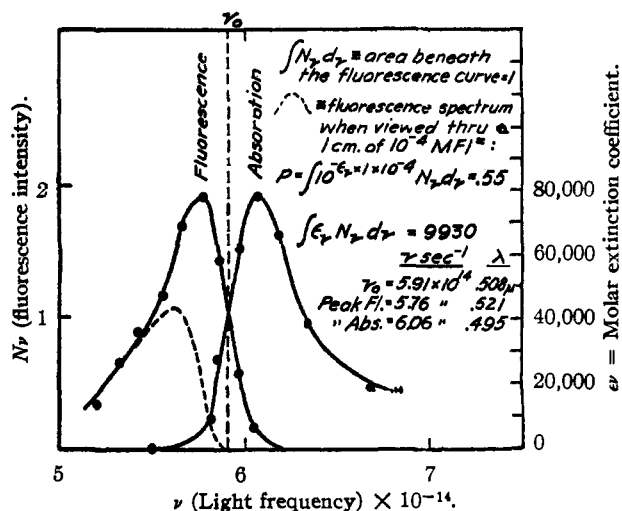


Fig. 3.—Mirror symmetry of absorption and fluorescence of fluorescein ion.^a

^a Lewschin, *Z. Physik*, **72**, 368 (1931).

proportional to the average life of the excited state of the molecules, provided the absorption remains constant, as proven by equation (22). If secondary factors, such as the partial absorption of the fluorescence, come into play, this proportionality is disturbed. For dilute aqueous solutions: let τ_0^0 = the idealized average life of the excited state with no solvent or internal quenching, *i. e.*, with 100% quantum efficiency; τ_0 = the actual average life of the excited state = 0.45×10^{-8} second for fluorescein ion,^{19,20} quantum efficiency of fluorescence = $\tau_0/\tau_0^0 = 0.85$; and τ = the average life of the excited state with quencher present, quantum efficiency of fluorescence = τ/τ_0^0 .

When a photon of fluorescence light is emitted by a fluorescein ion somewhere in the body of the solution, there is a probability p' that it will be transmitted through the solution and the filter to the photocell. Define p as the probability of transmission through the solution, only, and hence $(1 - p)$ is the probability of absorption by the solution. Now compare two excited fluorescein ions, the first in the absence and the second in the presence of a quencher. The probabilities of fluorescence are the respective efficiencies τ_0/τ_0^0 and τ/τ_0^0 . The apparatus, however, responds only to those photons which reach the photocell, and the response of the photocell is therefore proportional to $\tau_0/\tau_0^0 p'$ for the unquenched solution. However, this is incomplete, because there is a probability, $(1 - p)\tau_0/\tau_0^0$, of the photon being absorbed by the solution and then being re-emitted, and the probability of this new photon reaching the photocell is again p' . This process of fluorescence, absorption, re-fluorescence, re-absorption, etc., continues "ad infinitum," *i. e.*

(19) E. Gaviola, *Z. Physik*, **42**, 853 (1927); W. Syzmaniowski, *ibid.*, **95**, 440 (1936).

(20) F. Perrin, *Ann. Phys.*, [10S], **12**, 169 (1929).

$$I_0 = \frac{\tau_0}{\tau_0^0} \left[p' + (1-p) \frac{\tau_0}{\tau_0^0} p' + (1-p) \frac{\tau_0}{\tau_0^0} (1-p) \frac{\tau_0}{\tau_0^0} p' + \dots \right] = \frac{\tau_0}{\tau_0^0} p' / \left[1 - (1-p) \frac{\tau_0}{\tau_0^0} \right]$$

where I_0 is the probability that a fluorescence photon, emitted by a fluorescein ion with no quencher present, will reach the photocell. If quencher is present

$$I = \frac{\tau}{\tau_0^0} p' / \left[1 - (1-p) \frac{\tau}{\tau_0^0} \right]$$

Therefore

$$I_0/I = \frac{\tau_0}{\tau} \left[1 - (1-p) \frac{\tau}{\tau_0^0} \right] / \left[1 - (1-p) \frac{\tau_0}{\tau_0^0} \right];$$

$$\frac{I_0}{\bar{l}} - 1/Q = k_Q = \tau_0/t[Q] / \left[1 - (1-p) \frac{\tau_0}{\tau_0^0} \right] \quad (24)$$

If the quenched and unquenched fluorescence intensities are measured with the same filter-photocell combination, the factor p' , expressing the specific effects of the filter-photocell combination, cancels out. $\tau_0/t[Q]$ is the ratio of the average time of fluorescence to the average time between quenching collisions in a solution of 1 M quencher, and is the true quenching constant, *i. e.*, the value extrapolated to zero concentration of fluorescein ion, $k_Q^{C=0}$.

If the fluorescence light were strictly monochromatic, $(1-p)$ would equal $1 - e^{-k_f \bar{l} C}$, the Beer-Lambert expression for the absorption of radiation of frequency ν , where \bar{l} is the average distance of photon transit through the solution. However, the fluorescence is a spectral band about 1 μ wide, and p must be averaged over the band, *i. e.*, $p = \int e^{-k_f \bar{l} C} N_\nu d\nu$, where $N_\nu d\nu$ is the probability that the fluoresced photon will be of frequency between ν and $\nu + d\nu$ and is obtained from the fluorescence intensity-frequency distribution curve of Fig. 3. If the concentration of fluorescein is low ($< 10^{-5} M$): $p \cong 1 - \bar{l} C \int k_f N_\nu d\nu$, and

$$\ln k_Q^C / k_Q^{C=0} = - \ln \left[1 - \frac{\tau_0}{\tau_0^0} \bar{l} C \int k_f N_\nu d\nu \right] \cong \frac{\tau_0}{\tau_0^0} \bar{l} C \int k_f N_\nu d\nu \quad (25)$$

This equation predicts a linear plot of $\log k_Q$ versus the concentration of dye, as was found experimentally for low concentrations of fluorescein ion.

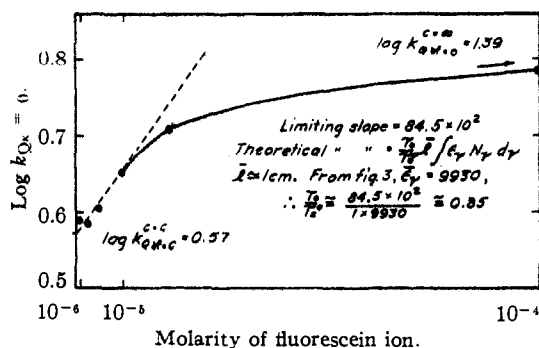


Fig. 4.—Quenching constants (zero ionic strength) vs. fluorescein concentration.

By plotting $\log k_Q^C$ against C , the concentration of dye, the limiting slope can be used to obtain τ_0/τ_0^0 . It is suggested that this procedure will prove to be a feasible method of avoiding the difficulties^{21,22} encountered in measuring fluorescence efficiencies directly. Taking the average photon transit distance through the solution to be 1 cm., it is found that if τ_0/τ_0^0 is 0.85 (85% quantum efficiency), equation (24) fits the experimental data from zero to $10^{-4} M$ fluorescein ion within a few per cent. Wawilow^{22,23} measured the ratio of emitted to absorbed energy directly and found 0.80 for fluorescein ion, which when converted to quantum efficiency becomes 0.84. This close agreement is fortuitous, for neither we nor Wawilow claim high accuracy for our respective quantum efficiency measurements.

From equation (24), it is seen that as the concentration of fluorescein ion increases indefinitely, k_Q approaches $k_Q^{C=0} / 1 - \frac{\tau_0}{\tau_0^0}$. This may prove to be the best method for obtaining the quantum efficiency.

The above treatment suffices as an explanation and method of correcting data for the dependence of the quenching constants on the concentration of dye as shown in Fig. 4, where $\log k_{Q_{\kappa=0}}$ is plotted against the concentration of fluorescein ion. The extrapolated iodide ion quenching constant is 3.7 at 23° for zero ionic strength. This value is low compared to previous measurements recorded in the literature, but we have found an independent experimental check in F. Perrin's²⁰ polarization measurements. He found the degree of polarization of the fluorescence of fluorescein ion to be 0.017 and 0.062 in pure water and 0.301 M aqueous potassium iodide, respectively. Perrin²⁰ derived a relationship between degree of polarization and average life of fluorescence, and from this we find $\tau_0/\tau = 4.1$. From equation (22) $k_Q = 10.3$, which when extrapolated to $\kappa = 0$ yields $k_{Q_{\kappa=0}} = 3.4$, as compared to our value 3.7. Values recorded in the literature are higher since they are not extrapolated to zero ionic strength and zero concentration of fluorescein.

III. Comparison of Experimental and Theoretical Encounter Frequencies.—In the next section it is shown that the experimentally observed self-quenching constant of fluorescein ion, when extrapolated to zero ionic strength and zero concentration of fluorescein ion, is $k_{Q_{\kappa=0}^{C=0}} = 8.2$. Now we calculate the self-quenching constant using the theoretical equation

(21) E. J. Bowen, "Chemical Aspects of Light," Oxford University Press, New York, N. Y., 1942, pp. 108-111.

(22) P. Pringsheim and M. Vogel, "Luminescence of Liquids and Solids," Interscience Publishers, Inc., New York, N. Y., 1943, pp. 28, 57-58.

(23) S. I. Wawilow, *Z. Physik*, **22**, 266 (1924).

$$k_Q = \frac{8N_A kT}{3000\eta} \tau_0 \left(\frac{2 + r_a/r_b + r_b/r_a}{4} \right) \left(1 + R/\sqrt{\tau_0 D} \right) / R \int_R^\infty \frac{e^{U/kT} dr}{r^2} \quad (26)$$

which, for $\kappa = 0$ and $r_a = r_b$, becomes

$$k_{Q\kappa=0} = 32 \left(1 + R/\sqrt{\tau_0 D} \right) \frac{L/R}{e^{L/R} - 1}$$

The volume of the fluorescein molecule is 500 cubic Å.²⁰ Assuming a spherical molecule, $r = 4.9$ Å., $R = 2r = 9.8$ Å., $L/R = 2.9$, $\sqrt{\tau_0 D} = 18$ Å., and therefore $k_{Q\kappa=0}$ (theoretical) = 8.1 as compared with $k_{Q\kappa=0}^{C=0}$ (experimental) = 8.2. This very close agreement is fortuitous. Cram²⁴ measured the average life of Fl^{*-} as a function of concentration using the Kerr effect. Rough analysis of his data yields $k_{Q\kappa=0} = 8.6$. This method should give accurate results, but the ionic strengths of the solutions are not listed in his paper.

Now turn to the quenching reaction between fluorescein ion and iodide ion. $k_{Q\kappa=0}^{C=0}$ (experimental) = 3.7. Estimating r_{I^-} to be 2.3 Å. (average of crystal and solution data), $R = r_{Fl^-} + r_{I^-} = 7.2$ Å., $\sqrt{\tau_0 D} = 25$ Å., and $L/R = 2.0$. These values when inserted in equation (26) yield $k_{Q\kappa=0}$ (theoretical) $\cong 15$ as compared with the experimental value 3.7. This large discrepancy is probably due to an orientation effect, to a small chemical activation energy of quenching, or to inaccuracy of the values used for the ionic radii and non-equality of diffusion and quenching radii.

IV. Self-quenching of the Fluorescence of Fluorescein Ion: Theory.—It has often been observed that as the concentration of fluorescein ion is increased beyond 10^{-4} M, the quantum yield of fluorescence decreases. This phenomenon has been explained by Lewschin,²⁵ for the case of rhodamine B, as due to an equilibrium between fluorescent monomers and non-fluorescent dimers. He contends that part of the exciting light is absorbed by dimers, which cannot fluoresce, and hence the ratio of fluoresced to absorbed light, or the efficiency of fluorescence, is decreased. F. Perrin²⁶ includes a second effect of dimers. He has calculated, by quantum mechanics, the dipole-induced dipole forces between similar molecules, one of which is excited by light absorption, and finds that even at distances of a hundred Angström units, this coupling induces a rapid transfer (in about 10^{-8} sec.) of the fluorescence photon from molecule to molecule. If a dimer is nearby, this coupling could induce the energy to be transferred to the dimer with resultant quenching. This theory of self-quenching is similar to Frenkel's²⁷ "exciton" theory of the transformation of light into heat in solids. Both of the above dimer

phenomena probably occur in concentrated solutions, but we shall now present evidence for no dimerization in 10^{-3} M Fl^- and more dilute solutions.

When 0.1 M potassium nitrate was added to 10^{-3} M Fl^- solution, a 12% decrease in fluorescence occurred. However, the absorption of the solution was unchanged ($\cong 0.2\%$) at the exciting wave length, $\lambda = 0.365 \mu$. Hence the phenomenon is interpreted as a primary salt effect on the frequency of self-quenching encounters of fluorescein ions and not as a salt effect on dimerization. This effect is not due to specific quenching by potassium nitrate, because the effect vanishes at low concentrations of fluorescein, when encounters between Fl^- are negligible. Potassium perchlorate was found to produce the same quantitative result as potassium nitrate. Also, Söderborg²⁸ found that the absorption peak due to fluorescein ion dimers in solution becomes appreciable at 0.15 M fluorescein which is 150 times greater than the highest concentration used in our experiments. Therefore we assume that at 10^{-3} M and more dilute solutions of fluorescein, quenching results mainly from encounters between fluorescein ions.

The effect of the absorption of the exciting and fluorescence light is not true quenching. Rollefson and Dodgen²⁹ have discussed these factors recently, but their equations do not allow for refluorescence, as in equation (24), or for the polychromatic nature of the fluorescence light.

Experimental Self-quenching.—The Brönsted salt effect was verified by measuring the intensity of fluorescence of 10^{-3} M fluorescein ion as a function of ionic strength by adding various amounts of potassium nitrate, a non-quencher. These values are listed in columns one and three of Table III. To calculate quenching constants

TABLE III
SALT EFFECT ON SELF-QUENCHING
Temp., 23°; 10^{-3} M Na_2FI ; 0.0032 M NaOH; "a," 7.3 Å.

$\sqrt{\mu}$	ΔI	I ($I_0, 6340$)	k_Q	log k_Q	log $k_{Q\kappa=0}$	log $k_{Q\kappa=0}$ ($I_0, 6470$)
0.00	0					
.08	311	6029	51.6	1.713	1.44	1.58
.13	436	5904	73.9	1.869	1.47	1.58
.15	500	5840	85.7	1.933	1.49	1.59
.18	615	5725	107	2.031	1.52	1.59
.24	787	5553	141	2.151	1.53	1.60
.33	973	5367	181	2.258	1.50	1.56

Weighted av. = 1.51

from these values of fluorescence intensity, the equation

$$(I_0/I) - 1/[Fl^-] = k_Q \quad (27)$$

is used. However, this entails knowing I_0 , the fluorescence intensity in the absence of self-quenching. A rough value of $I_0 = 6340$ is ob-

(24) S. W. Cram, *Z. Physik*, **103**, 551 (1936).
(25) V. L. Lewschin, *Acta Physicochim. U.R.S.S.*, **1**, 685 (1935); **4**, 221 (1936).
(26) F. Perrin, *Ann. Phys.*, [10 S], **17**, 283 (1932).
(27) J. Frenkel, *Phys. Rev.*, [2] **17**, 17 (1931).

(28) B. Söderborg, *Ann. Physik*, **41**, 381 (1913).
(29) G. K. Rollefson and H. W. Dodgen, *J. Chem. Phys.*, **12**, 107 (1944).

tained by extrapolating the fluorescence intensity to zero ionic strength as shown in Fig. 5.

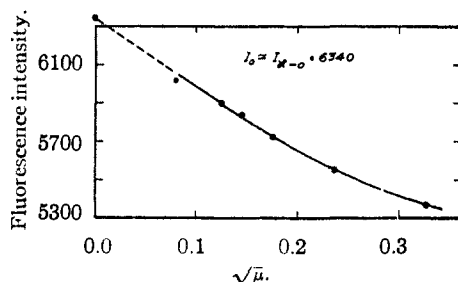


Fig. 5.—Determination of I_0 by extrapolation to zero ionic strength.

The data are fitted by the equation

$$\log k_Q = \log k_{Qx=0} + 4.1\sqrt{\mu}/(1 + 2.4\sqrt{\mu}) \quad (28)$$

as indicated by the constancy of $\log k_{Qx=0}$ in the sixth column of Table III, thus proving that self-quenching results from encounters of two divalent ions in support of the mechanism $\text{Fl}^{*2+} + \text{Fl}^- \rightarrow 2\text{Fl}^- + \text{heat}$. Having proved that equation (28) expresses the salt effect on the self-quenching constants, I_0 may in the future be determined by choosing that value which when inserted in equation (27) produces quenching constants with the correct salt effect, as determined by equation (28). In this way an improved value of $I_0 = 6470$ is obtained, and the resulting quenching constants are listed in column seven of Table III.

Further experiments on self-quenching were performed at progressively lower fluorescein ion concentrations, and extrapolation to zero concentration of fluorescein eliminates the effect of the absorption of fluorescence. In Table IV are listed the results of these experiments, and in Fig. 6, $\log k_{Qx=0}$ is plotted against the concentration of fluorescein ion. The extrapolated self-quench-

$\sqrt{\mu}$	I	$\log k_{Qx=0}$	Av.
$6 \times 10^{-4} M \text{Na}_2\text{Fl}, 0.01 M \text{NaOH}$			
0.0	$I_0 = (4810)$		
.11	4642	1.423	
.33	4404	1.421	1.42
$4 \times 10^{-4} M \text{Na}_2\text{Fl}, 0.01 M \text{NaOH}$			
0.0	$I_0 = (5384)$		
.11	5276	1.362	
.33	5113	1.358	1.36
$2 \times 10^{-4} M \text{Na}_2\text{Fl}, 0.01 M \text{NaOH}$			
0.0	$I_0 = (5646)$		
.10	5602	1.264	
.33	5529	1.263	1.26
$1 \times 10^{-4} M \text{Na}_2\text{Fl}, 0.01 M \text{NaOH}$			
0.0	$I_0 = (4706)$		
.10	4692	1.122	
.33	4670	1.119	1.12

ing constant is $\log k_{Qx=0}^{C=0} = 0.91$. These self-quenching experiments were performed only once, and hence it is difficult to estimate their precision. Because of the smallness of self-quenching, the data are not as precise as Fig. 6 might indicate. This explains the discrepancy of the slope of Fig. 6 as compared to Fig. 4 at $10^{-4} M$ fluorescein.

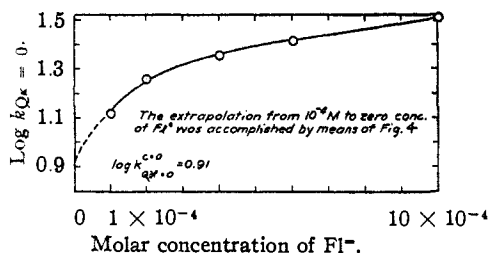


Fig. 6.—Removal of the effect of fluorescence absorption, on the self-quenching constants of fluorescein, by extrapolation to zero concentration of fluorescein.

V. Iodide Ion Quenching.—The salt effect data for the iodide ion quenching of $10^{-5} M$ fluorescein ion are fitted by the Debye-Hückel parameter " a " = 7.3 Å. It is important to see whether this value is also valid for iodide quenching data at other concentrations of fluorescein ion. Proof is seen in Table V of quenching data at 10^{-4} and $2 \times 10^{-5} M$ fluorescein ion. The constancy of $\log k_{Qx=0}$ proves that the value of " a " is independent of the concentration of fluorescein ion. This is in accordance with equation (24), which proves that the absorption of fluorescence does not change the Brönsted salt effect.

$\sqrt{\mu}$	$\log k_Q$	$\log k_{Qx=0}$
$10^{-4} M \text{Na}_2\text{Fl}; "a" = 7.3 \text{ \AA}; \text{Temp.}, 23^\circ$		
0.074	0.926	0.796
.103	.958	.790
.125	.981	.786
.160	1.023	.788
.225	1.083	.786
.317	1.156	.790
$2 \times 10^{-5} M \text{Na}_2\text{Fl}; "a" = 7.3 \text{ \AA}; \text{Temp.}, 23^\circ$		
.101	0.875	.710
.123	.900	.707
.159	.942	.708

The experiments at lower concentrations of Fl^- are less precise, the average values of the quenching constants being plotted against the concentration of fluorescein ion in Fig. 4.

$10^{-5} M$ fluorescein ion was quenched by potassium iodide in solutions of pH 10, 11 and 12, and no effect of pH was detected. However, upon using pure di-sodium fluorescein salt, without the addition of any sodium hydroxide, results were obtained which indicated incomplete ionization of the fluorescein. The addition of iodide

ions to fluorescein solutions produces a large quenching of fluorescence while absorption changes are negligible. Reducing the intensity of the light source by one-half and changing the wave length from 0.365 to 0.43 μ produced negligible effects on the quenching constants.

Appendix

I. **Purity and Stability of Materials.**—Eastman Kodak Co. uranin (di-sodium fluorescein salt) was used without further purification. This was found to be 2.1% less fluorescent than E. K. Co. fluorescein (recrystallized once). The molar extinction coefficient of the unpurified E. K. Na_2FI was 76.9×10^3 at the absorption peak $\lambda = 0.49 \mu$. Increasing this value by 2.1%, one obtains 78.5×10^3 . Lewschin³⁰ found 78.2×10^3 , and Orndorff, Gibbs and Shapiro³¹ found 76.2×10^3 . Check experiments were performed with the purified fluorescein to guarantee that any anomalous results were not caused by the 2% impurity in the E. K. Co. uranin. Bleaching and quenching of FI^{*-} by OH^- were found to be negligible. The c. p. grade of potassium iodide of several sources was used—all with the same results. Recrystallization produced no perceptible differences.

The stability of the apparatus and solutions is demonstrated by the data of Table VI. Run 13 was made one week after run 12 on the same solutions stored in non-sol bottles in diffuse light—proving that mixed solutions of fluorescein ion and iodide ion are stable. The agreement between these experiments corresponds to measuring 10% quenching to within one part in a hundred.

TABLE VI

THE STABILITY OF IODIDE ION QUENCHING OF FI^{*-}
 $10^{-4} M \text{Na}_2\text{FI}$; $0.0004 M \text{OH}^-$; Temp., 23°

KI, <i>M</i>	$\sqrt{\mu}$	$\log k_q$	
		Run 12	Run 13
0.005	0.074	0.912	0.940
.010	.103	.957	.958
.015	.123	.982	.979
.025	.160	1.023	1.022
.050	.225	1.084	1.081
.100	.317	1.159	1.153

II. **Characteristics of the Apparatus.**—To make quantitative investigation of salt effects on the quenching of fluorescence in solutions of ionic strength 0.005 to 0.1, it was necessary to design and construct a fluorimeter which is more sensitive than any described in the literature. To eliminate the effect of light source and temperature fluctuations, the apparatus is composed of twin halves which can be matched by suitable adjustment. The linearity, precision, and matching of the two halves of the apparatus are demonstrated by the data of Table VII on the fluorescence intensity of aqueous fluorescein ion solu-

(30) V. L. Lewschin, *Z. Physik*, **73**, 368 (1931).

(31) W. R. Orndorff, R. C. Gibbs and C. V. Shapiro, *THIS JOURNAL*, **50**, 819 (1928).

TABLE VII

TESTING THE APPARATUS

Fluorescein ion, <i>M</i>	Fluorescence (av. of 5 readings), % a.d.		Fluorescence per mole	
	Left side used	Right side used	Left side	Right side
10×10^{-7}	12658 = 0.13	12658 = 0.09	1266×10^{-7}	1266×10^{-7}
8	10140 .13	10129 .13	1268	1266
6	7568 .14	7586 .08	1261	1264
4	5075 .09	5066 .3	1269	1267
2	2527 .4	2528 .4	1264	1264

tions as a function of concentration. The apparatus was adjusted once and remained so for the whole run, which lasted about one and one-half hours. The concentration of OH^- in all solutions was 0.01 *M*. The linearity is reflected in the constancy of the fluorescence yield per mole, and the equivalence of the two halves of the apparatus is proved by the equal response of the two sides to the same solutions, as shown in the last two columns. The intensities are expressed in μ volts, *i. e.*, the voltage drop of the photocurrent across the photload resistor of 20 meg Ω .

III. **Schematic Representation of the Apparatus.**—From right to left of Fig. 7: B is 45-volt heavy duty "B" battery (negative pole to ground and positive pole to the anodes of the photocells); S is light source. General Electric alternating current, A H-4 100 watt mercury vapor lamp; (\emptyset), lens, focal length about one and one-half inches; D, D' , iris diaphragms; C, C' , 25-cc. glass absorption cells (inside dimension $6 \times 5 \times 1$ cm.) to hold the fluorescent solutions; P, P' , photocells, R.C.A. no. 926; i, i' , photocurrents; G, G' , control grids of amplifier tubes (R.C.A. 6J7-GT); R, R' , photload resistors of constant, but not necessarily matched, resistance; $S.B.P.$, slide back potentiometer, Leeds and Northrup type K_1 potentiometer used to slide back the grid potential to balance. To measure the intensity of the fluorescence of fluorescein, ultraviolet light filters (combination of Corning Pyrex filters 5860 and 738 to transmit the mercury line $\lambda = 0.365 \mu$) are inserted in the light path between the source S and the cells C. Green filters (4010 half-thickness) are glued onto the faces of the absorption cells next to the photocells to absorb any stray ultraviolet light.

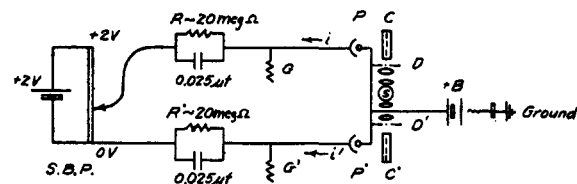
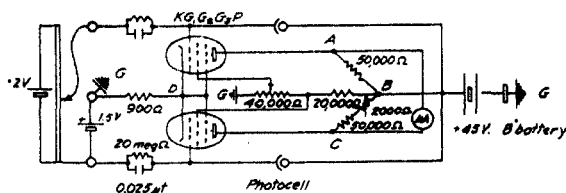


Fig. 7.—Schematic representation of the apparatus (galvanometer and amplifier not shown).

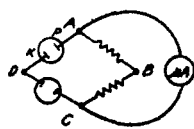
IV. **Operational Adjustments of the Apparatus.**—(1) With no light on the photocells, adjust the rheostats on the amplifier until the galvanometer reads zero. At this point, the plates of the two 6J7-GT amplifier tubes are at the same

potential. (2) Fill both absorption cells with a solution of fluorescent dye, and excite fluorescence with light from the source S. If the upper and lower halves of the apparatus are identical, the potentials of both control grids will increase by the same amount, and there will be no galvanometer deflection. All sources of asymmetry (photocells, light paths, amplification) are corrected by adjusting one of the iris diaphragms until the two plates again reach equal potentials, with the galvanometer again reading zero. This completes the matching of the two halves of the apparatus. (3) Replace the fluorescent solution in the upper absorption cell with an identical dye solution plus quenching salts. The setting is thrown off balance, and the deflection of the galvanometer is a measure of the quenching. (4) Slide back the potential on the upper control grid until the current through the galvanometer is again equal to zero. The reading, ΔV , on the potentiometer is directly proportional to the difference in fluorescence intensity due to quenching. (5) Without disturbing the adjustment, darken the upper photocell and apply a slide back potential, V_0 , to the upper control grid to restore the balanced condition. (6) $I_0/I_Q = 1$, the quantity desired, equals $\Delta V/(V_0 - \Delta V)$. I is the intensity of the fluorescence emission, and the subscripts 0 and Q refer to the unquenched and quenched solutions, respectively.

V. Details of the Balanced Type Amplifying Circuit.—The modified R. C. A. amplifying circuit shown in Fig. 8 was chosen for its stability. It is a balanced Wheatstone bridge type, and hence the de-stabilizing effects of fluctuations in heater voltage, etc., are reduced. The heaters of the amplifier tubes are connected in parallel and are supplied with 4.9 volts from a 6-volt lead storage battery in series with a 3 Ω resistor. The current drain on the battery is reduced to 0.1 amp. by charging at a rate (0.4 amp.) just under the discharge rate (0.5 amp.), *i. e.*, the storage battery is used as a stabilizer of the unsteady direct current used for charging.



μ A, Leeds and Northrup reflecting galvanometer, Type R, sensitivity = 0.0024 μ amp./mm.; coil resistance = 500 Ω . G, ground; K = cathode; G_1 = control grid; G_2 , screen grid; G_3 = suppressor grid; P, plate.



Schema showing the arms of the bridge type amplifier.

Fig. 8. —Details of the balanced type amplifying circuit.

The 1.5 volt "A" battery is placed between the control grids and ground to reduce the cathode-to-grid current. This can be omitted. All conductors leading to ground through a high resistance (from photocathode to photoload resistor) consist of shielded co-axial cable. The amplifier is housed in a grounded all-metal box, and the amplifier tubes are coated with cerusin wax to prevent surface conduction from the control grid-cap to the base.

VI. Miscellaneous.—The effect of vibrations was eliminated by screwing down the optical bench (built of 0.25-in. pipe) to a large piece of soapstone, which in turn was "floated" on a layer of spongy absorbent paper 2 inches thick.

The light source is operated on 120 = 0.5 a. c. volts from a Coleman model U-11-MT voltage stabilizer. The A H-4 mercury vapor lamp has a roughly linear light output versus voltage characteristic, a 1% fluctuation in the a. c. voltage producing only 1% change in light output of the lamp. This lamp emits an intermittent beam of light of frequency 120 per sec., thus producing a photocurrent with a wave form similar to a full-wave rectified alternating current. However, with 20 megohms and 0.025 microfarad in parallel, the voltage across the photo-load resistor is at all times (even when there is no light on the photocells) within 99% of the peak voltage.

Acknowledgments.—We wish to express our appreciation to Professor F. Perrin of the University of Paris for many conferences on fluorescence during his stay at Columbia University. We also wish to thank Professor P. Debye of Cornell University for helpful discussions on the effect of ionic charges on encounter frequencies.

Summary

1. The Brönsted primary kinetic salt effect is not evidence for the existence of a rate-controlling activated complex intermediate between the reactants and the products of a reaction.

2. The Smoluchowski diffusion model for calculating the frequency of encounters of colloid particles can be extended to include particles of molecular dimensions, as proved by the agreement between the experimentally measured rates of fluorescence quenching and the calculated encounter frequencies.

3. The quenching of fluorescence, though a diffusion controlled reaction, is not a linear function of D , the relative diffusion coefficient of quencher and quenched, but instead $k_Q = 3P(1 - \alpha)V(g + \sqrt{g})/f$ over a wide range. V is the volume in liters per mole of the "sphere of effective quenching," $g = \tau_0 D/R^2$, f expresses ionic charge effects, and $P(1 - \alpha)$ is the probability of any encounter resulting in quenching.

4. Fluorescence quenching is measured at lower ionic strengths than any previous work. The Brönsted primary salt effect on the quench-

ing constants of fluorescein and iodide ions is accurately described by the equation $\log k_Q = k_{Q_{x=0}} + 2.0\sqrt{\mu}/(1 + 2.4\sqrt{\mu})$. An unpredicted dependence of the quenching constants on the concentration of fluorescein is observed. This effect, which is caused by the absorption of the fluorescence light in its transit from the body of the solution to the photocell, is eliminated by extrapolation to zero concentration of fluorescein.

5. A new method for measuring the quantum efficiency of fluorescence of dyes in solution has been developed. The quantum efficiency for fluorescein ion is 84 to 85%.

6. Self-quenching of fluorescence results from an encounter between an excited and an unexcited fluorescein ion involving dipole-induced dipole forces.³² Each encounter results in quenching, *i. e.*, $P(1 - \alpha) \simeq 1$.

7. Iodide ion quenching of fluorescence results from an encounter between an excited fluorescein ion and an iodide ion involving shared-electron and electron-transfer³² forces. Quenching occurs in, roughly, one out of four encounters, *i. e.*, $P(1 - \alpha) \simeq 1/4$.

(32) To be described in a later paper.

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Moving Boundary Studies on Salt Mixtures

BY L. G. LONGSWORTH

Introduction.—The moving boundary studies previously reported from this Laboratory on salt mixtures¹ were restricted to solutions of two binary salts having a common ion in which the unlike ions have large differences of mobility. Only under these conditions were the boundaries sufficiently sharp to be located with the simple optical arrangements then in use. With the new procedures,^{2,3} in which the refractive index gradients in the boundaries are photographed with the schlieren scanning camera during substantially convection-free electrolysis at 0° in the Tiselius cell, this restriction is removed and mixtures of several species of ions having similar mobilities can now be studied. If disturbances due to gravity are avoided, an initially sharp boundary between two such mixtures will generally split, on passage of an electric current, into a number of separate boundaries, each moving at a different rate. This is illustrated by the pattern of Fig. 1. In this experiment a boundary was formed initially between a mixture of lithium chloride and sodium chloride, 0.08 *N* in each, and a mixture of the bromate and iodate of potassium, 0.1 *N* in each. The gradients in this boundary just prior to the passage of the current are indicated by the traced line in Fig. 1. The shaded peaks of this pattern are due to the boundaries that separated on electrolysis.⁴

Although transference data for the salt mixtures have been obtained in the course of this research the chief purpose of the investigation has been to use the new tool for the measurement of the concentration changes accompanying the separation of the boundaries and to show that

(1) Longworth, *THIS JOURNAL*, **52**, 1897 (1930); **57**, 1698 (1935).

(2) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(3) Longworth, *THIS JOURNAL*, **65**, 1755 (1943).

(4) The vertical line, h_0 , in this and other patterns of this paper is due to a graduation on the cell from which boundary displacements are measured. Except for the tracing of the initial gradients, the patterns are exactly as photographed.

these changes are in accord with the theory of the process. At the time this investigation was begun the available theory, due to Kohlrausch⁵ and Weber,⁶ was restricted to ternary ion systems. As more complex systems were studied the need for an extension of the theory became evident. While this work was proceeding, Dr. Vincent P. Dole of these Laboratories developed a general solution, reported in the following paper,⁷ for the moving boundary equation and his results have been adapted, where necessary, to the systems described below.

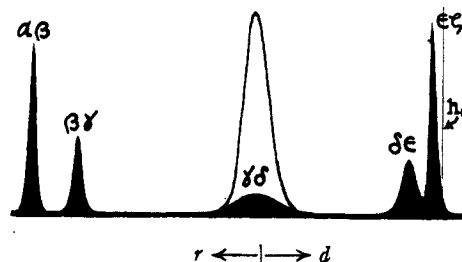


Fig. 1.—Schlieren scanning pattern of the system 0.08 *N* LiCl, 0.08 *N* NaCl–0.1 *N* KBrO₃, 0.1 *N* KIO₃.

The work reported here is also proving of value in the interpretation of the electrophoretic patterns of proteins. Although salt and protein ions differ in many respects, the principles underlying the moving boundary studies of these materials are essentially the same. Since the equivalent weights and mobilities of the simple electrolytic ions are well established, mixtures of these ions are better adapted for the experimental tests of the moving boundary theory than are protein mixtures.

Not only do the patterns yield data as to the

(5) Kohlrausch, *Ann. Physik*, **62**, 209 (1897).

(6) H. Weber, "Die partiellen Differential-Gleichungen der mathematischen Physik," Braunschweig, 1910, 5th edition, chapter 24.

(7) Dole, *THIS JOURNAL*, **67**, 1119 (1945).