The Kinetics of Activation–Diffusion Controlled Reactions in Solution. The Temperature Dependence of the Quenching of Fluorescence[1,2]

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The quenching of fluorescence in solution is generally considered today to be a diffusion controlled reaction. The most conclusive evidence for this view is the dependence of the empirical rate constant upon the viscosity of the medium. By developing an equation with which they calculated the encounter frequency of ions in solution, and by measuring the quenching constant corrected for various interfering phenomena, Umberger and La Mer[4] showed that only about one encounter in four results in quenching for the reaction involving the quenching of the fluorescence of uranin (the basic form of fluorescein) by iodide ion. Hence the rate of diffusion of the reactants toward each other is the primary, but not the sole factor governing the rate of this and presumably other quenching reactions. It seemed probable that a chemical energy of activation might also be involved in the quenching process and an investigation of this aspect of the problem is the principal objective of this research.

Theory

The development of a rate equation which includes both the energy of activation and the collision frequency is not as simple for liquids as it is for gases. Rabinowitch[5] has shown that collisions in liquids occur in sets, called co-ordinations or encounters. Each encounter consists of many collisions, and the duration of an encounter depends upon the viscosity and the temperature of the liquid under consideration. This concept of diffusion in liquids will be used with the further general provision, namely, that collisions between two reactants, A and B, with each other and with the solvent molecules are not different kinetically when A and B are in an encounter together than when each is alone in an "encounter" with only solvent molecules. The presence of A and B in an encounter thus places no restriction upon either their electronic or thermal activation.

The molar rate constant, k, of a bimolecular reaction in solution can accordingly be represented as the product of the encounter frequency or the rate of diffusion of the reactants together, k_D, times the probability of reaction per *encounter*, p.

$$k = pk_D \qquad (1)$$

The Rate of Diffusion.—An exact and general expression for the encounter rate of molecular solute particles in solution, analogous to gaseous collison frequencies, is not derivable because of the limitations of our knowledge of the nature of liquids. Two different approaches to this problem have been used. Rabinowitch[5] and Fowler and Slater[6] have derived equivalent expressions by considering liquids to have a quasi-crystalline structure, and calculating the probability that two solute particles will occupy adjacent lattice points. Their expressions are especially approximate when the sizes of the solute and solvent molecules differ.

The second approach, and the one that has been used by Sveshnikoff and Wawilow[7] and by Umberger and La Mer[4] in their work with the quenching of fluorescence in solution is the application of Smoluchowski's theory[8] for the coagulation of colloids. Although there are objections to the derivation and to the use of Smoluchowski's expressions in the calculation of encounter frequencies, ample experimental agreement between the rapid coagulation of colloids and Smoluchowski's stationary state solution has been obtained. The stationary state solution, $4\pi DR$, gives very nearly the same numerical values as are obtained from the quasi-crystalline formula and will be used in this paper, i. e.

$$k_D = 4\pi DRN/1000 f \qquad (2)$$

Sveshnikoff and Wawilow included the transient state term, $R/\sqrt{\tau_0 D}$, of Smoluchowski's theory in their theoretical treatment, substituting τ_0, the mean life of the excited state of the fluorescent molecule for Smoluchowski's general parameter, t. Umberger and La Mer also included the transient state term in their recent work, as has Montroll.[9] The full physical significance of this term needs further clarification. Smoluchowski's boundary conditions are not realized in physical processes, even in the case of colloidal coagulations, for very small or for very large values of t.

The equality of the quenching and diffusion radii also means that static quenching, as discussed by Frank and Wawilow,[10,11,12] will be absent. Static quenching also results from the electronic excitation of a fluorescent molecule while in an encounter with a quencher molecule. This effect is compensated, however, by fluorescence during an

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(4) J. Q. Umberger and V. K. La Mer, THIS JOURNAL, 67, 1099 (1945).

(5) E. Rabinowitch, Trans. Faraday Soc., 33, 1225 (1937).

(6) R. H. Fowler and N. B. Slater, ibid., 34, 81 (1938).

(7) B. I. Sveshnikoff, Acta Physicochim., U. R. S. S., 3, 257 (1935).

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

(9) E. W. Montroll, J. Chem. Phys., 14, 202 (1946).

(10) B. I. Sveshnikoff, Compt. rend. Acad. Sci., U. R. S. S., 3, 61 (1936).

(11) J. M. Frank and S. I. Wawilow, Z. Physik, 69, 100 (1931).

(12) E. J. Bowen, Trans. Faraday Soc., 35, 17 (1939).

encounter. As static quenching is a function of the concentration of quencher, it is eliminated beyond doubt in this work by the necessary extrapolation of the quenching constants to zero concentration quencher.

There remains the problem of the proper values of the diffusion constant to be used in the calculation of k_D. If the Stokes–Einstein expression, $D = kT/6\pi\eta r$, is used the question reduces to that of the proper radius to use. This difficulty has been minimized by Hodges and La Mer[13] who have measured the diffusion constants of the reactants involved using the diaphragm cell method.

The Probability of Reaction per Encounter.— Because collisions in liquids occur in sets, the probability of reaction depends not only upon an energy or an entropy of activation, but also upon the distribution of the collisions, for if a reaction occurs early in an encounter the subsequent collisions of that encounter will be wasted, i. e., they will not be reflected in the reaction rate. To consider these circumstances, let w be the probability of reaction upon collision of reactants A and B, and let x be the probability of the encounter being broken by diffusion between collisions of A and B. If one assumes that an encounter begins with a collision of A and B, the probability of reaction per encounter, p, may be expressed as

$$p = w + (1 - w)(1 - x)p \qquad (3)$$

which, if $xw \ll x$ or w, reduces to

$$p = w/(x + w) \qquad (4)$$

If instead one assumes that diffusion away may occur before the first collision of A and B, equation (4) is derivable directly without the approximation that $xw \ll x$ or w.

The general equation for k, the reaction rate constant in solution, or in media where collisions occur in sets, is then

$$k = (w/x + w)k_D \qquad (5)$$

i. e., the rate of reaction is equal to the rate of formation of encounters times the probability that reaction will occur before the encounter is broken by diffusion away.

Equation (5) is a more general form of equations that have been developed by Rabinowitch[5] and by Fowler and Slater[6] from considerations of the quasi-crystalline structure of liquids.

The probability of reaction per collision may be represented as

$$w = Pe^{-E_a/RT} \qquad (6)$$

where P is an orientation or entropy factor, and E_a is the usual chemical energy of activation.

The probability of a molecule escaping by diffusion between collisions of A and B may also be represented by an exponential term

$$x = Ce^{-E_d/RT} \qquad (7)$$

where E_d is an energy of activation of diffusion, and C consists of an orientation factor together

with other small numerical factors to be discussed later.

The relative influence of the chemical energy of activation and the diffusional energy of activation is also shown by rearranging Eq. (4) to the form

$$(1/p) - 1 = x/w \qquad (8)$$

Substituting equations (6) and (7) into the logarithmic form of equation (8) gives

$$\log (1/p - 1) = \log C/P + (E_a - E_d)/2.3RT \qquad (9)$$

A plot of $\log(1/p - 1)$ against $1/T$ will have a positive slope if $E_a > E_d$, a negative slope if $E_a < E_d$, and a zero slope if $E_a = E_d$. The intercept of the curve will be a measure of the relative magnitude of the steric factors of diffusion and reaction, and if one is known, the other may be determined.

An expression for the probability, x, of the termination of an encounter between collisions of A and B may be developed and E_a identified with the experimental energy of activation of diffusion. Let x' be the probability that any single attempt at diffusion will be successful, i. e., a collision of a molecule with its cage wall will result in displacement to a new equilibrium position. Diffusion by either of the reactants to a new equilibrium position not adjacent to that occupied by the other reactant will break the encounter so that

$$x = (2n\gamma)x' \qquad (10)$$

where x is the probability that the new equilibrium position will not be adjacent to the other reactant, and $2n$ is the number of attempts at diffusion by the two reactants between collisions with themselves (assuming $n_a = n_b = n/2$).

The number of diffusive collisions per second is $x'Z_1 = 1/\theta$, Z_1 being the collision frequency in liquids and θ is the average time between diffusive collisions. From the Einstein[14] expression for the diffusion constant, the number of diffusive collisions per second in terms of the distance between equilibrium positions in the liquid, λ, is

$$1/t = 2D/\lambda^2 \qquad (11)$$

The relationship between x' and the diffusion constant is then given by equation (12)

$$x' = 2D/\lambda^2 Z_1 \qquad (12)$$

The probability, x', may now be expressed in terms of the experimental energy of activation of diffusion by substituting into Eq. (12) Eyring's[15] expression for D (Eq. 13) and Fowler's[6] expression for Z_1 (Eq. 14)

$$D = (\lambda^2/v_f^{1/3})(k_BT/2\pi m)^{1/2} e^{-E_d/RT} \qquad (13)$$

$$Z_1 = 3(2k_BT/\pi m)/a \qquad (14)$$

The factor a is defined as the diameter of the cage, i. e., the diameter of the free volume allowed to any particle, so that a is approximately equal to $v_f^{1/3}$, and Eq. (12) reduces to

$$x' = 1/6e^{-E_d/RT} \qquad (15)$$

(13) K. C. Hodges and V. K. La Mer, THIS JOURNAL, **70**, 722 (1948).

(14) A. Einstein, *Ann. Physik*, **17**, 549 (1905).

(15) A. E. Stearn, E. M. Irish and H. Eyring, *J. Phys. Chem.*, **44**, 981 (1940).

Equation (10) now becomes

$$x = (2n\gamma/6)e^{-E_d/RT} \tag{16}$$

The factor n will vary from unity in the case of vibrations of A and B restricted to one coördinate to 11 for random movement in a hexagonal lattice, and γ is of the order of magnitude of $1/2$. Orientation or steric factors influencing the diffusion of a molecule from one equilibrium position to the next have not been considered above, and a probability factor, α, which will be unity for spherical molecules should be included in Eq. 16. The constant of Eq. (7) is now seen to be

$$C = \alpha\gamma m/3 \tag{17}$$

and is a small number approximately equal to unity for near spherical molecules. This means that for proper geometrical cases, log C/P, the intercept of equation (9) plotted against $1/T$, will give the order of magnitude of the reaction orientation factor P.
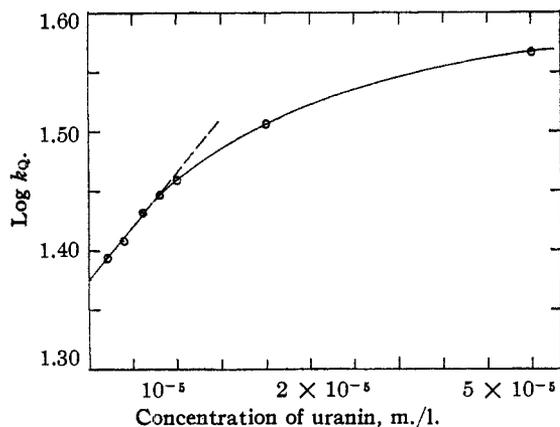

Fig. 1.—The molar quenching constant as a function of the concentration of uranin.

The probability, x', may also be derived as a function of temperature and viscosity, η, by substituting into Eq. (12) another form of Eyring's formula for D,[16] namely

$$D = \frac{\lambda_1}{\lambda_2\lambda_3}\frac{kT}{\eta} \tag{18}$$

The resulting expression for x is

$$x = C_1\sqrt{T/\eta} \tag{19}$$

where C_1 is of the order of magnitude of 10^{-6} to 10^{-5}, depending for the most part upon the mass of the solute particle. Equation (19) is useful because if C_1 can be determined, it will predict more accurately the dependence of x upon T and η than will Equation (7).

The Uranin–Aniline Quenching Reaction.—The quenching of the fluorescence of uranin, Fl⁻, by aniline, An, has been measured as a function of the concentration of both uranin and aniline, as well as temperature, in order to obtain rate constant values free from absorption effects. The apparatus and procedure used is very similar to that of Umberger and La Mer. The photocells and cuvettes

were enclosed in water jacketed housings for temperature control. The Stern–Volmer equation[17] was employed in the initial calculation of the quenching constants.

The decomposition of aniline in basic solution is serious in this work and the solutions, kept in blackened flasks, must be basic no longer than one hour at room temperature before intensity readings are obtained. The decomposition rate increases with temperature, and limits the temperature range employable.

The reabsorption of its own fluorescence by uranin in the presence of 0.01 M aniline at 27° is shown in Table I and Fig. 1, and is in good agreement with the theory and data of Umberger and La Mer.[4]

TABLE I

THE EXPERIMENTAL QUENCHING CONSTANT AS A FUNCTION OF THE CONCENTRATION OF URANIN

Concentration of uranin, m./l.	k_Q	log k_Q
1×10^{-4}	40.5	1.608
2×10^{-5}	32.0	1.505
1×10^{-5}	28.8	1.459
8×10^{-6}	28.0	1.447
6×10^{-6}	27.0	1.431
4×10^{-6}	25.6	1.408
2×10^{-6}	24.8	1.394
zero	(23.7)	(1.375)

At concentrations of aniline above 0.005 M, the quenching constant was found to increase with increasing concentration, Table II and Fig. 2. This

TABLE II

EXPERIMENTAL QUENCHING CONSTANT VALUES FOR THE URANIN–ANILINE REACTION AS A FUNCTION OF THE CONCENTRATION OF ANILINE

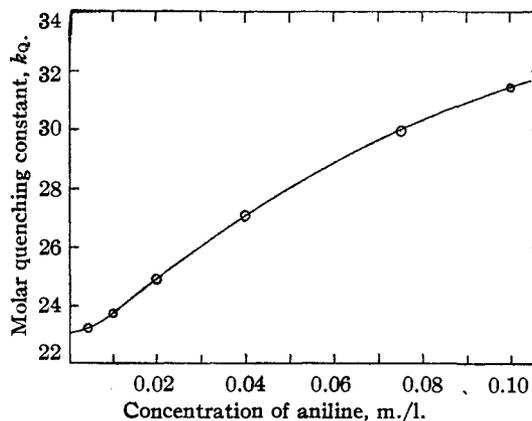| Concn. of aniline, m./l.	Quenching, %	k_Q $10^{-5} M$ Fl⁻	k_Q Extrap., $C_{Fl^-} = 0$
0.100	70	37.9	31.4
.075	63	36.1	29.9
.040	48	32.7	27.1
.020	31	30.1	24.9
.010	19	28.8	23.7
.004	8	28.1	23.3
zero		(28.0)	(23.2)

Fig. 2.—The molar quenching constant as a function of the concentration of aniline.

(16) H. Eyring. *J. Chem. Phys.*, **4**, 283 (1936).

(17) O. Stern and M. Volmer, *Z. wiss. Phot.*, **19**, 275 (1920).

has been predicted by Sveshnikoff,[10,18] and constitutes the so-called deviations from the Stern-Volmer equation. Some absorption by aniline of the incident and fluorescent light also contribute. The increase is not due to a salt effect on the reaction as evidenced by no change in the quenching constant in the presence of 0.1 M NaOH or 0.1 M NaCl.

The quenching constant extrapolated to zero concentration of both uranin and aniline is 23.2 ± 0.3 at 27.0 ± 0.5°. The rate of quenching is hence 23.2 times as great as the rate of fluorescence on a molar basis. Using Equation (2), with

$$D = \frac{k_B T \ (1/r_{Fl^-} + 1/r_{An})}{6\pi\eta}$$

the number of encounters between uranin and aniline molecules in the time τ_0 is found to be 43.5 at 27.0°.[20] The probability of quenching per encounter is therefore 0.53 and approximately one out of every two encounters results in quenching.

The Temperature Dependence of the Uranin–Aniline Reaction.—Although much work has been done on the quenching of fluorescence in solution, the temperature dependence of a quenching reaction independent of viscous and other effects has not been determined. Stoughton and Rollefson[19] have been the most recent investigators of the temperature dependence of quenching, but since they did not employ an equation predicting the dependence of the quenching constant upon viscosity they were unable to treat the effect of temperature independently.

In this work the quenching constant of the uranin–aniline reaction has been measured at temperature intervals of five degrees from 7° to 54° using 0.01 M aniline and 10^{-5} M uranin. The results are given in Table III and Fig. 3. In order to obtain molar quenching constants from the experimental values, the per cent. correction, 19.5%, determined from the two necessary extrapolations at 27° was used at all temperatures. The validity of this procedure was established by measuring the effects of the aniline and uranin concentrations at 40°. The correction was found to be the same per cent. of the experimental value as at 27°.

The intensity of the fluorescence of a uranin solution in the absence of a quencher was found to decrease between 1–2% per 10° increase in temperature. From the work of Lewschin,[20] Jenness[21] and Speas[22] this effect may be shown to be due to a decrease in the extinction coefficient of the dye with increasing temperature. The fluorescence

(18) B. I. Sveshnikoff, *Acta Physiochim., U. R. S. S.*, **4**, 453 (1936).
(19) R. W. Stoughton and G. K. Rollefson, THIS JOURNAL, **62**, 2264 (1940).
(20) The values used in this computation are:

k_B	= 1.38 × 10⁻¹⁶	t_0	= 0.45 × 10⁻⁸ sec.
N_a	= 6.02 × 10²³	r_{Fl^-}	= 6.6 × 10⁻⁸ cm.
T	= 300°	r_{An}	= 2.5 = 10⁻⁸ cm.
η	= 0.00854 poise		

(20) V. L. Lewschin, *Z. Physik*, **72**, 368 (1931).
(21) J. R. Jenness, *Phys. Rev.*, **34**, 1275 (1929).
(22) W. E. Speas, *ibid.*, **31**, 569 (1928).

ANILINE QUENCHING OF THE FLUORESCENCE OF URANIN AS A FUNCTION OF TEMPERATURE

Temp., °C.	k_Q. exptl.	k_Q. cor.	k_D. calcd.	p	log $(1/p - 1)$
6.5	17.5	14.0	24.2	0.578	−0.138
7.0	18.5	14.9	24.6	.606	− .187
7.4	18.3	14.7	24.9	.590	− .159
8.1	18.8	15.1	25.5	.592	− .161
9.5	18.9	15.2	26.6	.571	− .125
9.8	19.3	15.6	26.9	.580	− .140
11.0	20.0	16.1	27.9	.577	− .134
11.8	20.5	16.5	28.6	.577	− .134
16.0	22.2	17.9	32.5	.551	− .089
19.6	24.4	19.7	36.0	.546	− .082
20.0	24.2	19.5	36.4	.536	− .062
25.0	27.3	22.0	41.7	.527	− .047
30.0	30.5	24.6	47.4	.520	− .035
35.0	34.0	27.4	53.2	.515	− .026
40.0	37.4	30.1	59.9	.503	− .005
44.3	40.5	32.6	66.0	.494	.010
45.6	41.5	33.4	67.4	.496	.007
49.0	44.0	35.7	72.1	.495	.009
52.3	46.8	37.7	77.0	.490	.017
54.0	47.8	38.5	79.4	.485	.026

intensity is shown to be a linear function of the extinction coefficient, and the mirror symmetry of the fluorescence and absorption spectra is also maintained over this range of temperature. The average life of uranin in water has been shown to be independent of temperature by Perrin,[23] using polarization measurements. These considerations also show why the correction due to the re-
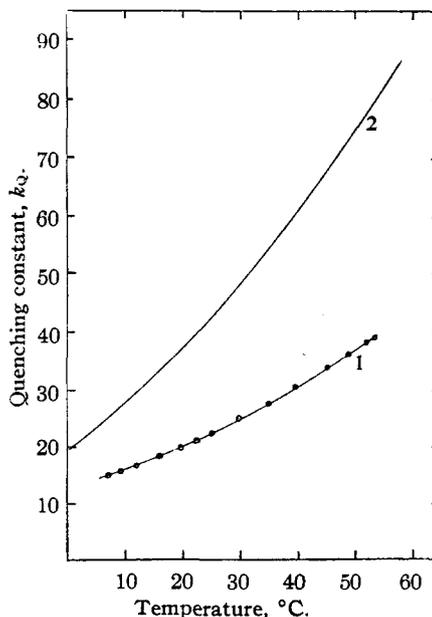

Fig. 3.—The calculated encounter rate (1), and the experimental quenching constant (2) as a function of temperature.

(23) F. Perrin, *Ann. Physik*, **108**, 12, 169 (1929).

absorption of fluorescence is independent of temperature.

Although the radii of these reactants have not been measured as a function of temperature it is expected that they will be constant. The radius of mannitol has been measured as a function of temperature in aqueous solution by Scheffer and Scheffer[24] and discussed by Stearn, Irish and Eyring.[15] Their data show the radius to be independent of temperature to within a few tenths of an Ångström unit. Variations of this order of magnitude are not significant in this work as the value of k_D is not sensitive to small changes in the radii.

Discussion of Temperature Results

The quenching constant measured over a temperature range is a function of two variables, the temperature, T, and the viscosity, η. Equation (2) in terms of these parameters with the constants evaluated for the uranin–aniline reaction is given below

$$k_D = 0.00125T/\eta \qquad (22)$$

The usual procedure of determining the energy of activation of a chemical reaction from measurements of the rate constant as a function of temperature is to plot $\log k$ vs. $1/T$. Such a procedure in this case cannot be used because of the dependence of the encounter frequency upon the temperature and the viscosity.

If Equation (9) is plotted against $1/T$ using the experimental values of p from Table III, the slope will be a measure of the difference in E_a and E_d. This plot is shown as Fig. 4. In accordance with Eq. (9) the slope of the curve shows that the energy of activation of the reaction is of the same order of magnitude as the energy of activation of diffusion, but is about 1000 calories per mole less, or approximately 3000 cal./mole.

The intercept of Fig. 4 is approximately 0.2, which means that $C = 1.5P$. Since C is approximately unity, P is also approximately unity, and hence the orientation and entropy requirements
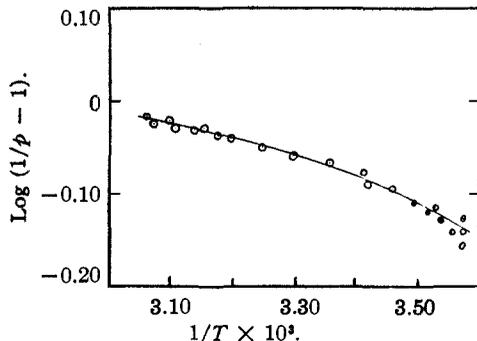

Fig. 4.—The probability of quenching per encounter as a function of temperature.

(24) J. D. R. Scheffer and F. E. C. Scheffer, Verslag Akad. Wetenschappen Amsterdam, 25, 67 (1916).

for the formation of an activated complex which will result in quenching are very slight.

The average number of collisions per encounter, n, is the reciprocal of x, the probability per collision of an encounter terminating by diffusion of the reactants. The influence of the temperature, and the accompanying viscosity changes, upon the number of collisions per encounter may be calculated from Eq. (7) or Eq. (19). The viscous effect is predominant with the result that while n is nearly 1000 at 0°, it decreases to about 100 at 100°.

A survey of the literature shows that of all the many quenching reactions investigated only a very few are as efficient as the aniline–uranin reaction. The self-quenching of dyes as reported by Umberger and La Mer[4] is the only other type of quenching efficient enough to be a possible exception to the argument being presented. The diffusion constants of all common quenching reactants in solution will be of the same order of magnitude, and it is thus reasonable to expect that the large majority of quenching reactions will require a chemical energy of activation equal to, and in most cases exceeding, that required by uranin–aniline reaction. The quenching of fluorescence in solution in general therefore may be expected to be activation as well as diffusion controlled.

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Summary

1. The kinetics of the quenching of the fluorescence of uranin by aniline has been investigated using a precision photoelectric fluorimeter. Molar quenching constants, corrected for reabsorption and other effects, have been measured as a function of temperature.

2. A general equation, utilizing the concept that collisions in liquids occur in sets and introducing an energy of activation term into Smoluchowski's diffusion equation, is presented for reactions in solution whose rates are governed by both an energy of activation and by the interdiffusion of the reactants.

3. Experimental quenching constants for the uranin–aniline reaction, measured as a function of temperature (and necessarily of viscosity also), are interpreted using this equation. Quenching occurs in approximately one out of every encounters (one out of every 1000 collisions) and hence diffusion alone is not sufficient to explain the observed rate of quenching. An energy of activation of the same order of magnitude as the energy of activation of diffusion suffices for agreement between theory and experiment. In general, the quenching of fluorescence in solution should be expected to be activation as well as diffusion controlled.