[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

Solvent Effects on the Quenching of the Fluorescence of Uranin by Aniline[1,2]

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The effects on the quenching of fluorescence produced by changes in viscosity, through the use of different solvents, were noticed early in experimental investigations of this subject.[4,5,6] The theoretical and experimental investigations of Umberger and La Mer[7] showed, however, that it was necessary to apply a number of corrections to the direct measurements of fluorescence quenching in order to convert these data to rate constants. Unfortunately, the necessity of making these corrections was not recognized in the early investigations so that none of the data on solvent effects in the literature can be interpreted unambiguously.

It is the primary purpose of this investigation to interpret rate constants for the quenching of the fluorescence of uranin (basic fluorescein ion) by aniline as a function of the viscosity of the medium. The presently accepted theoretical equations for the calculation of encounter frequencies involve the diffusion coefficients of the reactants. In order to compare the encounter frequencies in liquids with the rate constants, it was necessary to measure these diffusion coefficients. It will be shown that the elementary view that quenching constants will be linear functions of the solvent fluidity is inadequate.

Theory

The application of the cage-model theory of liquids to the study of reaction rates, and the development of rate equations, have been discussed in the previous paper[8] and will not be repeated.

One method of approach to the calculation of encounter frequencies in liquids has been from a model which considers liquids as possessing a lattice structure. Calculations of this type have been made by Rabinowitch,[9] Fowler and Slater,[10] and very recently by Kimball.[11] The molar encounter frequency derived by Kimball is

$$k_{\mathrm{D}} = 4\,\pi R^2 D\,\frac{1}{a}\,\frac{(\mathrm{N})}{1000}\ \text{liters moles}^{-1}\ \text{sec.}^{-1} \qquad (1)$$

where "a" is the distance between lattice points, and all other symbols have their usual values. Rewriting equation (1) as

$$k_{\mathrm{D}} = \frac{4\pi DR(\mathrm{N})}{1000}\,\frac{R}{a}$$

it is evident that k_{D} will be larger than the value given by the stationary state term of Smoluchowski's equation (ref. 8, eq. 2) since in general R will be larger than "a." The dependence of the encounter rate on the viscosity of the medium, eta, is evident if the relation

$$D = \frac{kT}{6\pi\eta}\left(\frac{1}{r_{\mathrm{d}}} + \frac{1}{r_{\mathrm{q}}}\right)$$

is substituted into either equation.

Equation 1 predicts a dependence of k_{D} on the solvent structure through the inclusion of the factor "a" which will be roughly proportional to the size of the solvent molecules. However, an exact value of "a" is not known. It is equivalent to λ, the distance between equilibrium positions of the molecules involved in viscous flow, as defined by Eyring.[12]

The results in this investigation are interpreted in terms of eq. 1. The effects of different solvents on the rate of fluorescence quenching reactions will be discussed on the basis of the relation presented for the probability of reaction per encounter by Williamson and La Mer.[8]

Experimental

Quenching Constants.—Quenching constants have been measured with the sensitive photoelectric fluorimeter developed by Umberger and La Mer,[7] and the experimental techniques described therein. The volume-molar quenching constants for the quenching of the fluorescence of uranin by aniline have been measured in the following solutions: water and glycerol, water and sucrose, water and methanol, and methanol, ethanol and isopropanol.

Uranin was chosen as the fluorescent material since its average life in the excited state, τ, was constant within the solvent ranges chosen.[13,14] Aniline was chosen as the quencher since its electrical neutrality in alkaline solution reduced very greatly primary kinetic salt effects. These have been shown to be zero by Williamson and La Mer.[8]

All quenching measurements were made within the temperature range 24.5–26.5°, with most falling between 24.5 and 25.5°. However, the actual temperature in the cell was recorded at the time of the measurement to ±0.2°, and the viscosity of the solution experimentally determined at this temperature with an Ostwald viscometer. The concentration of quencher used was 0.0100 molar, and the concentration of uranin 10^{-5} molar, with the *p*H of all solutions approximately 12. The results are given in Table I, and are plotted in Fig. 1 as a function of the solvent fluidity.

To correct for the reabsorption of the fluorescent light,[7] it was necessary to obtain the value of the quenching con-

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

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

(6) R. W. Stoughton and G. K. Rollefson, THIS JOURNAL, **62**, 2264 (1940).

(7) J. Q. Umberger and V. K. La Mer, *ibid.*, **67**, 1099 (1945).

(8) B. Williamson and Victor K. La Mer, *ibid.*, **70**, 717 (1948).

(9) E. Rabinowitch, *Trans. Faraday Soc.*, **33**, 1228 (1937).

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

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

(13) F. Perrin, *Ann. Physik*, [10S] **12**, 169 (1929).

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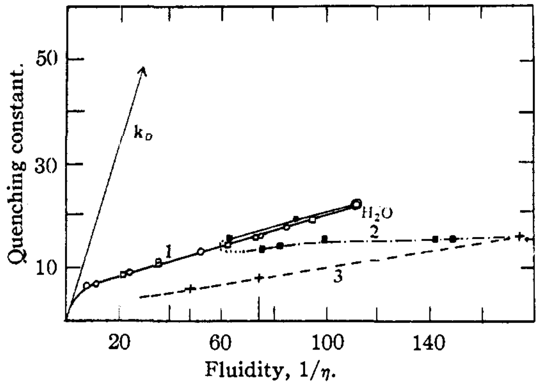

Fig. 1.—Quenching constants as a function of solvent fluidity: ○, glycerol–H_2O; □, sucrose–H_2O; ■, methanol–H_2O.

stant extrapolated to zero uranin concentration. A plot of log k_Q against the concentration of dye shows a linear relation between 10^{-5} and 2×10^{-6} molar uranin. Extrapolation to the zero concentration intercept gives the quenching constant corrected for reabsorption. The slope of this line for the quenching reactions uranin and aniline and uranin and potassium iodide was determined in water at 25° as 8500 ± 500 l./m. In the glycerol–water and sucrose–water mixtures the slopes were found to be less than that in water; for example, 5600 ± 500 for 36.8% glycerol, and 6400 ± 600 for 18.6% sucrose, respectively. This decrease is probably due to a difference in the amount of overlap of the fluorescence and absorption spectra as the solvents are changed. Hence the correction to be applied to the 10^{-5} molar quenching constant decreased as the concentration of the glycerol and the sucrose increased. The slopes in the alcohols were the same as in water.

TABLE I

ANILINE QUENCHING OF THE FLUORESCENCE OF URANIN AS A FUNCTION OF SOLVENT FLUIDITY

Solvent composition Wt. % glycerol	Viscosity (poises × 10³)	Fluidity (poise⁻¹)	k_Q exp. (l./m.)	k_Q corr. (l./m.)
0.00	8.95	112	26.9	22.0
9.8	11.7	85.5	21.5	17.7
14.6	13.1	76.5	19.6	16.2
28.2	19.2	52.0	15.8	13.3
36.8	28.2	35.4	13.0	11.4
45.4	39.8	25.1	11.0	9.8
61.5	81.3	12.3	8.1	6.9
69.0	120	8.25	7.8	6.8
Wt. % sucrose				
9.65	10.6	94	22.6	18.5
14.2	13.6	73.5	19.4	16.0
18.6	16.1	62.1	17.3	14.4
31.1	28.6	35.0	13.0	11.2
39.2	45.5	22.0	10.0	8.7
Wt. % methanol				
8.1	11.2	89.3	24.1	19.6
43	15.9	63.0	19.5	15.8
66	13.15	76.0	16.8	13.4
76	12.0	83.5	17.6	14.2
87	10.0	100	19.4	15.6
94	7.04	142	19.1	15.4
95.5	6.75	148	19.1	15.4
Methanol	5.71	175	19.8	15.9
Ethanol	13.4	74.5	10.3	8.1
Isopropanol	20.8	48.1	8.0	6.2

A small correction was also necessary for the absorption of the incident light by the aniline, as reported by Williamson and La Mer.[8] Their experiments were repeated in various solvents and the absorption remained constant. Hence, as the k_Q decreased, the correction became relatively greater. Quenching independent of diffusion, known as static quenching, may occur when the uranin is excited while in an encounter.[15] This complication is removed by extrapolation to zero concentration of quencher.

Since the addition of sodium hydroxide to aniline hydrochloride produced a precipitate of sodium chloride in the alcoholic solvents, it was necessary to use freshly distilled aniline as quencher in these solvents. In 10^{-5} molar uranin, and 0.01 molar sodium hydroxide in excess of the aniline hydrochloride concentration, the same value of the quenching constant was obtained whether aniline or aniline hydrochloride was used as the quencher.

Diffusion Coefficients.—In order to calculate the molar encounter frequencies for the reaction, values of the diffusion coefficients D, of both dye and quencher are required. A search of the literature revealed that no values were recorded for uranin and aniline at 25°, in the solvents used. Hence, it was necessary to determine the diffusion coefficients in systems closely approximating those occurring during quenching measurements. The "diaphragm cell" method of measurement was employed,[16] using as a standard that value proposed by Gordon, $D = 1.838 \times 10^{-5}$ sq. cm./sec. for 0.10 molar potassium chloride for which $\Delta c_t/\Delta c_0$ is almost unity.

Analysis of the solutions in the upper and lower compartments was carried out as follows:

Potassium Chloride and Hydrochloric Acid.—Volumetric titration using standard procedures ("Elementary Quantitative Analysis" by Willard and Furman).

Uranin.—The uranin concentrations were measured in the fluorimeter after appropriate dilutions with solvent, until upper and lower intensities were comparable and in a range where reabsorption effects did not interfere.

Aniline.—Because of the decomposition which occurs in basic aniline with time, and the consequent shifting of absorption bands, it was necessary to do considerable trial work with basic aniline solutions, making periodic analyses at different wave lengths in the Beckman Ultraviolet Spectrophotometer. The absorption of aniline over a seventy-two hour period at wave lengths between 240 and 300 mμ was found to be sufficiently constant if the diffusion cells were painted black and the solutions protected as well as possible from the light.

The results for uranin and aniline in different solvents are given in Table II. The diffusion coefficients of 10^{-5} molar uranin in glycerol–water and sucrose–water solutions are plotted in Fig. 2 as a function of the weight percentage and mole fraction of glycerol or sucrose.

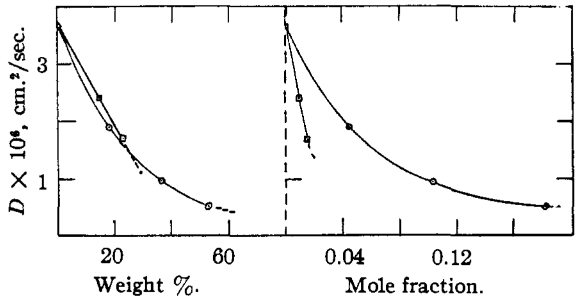

Fig. 2.—Diffusion coefficients of 10^{-5} M uranin in glycerol-water, ⊙, and in sucrose–water, ⊡, solution.

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

(16) A. R. Gordon, *Ann. N. Y. Acad. Sci.*, p. 285 (1945).

TABLE II

DIFFUSION COEFFICIENTS, D, OF URANIN AND ANILINE IN VARIOUS SOLVENTS AT 25°

Solvent composition[a]				
Wt. % glycerol	M./l.	Mole fraction	$D \times 10^6$ (cm.²/sec.)	$r = kT/6\pi\eta D$ (Å. units)
Solute, 10^{-5} M Uranin				
0.0	0.0	0.0	3.67	6.6
19.2	2.18	.044	1.91	7.7
36.8	4.35	.103	0.95	8.8
53.1	6.52	.182	0.51	8.6
Sucrose				
14.2	0.44	0.0087	2.40	6.65
22.9	.73	.0153	1.71	6.7
43% Methanol			2.08	6.6
Methanol			5.64	6.5
Isopropanol			2.18	4.7
Ethanol			3.20	5.0
10^{-4} M Uranin in water			3.60	6.8
Solute, 0.010 M aniline				
Water			9.7	2.5
19.2% Glycerol			5.8	2.5
Methanol			19.3	2.0
Ethanol			10.0	1.6
Isopropanol			6.6	1.6

[a] All solutions pH 12.

Discussion of Results

Diffusion Coefficients.—Kinetic theory when applied to suspended particles in a medium yields,[17] $D = RT/NaF$. Here R, T, and Na, have their usual meanings and F is the frictional resistance, to movement of a particle. According to Stokes' law the frictional resistance for spherical particles, large compared to the molecules of solvent, is $F = 6\pi\eta r$, where "r" is the radius of the particle, and eta is the viscosity of the medium. Combining the equations yields

$$D = \frac{RT}{Na} \times \frac{1}{6\pi\eta r} \tag{2}$$

The methods of testing the reliability of this equation have been to observe the constancy of the products $D\eta$, and Dr, as the other variables are changed. Excellent constancy is obtained when the viscosity is varied by changing the temperature in a fixed solvent. On the other hand, less quantitative agreement is obtained when the viscosity is varied by changing the solvent at a given temperature. The explanation, very likely, is that the frictional resistance given above is less applicable when the solute molecules approach the size of the solvent molecules. In the limit, for very small solute molecules, Sutherland[18] expressed the frictional resistance as $4\pi\eta r$. The radius for uranin is consequently more reliable than that for the smaller aniline molecule.

The effective diffusion radius of the uranin and the aniline, calculated from the Stokes–Einstein relation(2), are reported in Table II. In the glycerol-water mixtures the values of "r" for uranin increase slightly with increasing glycerol content. Solvation of the uranin by the glycerol could account for this increase. It is believed that the smaller values of the radii obtained in the alcohols may be due to the inadequacies of the Stokes–Einstein relation rather than to an actual radius decrease. Hence, in calculating encounter frequencies in the alcohols, the values of the radii obtained in water have been used for the reaction radius, R.

The radius value obtained for uranin in water, 6.6 Å. (pH 12), checks well with the value, 6.8 Å., reported by Marenesco[19] for neutral fluorescein, but differs somewhat from his value, 5.5 Å., for uranin in 0.75 molar sodium hydroxide. Both of Marenesco's values were obtained from viscosity measurements.

Marenesco measured the diffusion coefficients for other dyes—Rhodamine B, Eosin—(see Table III) which are similar in structure to uranin, in solutions of pH 6.2–7. In this range of pH these dyes are not appreciably ionized. There is disagreement between the results of Marenesco and the authors in respect to the trend in radii resulting from the change of solvent from water to alcohols. This divergence probably results from the fact that the two sets of measurements involve substances of different electric charge type, namely, Marenesco's are on a molecule, and these upon ion. Likewise, all of the present measurements were carried out in solutions of 0.01 M sodium hydroxide concentration, and the dye was several hundred-fold more dilute. In addition, the differences of the dielectric constants in the solvents used may effect the diffusion in the case of the uranin.

TABLE III

COMPARISON OF DIFFUSION COEFFICIENTS IN DIFFERENT SOLVENTS

Solvent	Marenesco, 20°C. Base concn. = 10^{-7}–10^{-8} M			This investigation, 25°C. Base concn. = 10^{-2} M		
	$D \times 10^6$ (sq. cm./sec.)		r in Å.	$D \times 10^6$ (sq. cm./sec.)		r in Å.
Water	3.3	Rhodamine B	6.5	3.7	Uranin	6.6
	3.3	Eosin	6.5			
Methanol				5.64	Uranin	6.5
Ethanol	2.4	Rhodamine B	7.5	3.20	Uranin	5.0
	2.1	Eosin	8.6			
Isopropanol				2.18	Uranin	4.7

Quenching Constants.—It may be seen from Table I and Fig. 1 that the results for the dependence of k_Q on η for the different types of solvents do not coincide with each other. The results show a decided dependence on the type of solvent used to obtain the macroscopic viscosity. Sveshnikoff[14] measured I_0/I values for the same reaction in a series of alcohols at various temperatures and reported no specific solvent effects.

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[17] A. Einstein, *Z. Electrochem.*, **14**, 235 (1908).

[18] W. Sutherland, *Phil. Mag.*, [6] **9**, 781 (1905).

[19] N. S. Marenesco, *J. Chim. Phys.*, **24**, 593 (1927).

Unfortunately, it is impossible to correct his measurements for the various interfering effects mentioned previously. Hence, we are unable to report properly corrected quenching constants from his data.

The present results follow three general paths. Starting with water and increasing the viscosity by additions of either glycerol or sucrose, the quenching constants decrease along what has been labelled—path 1—in Fig. 1. The results in methanol, ethanol and isopropanol fall in a series by themselves—path 3—running more or less parallel to the water–glycerol series. However, in changing solvent from methanol to water through a series of methanol–water mixtures, the quenching constants follow path 2. The essentially different nature of methanol-rich mixtures and water-rich mixtures of the same macroscopic viscosity accounts for the effect of these solutions on the quenching constants.

The effect of solvent on the quenching constant, other than that caused by the macroscopic viscosity, may be partially interpreted by the effect of the solvent changes on the lattice distance "a." The larger value of "a" which one would expect in alcohols over that value in water will reduce k_D, the encounter frequency. For example, if the lattice distance be assumed equal to the cube root of the volume per molecule in the following solvents, the decreases in k_D are evident:

Solvent	Molar vol.	"a" in Å.	k_D ("a" const.)	k_D ("a" from column 3)
Water	18	3.1	122 (a = 3.1)	122
Methanol	40	4.1	226 (a = 3.1)	171
Ethanol	58	4.6	120 (a = 3.1)	81
Isopropanol	77	5.0	79 (a = 3.1)	50

Part of the specific solvents effects on the quenching of fluorescence may not have been recognized in the calculation of k_D, the encounter frequency, because the method of measurement of the diffusion coefficients in the various solvents involves the determination of large concentration differences. The true driving force of diffusion is not concentration gradient, but rather gradient of chemical potential or its index, the activity. This may necessitate corrections in k_D which cannot be made easily and it has been necessary to ignore them when treating the data.

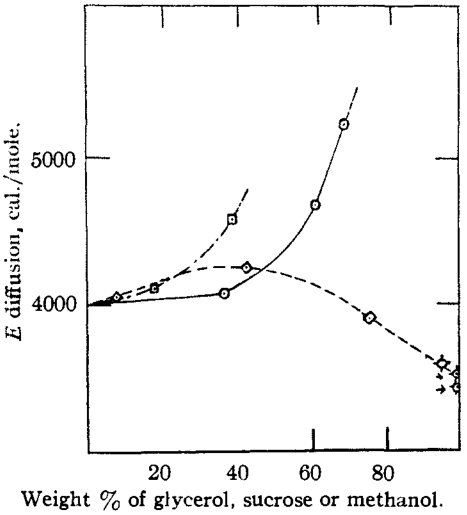

Fig. 3.—Energy of activation of diffusion as a function of solvent: —, ⊙, glycerol-water; – – –, ⊡, sucrose-water; — — —, ◇, methanol-water.

The probability of reaction per encounter, "p"[8] may change with changing solvent. This probability may be equated to the ratio of the probability of reaction per collision to the sum of the probabilities of reaction per collision plus the probability of diffusion out of the encounter by either reactant between collisions

$$p = \frac{P \exp.\,(-E\ \text{act.}/RT)}{P \exp.\,(-E\ \text{act.}/RT) + C \exp.\,(-E_{\text{diff.}}/RT)} \quad (3)$$

It seems reasonable to assume that the solvent forming the cage will have only a small effect on the chemical energy of activation. However, it is well known that the energy of activation of diffusion, $E_{\text{diff.}}$, varies considerably in different solvents. It is at once apparent that the lower the value of the energy of activation of diffusion, the more likely an encounter is to be broken by diffusion between collisions. Thus the probability of reaction per encounter must diminish.

From the "p" values obtained from comparison of measured quenching constants with the calculated encounter frequencies, the energies of activation of diffusion have been calculated. The chemical energy of activation has been calculated to be 3900 cal./mole by the method of Williamson and La Mer[8] with k_D from equation 1. The energy of activation of diffusion in water was chosen as 4000 cal./mole at 25°. The ratio C/P (equation 3) equals 5, and for the $E_{\text{diff.}}$ calculations the steric factor P has been assumed unity. Selected $E_{\text{diff.}}$ values are given in Table IV. The energies of activation of diffusion of the solvents used are plotted as a function of solvent composition in Fig. 3.

The glycerol–water and sucrose–water solutions yield values of the energy of activation of diffusion of 4000 cal./mole or more in all cases. The solvents methanol, ethanol and isopropanol give values around 3500 cal./mole. The energies of activation of diffusion pass through a maximum in the methanol–water mixtures.

The literature gives the following information on energies of activation of diffusion. Quantitative data on energies of activation of diffusion are relatively scarce due to the difficulties of measurement. Scheffer and Scheffer's[20] data on the diffusion of mannitol in water yield an $E_{\text{diff.}}$ of 4460 cal./mole in the temperature range 20–30°. Other data calculated from the "International Critical Tables" for the solvent water gives $E_{\text{diff.}}$

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[20] J. Scheffer and F. Scheffer, *Proc. Acad. Sci. Amsterdam*, **19**, 148 (1916).

at 15° as about 5000, while for iodine in methanol at 15° $E_{\text{diff.}}$ is 4100, and iodine in ethanol in the range 8–18° is 3700 cal./mole. In general when the solute molecules are large, $E_{\text{diff.}}$ is a function of the solvent rather than the solute.

Eyring[21] states that the energy of activation of diffusion is related to the energy of evaporation by a factor ranging between 3 and 4. For water and the alcohols these values have been calculated and are given in Table IV, assuming a constant factor of 3. The energy of activation of viscosity has been calculated from the temperature dependence of measured viscosities.

TABLE IV

OBSERVED AND CALCULATED VALUES OF THE ENERGY OF ACTIVATION OF DIFFUSION

Solvent	k_D (calcd.)	k_Q (meas.)	p	E_D (obs.) (cal./mole)	$E_{\text{visc.}}$ (calcd.) (cal./mole)
36.8 Wt. % glyc.	55.9	11.4	0.20	4070	20% 4620 40% 5570 60% 7110
61.5 Wt. % glyc.	16.8	6.9	.41	4670	
69 Wt. % glyc.	10.9	6.8	.63	5210	
18.6 Wt. % sucrose	67.8	14.4	.21	4110	20% 4620
39.2 Wt. % sucrose	23.6	8.7	.37	4590	40% 6110
8.1 Wt. % methanol	98	19.6	.20	4070	47.4% 4560 71.6% 3700
43 Wt. % methanol	62.4	15.8	.25	4260	
76 Wt. % methanol	87.0	14.2	.16	3910	
Methanol	171	15.9	.09	3540	2430
Ethanol	81	8.1	.10	3580	$E_{\text{vap.}}/3$-3140
Isopropanol	79	6.2	.08	3440	$E_{\text{vap.}}/3$-3170
Water	122	22.0	.18	4000	$E_{\text{vap.}}/3$-3520 $E_{\text{visc.}}$ 3700

The trends shown are all in agreement with the experimental results. Better quantitative agreement would be fortuitous, considering the original assumptions. Note that in Table IV, when p is small, the $E_{\text{diff.}}$ is small. This means that the probability of reaction during an encounter will be less the more easily the reactants can diffuse out of the encounter between collisions.

The dependence of quenching constants on the viscosity of the solvent will vary depending on the choice of solvent, and the chemical energy of activation for the particular fluorescent material and quencher. A linear relationship between quenching constant and fluidity will be the exception.

The data of this investigation have been recalculated on the basis of the normalized mole fraction concentration scale.[22] No advantage has been gained by such a change in this reaction.

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Summary

1. The kinetics of the quenching of the fluorescence of uranin by aniline has been investigated as a function of the viscosity of the solvent in which the reaction is carried out.

2. The diffusion coefficients, D of uranin and aniline have been measured using the diaphragm-cell method in the solvents water, methanol, ethanol, isopropanol, water–glycerol and water–sucrose. They are reported and discussed in terms of the theories pertaining to diffusion.

3. The molar quenching constants are dependent on properties other than the macroscopic viscosity of the solvent. Hence, the previously expected linear relationship between quenching constants and the reciprocal of viscosity need not hold. The data are interpreted in terms of both the diffusion of the reactants together and the probability of reaction when the two are in an encounter. This latter quantity is controlled by an energy of activation of diffusion. The functional dependence of quenching constants on viscosity will depend on the choice of solvent used to vary the viscosity.

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[21] Glasstone, Laidler and Eyring, "Theory of Rate Processes," The McGraw-Gill Book Co., New York, N. Y., 1941, p. 523.

[22] H. G. Davis and V. K. La Mer, *J. Chem. Phys.*, **10**, 585 (1942).