[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF MARYLAND]

The Quenching of the Fluorescence of the Eosin Ion[1]

BY W. J. SVIRBELY AND N. E. SHARPLESS[2]

RECEIVED AUGUST 19, 1953

An improved sensitive fluorimeter with a high degree of stability has been constructed and used in studying the quenching of fluorescence of eosin by iodide ion in water and in water–organic solvent mixtures. Both the self-quenching of eosin and the iodide ion quenching of eosin show a primary salt effect which is accountable for in terms of the Brönsted–Christiansen–Scatchard rate equation. Quenching constants increase linearly with increasing concentration of iodide ion at constant ionic strength. The rate of change of the quenching constant, k_Q, with quencher concentration is higher at higher ionic strengths. Comparison of observed and theoretical quenching constants in water shows that quenching of eosin may occur in about one out of every eight encounters with an iodide ion. In isodielectric media, the quenching constants increase with increasing fluidity of the system. It is shown that at constant ionic strength, the quenching constant can be expressed in terms of dielectric constant, D, viscosity η, and absolute temperature, T, by means of the equation $k_Q = (A'D^{1/2}T^{3/2})/\eta$. In isodielectric mixtures, the predictions of the above equation are met in case the organic solvent–water mixtures do not show a maximum in the viscosity–composition curve. However, if the organic solvent–water mixture shows a maximum in the viscosity–composition curve, deviations occur if the solvent mixture being used has a composition near the maximum in the viscosity–composition curve. The eosin–iodide quenching reaction has a positive temperature coefficient in isodielectric glycerol–water mixtures. The data are analyzed in terms of the Williamson–La Mer equation for activation and diffusion controlled reactions.

Introduction

Recently La Mer and his co-workers[3-5] have studied the quenching of uranin as affected by ionic strength, temperature and viscosity of the solvent. Modifications in the theory for the kinetics of diffusion controlled ionic reactions were found necessary in order to explain the observed results.

Eosin (disodium tetrabromofluorescein) has a moderate degree of fluorescence between 5200 and 6000 Å., with a maximum at 5400 Å.[6] Measurements of the fluorescent intensity showed that its fluorescence was, however, only about one-tenth as intense as that of an equivalent concentration of uranin (disodium fluorescein).

Comparatively little work has been published on the iodide quenching constants of eosin. Pringsheim[7] gives a value of 3.7 for k_Q. No conditions are given. Weber's[8] data lead to iodide quenching constants varying from 3.70 in a 0.03 M potassium iodide solution to 11.0 in a 1.208 M potassium iodide solution for the quenching of a 4.34×10^{-5} M eosin solution at 15°. Weber's ionic strengths varied with the potassium iodide concentration and thus the increase in k_Q due to increasing quencher concentration was superimposed on the ionic strength effect.

The purpose of this research was to investigate the reaction

$$^*E^{-2} + I^- \longrightarrow E^{-2} + I^- + \text{heat}$$

and to interpret the rate constants for the quenching as a function of ionic strength, dielectric constant, viscosity and temperature.

Experimental

Materials. Eosin.—National Aniline Co. eosin was recrystallized from n-butyl alcohol until it was free from inorganic halides.

Potassium Nitrate and Potassium Iodide.—Mallinckrodt C.P. products were used without further purification.

Organic Solvents.—Methyl alcohol, ethyl alcohol, dioxane, glycerol, acetone and ethylene glycol were purified by methods in the literature.[9]

Apparatus.—A design of a balanced type amplifying circuit for making quenching studies has been described by Umberger and La Mer.[3] In the interest of greater stability, it was found necessary to modify the circuit.[10] The amplifying circuit for this research is shown in Fig. 1. The amplifier was housed in a grounded, light-tight, all metal box. The box was mounted on $^3/_4$ inch of cotton backed by 1.5 inches of paper in order to minimize shock to the filaments of the electrometer tubes. During operation, the resistor R was adjusted to give a filament current of 10 ma. through each tube. The potentiometer, H, was used to adjust the plates of the tubes to the same potential. The leads from the photocell cathodes to the photocell resistors (B, B′) were of coaxial cable. All other external leads were of shielded wire.

The optical bench consisted of a thirty-inch length of channel iron slotted to accept the ultraviolet light mounting, the photocell-cuvette housings and the lens-diaphragm assemblies. The bench with its accessories is shown schematically by the right-hand section of Fig. 7 in ref. 3. The ultraviolet light source was a G.E. AH4 lamp operating off a Raytheon VR-4 voltage regulator.

Each glass absorption cell (75 ml. capacity, 6 × 6 × 2 cm.) had an isolating filter (Corning 586–738 to transmit the mercury line, $\lambda = 0.365\ \mu$) cemented over a 1-inch hole drilled in one of the faces of the cell. Each cell was housed in the larger chamber of a double unit brass box; the small chamber contained the photo cell. A yellow filter (Corning 038) was cemented over a hole drilled in the wall separating the two chambers. Each cuvette was mounted flush against the filter opening and a 1.5 inch tube led from the ultraviolet light source to the isolating filter of the cuvette. Water at constant temperature could thus be circulated around the cuvette. All joints between filter and box were sealed with litharge-glycerol cement. For runs made at 25, 35 and 45°, the temperatures were constant within 0.05, 0.1 and 0.2°, respectively.

The operational adjustments of the apparatus were the same as those of Umberger and La Mer.[3] The potentiome-

---

[1] (a) Abstracted fron the Ph.D. thesis of N. E. Sharpless, June, 1951. (b) Presented in part at the New York Meeting of the International Congress of Chemistry, September, 1951.

[2] National Institutes of Health pre-doctorate Fellow for 1948 and 1949.

[3] J. Q. Umberger and V. K. La Mer, THIS JOURNAL, **67**, 1099 (1945).

[4] B. Williamson and V. K. La Mer, *ibid.*, **70**, 717 (1948).

[5] K. C. Hodges and V. K. La Mer, *ibid.*, **70**, 722 (1948).

[6] E. L. Nichols and E. Meritt, *Phys. Rev.*, **30**, 328 (1910); **30**, 376 (1910).

[7] P. Pringsheim, "Fluorescence and Phosphorescence," Interscience Publishers, Inc., New York, N. Y., 1949.

[8] K. Weber, *Trans. Faraday Soc.*, **XLIV**, 785 (1948).

[9] (a) N. Bjerrum and L. Zechmeister, *Ber.*, **56**, 894 (1923); (b) L. F. Fieser, "Experiments in Organic Chemistry," 2nd edition, D. C. Heath and Co., New York, N. Y., 1941, p. 359; (c) *ibid.*, p. 369; (d) G. Åkerlöf, THIS JOURNAL, **54**, 4125 (1932).

[10] We acknowledge our indebtedness to Dr. Howard L. Andrews of the National Institutes of Health for designing the amplifier circuit used.

ter readings for conditions of balance lead to values of $((I_0/I_Q) - 1)$ where I_0 and I_Q are the intensities of the unquenched and quenched solutions, respectively.

Solutions.—The following procedure was employed in preparing solutions: (1) stock solutions of 10^{-3} *M* eosin and 1 *M* potassium nitrate and 1 *M* potassium iodide were prepared. (Solutions of potassium iodide were prepared fresh for each run); (2) required volumes of eosin, potassium iodide and potassium nitrate stock solutions were mixed when preparing a quenched aqueous solution of finite composition and ionic strength; when a mixed solvent was employed, the appropriate volume of pure organic solvent was added to an aqueous solution of dye and salts. The whole was then made up to volume with stock solutions of the required dielectric constant[9d]; (3) required volumes of eosin and potassium nitrate stock solutions were mixed to give an unquenched solution of the same composition and ionic strength as the quenched solution with which it was to be compared.

Viscosities.—Viscosities were determined with an Ostwald viscometer.

Miscellaneous.—Although many fluorescent compounds are quenched by molecular oxygen, eosin is unaffected.[11] Special precautions to eliminate air from our solutions were, therefore, considered unnecessary.

Experimental Results and Discussions

Miscellaneous.—The fluorescent intensity of a 10^{-4} *M* eosin solution remained constant on increasing the *p*H to a maximum of 12.7 with sodium hydroxide. These results indicate that both the hydrolysis of eosin to the neutral molecule and the quenching of eosin by OH^- are experimentally undetectable.

The possibility that quenching action is due to a non-fluorescent complex formed by eosin and iodide ion was investigated by determining the absorption spectra from 400 to 590 mμ of two solutions containing 2×10^{-5} *M* eosin. One solution was made up to 0.012 *M* in potassium nitrate (a non-quencher) and the other to 0.012 *M* in potassium iodide (a quencher). Within the limits of experimental error, the two spectra are identical in regards to both the intensity and the position of the principal maximum.

Performance of Apparatus.—Quenching constants, k_Q, can be calculated by use of the Stern–Volmer[12] equation

$$k_Q = \left(\frac{I_0}{I_Q} - 1\right) \Big/ [C_Q] \qquad (1)$$

where $[C_Q]$ is the concentration of the quencher. To check the performance of the apparatus, runs were made using 10^{-4} *M* uranin quenched with iodide ion and the quenching constants were compared with the data in Table VI of Umberger and La Mer.[3] Agreement within 1% was obtained.

Self-quenching of the Fluorescence of Eosin.—Umberger and La Mer[3] have discussed self-quenching in the case of uranin. They interpreted the decrease in the fluorescence of uranin on the addition of potassium nitrate (a non-quencher) as a primary salt effect on the frequency of self-quenching encounters of fluorescein ions.

The same effect was investigated in this study of eosin. Due to the low fluorescent intensity of eosin and the small changes in the fluorescent intensity with added potassium nitrate, the results did not lend themselves to a very high degree of precision.

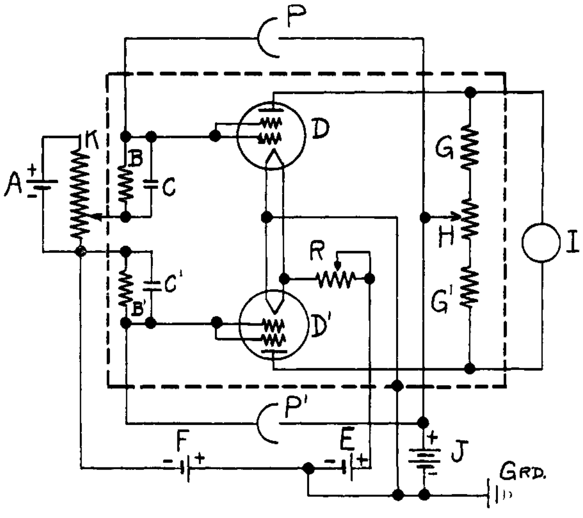


Fig. 1.—Diagram of circuit: A, 2 v. storage battery; B,B', 10 megohm matched resistors; C,C', 0.03 μf. condensers; D,D', Victoreen 41 B electrometer tubes; E, 2 v. Everready air cell; F, 1.5 v. "C" battery; G,G', 75,000 ohm wire wound resistors; H, 5,000 ohm wire wound potentiometer; J, 90 v. d.c. source; I, L and N type R reflecting galvanometer; K, L and N type K potentiometer; P,P', RCA 926 photocells; R, variable resistor.

The self-quenching constants, $k_Q{}^s$, for eosin can be calculated by use of equation 2

$$k_Q{}^s = \left(\frac{I_0}{I} - 1\right) \Big/ [E^{-2}] \qquad (2)$$

I_0, the intensity of the unquenched solution, is not directly measurable in self-quenching experiments. The extrapolation of an intensity *vs.* $\sqrt{\mu}$ curve to zero ionic strength will give a provisional value of I_0. Since the self-quenching reaction is one involving two ions, where $Z_A = Z_B = -2$, the Brönsted–Christiansen–Scatchard rate equation in water at 25° becomes

$$\log k_Q{}^s = \log k_Q{}^s{}_{(\mu=0)} + \frac{4.0\sqrt{\mu}}{1 + \beta a\sqrt{\mu}} \qquad (3)$$

Using the provisional value of I_0 obtained graphically, provisional self-quenching constants were first calculated by equation 2. Then by trial and error, a value of βa was determined by use of equation 3 and the provisional self-quenching constants which gave the most consistent value of $k_Q{}^s{}_{(\mu=0)}$ over the complete ionic strength range. The resulting equation was

$$\log k_Q{}^s = \log k_Q{}^s{}_{(\mu=0)} + \frac{4.0\sqrt{\mu}}{1 + 2.5\sqrt{\mu}} \qquad (4)$$

A combination of equations 2 and 4 gives equation 5

$$\log\left(\frac{I_0}{I} - 1\right) - \log [E^{-2}] = \log k_Q{}^s{}_{(\mu=0)} + \frac{4.0\sqrt{\mu}}{1 + 2.5\sqrt{\mu}} \qquad (5)$$

An improved value of I_0 was obtained by use of equation 5 and the experimental data. This improved value of I_0 was now used to calculate self-quenching constants by equation 2. These final values of $k_Q{}^s$ were found to fit equation 4 without further change in the parameter value of 2.5 for each concentration of eosin investigated. The final results of all calculations are given in Table I.

---

(11) H. Weil-Malherbe and J. Weiss, *Nature*, **149**, 471 (1942).

(12) O. Stern and M. Volmer, *Physik. Z.*, **20**, 183 (1919).

TABLE I

THE SALT EFFECT ON SELF-QUENCHING OF EOSIN AT 25°

Eosin, mole/l.	$\sqrt{\mu}$	I	$\log k_Q^s$	$\log k_{Q^s(\mu=0)}$
		(I_0 = 430)		
1 × 10⁻⁴	0.079	410	2.68	2.41
	.106	405	2.78	2.44
	.146	402	2.84	2.41
	.177	398	2.90	2.40
	.225	391	3.00	2.41
	.276	387	3.04	2.38
	.318	380	3.12	2.40
			Av.	2.41
		(I_0 = 1098)		
5 × 10⁻⁵	0.224	1035	3.08	2.51
	.317	1016	3.21	2.51
	.388	999	3.30	2.51
	.448	987	3.35	2.49
	.500	976	3.40	2.49
			Av.	2.50
		(I_0 = 368)		
2 × 10⁻⁵	0.224	362	2.90	2.32
	.448	355	3.27	2.42
	.548	354	3.29	2.36
			Av.	2.37

On theoretical grounds[3] it is predicted that a plot of $\log k_{Q^s(\mu=0)}$ against the concentration of the dye should be linear and that the extrapolated value of $\log k_{Q^s(\mu=0)}$ at zero concentration of the dye would be the "true" self-quenching constant, *i.e.*, the value corrected for fluorescence absorption. While our data for each concentration of eosin are consistent among themselves, nevertheless, they are not consistent regarding $\log k_{Q^s(\mu=0)}$ when compared as a function of the concentration of eosin. This may be the case either because the data are not as precise as they appear to be or because within experimental error, "the true" self-quenching constant has been reached in each of the concentrations studied. If the latter is the case, the average value of $k_{Q}{}^{s}{}^{(c=0)}_{(\mu=0)}$ for eosin is 270. The self-quenching of eosin is thus more efficient than that of uranin ($k_{Q}{}^{s}{}^{(c=0)}_{(\mu=0)}$ = 8.2).[3]

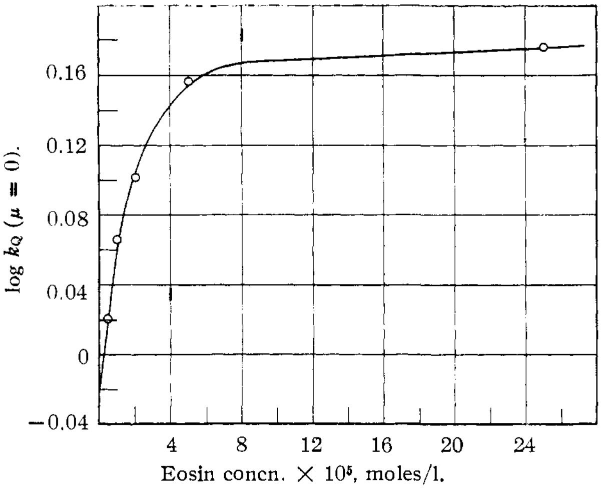


Fig. 2.—Iodide quenching constants at zero ionic strength *vs.* eosin concentration.

Iodide Ion Quenching.—The salt effect on the iodide quenching of eosin was investigated both as a function of the eosin concentration and of the ionic strength at 25° in aqueous solution using potassium nitrate (a non-quencher). Due to the low fluorescent intensity of eosin, quenching constants for eosin concentrations less than 5 × 10^{-6} M could not be obtained.

Since the quenching reaction in this case is between a divalent and a univalent ion of like charge sign, the B–C–S rate equation in water at 25° becomes

$$\log k_Q = \log k_{Q(\mu=0)} + \frac{2.0\sqrt{\mu}}{1+\beta a\sqrt{\mu}} \qquad (6)$$

After obtaining values of k_Q by use of equation 1, ($C_Q = C_{I^-}$), trial and error calculations showed that constancy of $\log k_{Q(\mu=0)}$ was obtained when βa was again equal to 2.5. The Debye–Hückel parameter "a" thus becomes 7.6 Å. for both self-quenching and iodide quenching of eosin and is independent of the concentration of eosin. The results are summarized in Table II for 0.1 M potassium iodide solutions. The $\log k_{Q(\mu=0)}$ term, as it does for uranin,[3] decreases as the eosin concentration decreases (Fig. 2). From Fig. 2, one obtains $\log k_{Q(\mu=0)}^{c=0} = -0.022$, from which $k_{Q(\mu=0)}^{c=0} = 0.95$. This is the "true" iodide quenching constant, corrected for salt effect and the reabsorption of the fluorescent light by the dye.

TABLE II

THE SALT EFFECT ON IODIDE QUENCHING OF EOSIN AT 25° IN 0.1 M POTASSIUM IODIDE SOLUTIONS

Eosin concn. × 10⁵, mole/l.	μ	k_Q	$\log k_{Q(\mu=0)}$	$\log k_{Q(\mu=0)}$ av.	$k_{Q(\mu=0)}$
0.5	0.100	2.32	0.022		
	.200	2.76	.018	0.020 ± 0.002	1.04
1.0	.100	2.62	.064		
	.150	2.85	.061		
	.200	3.10	.069		
	.250	3.30	.073		
	.500	3.77	.065	.066 ± 0.004	1.17
2.0	.100	2.79	.092		
	.200	3.38	.107		
	.300	3.67	.103		
	.500	4.08	.100	.101 ± 0.004	1.26
5.0	.100	3.24	.157		
	.150	3.46	.145		
	.200	3.78	.156		
	.250	4.00	.158		
	.300	4.16	.157		
	.500	4.74	.165	.156 ± 0.004	1.43
25.0	.201	3.95	.174		
	.251	4.19	.177	.176 ± 0.002	1.50

The application of equation 12 to the iodide quenching of eosin in water at 25° yields a value of 8.0 for $k_{Q(\mu=0)}^{c=0}$ theoretical. This value on being compared with the experimental value of 0.95 indicates that a quenching action occurs in approximately one out of eight encounters. This calculated result obviously depends on the correctness of the values of the average life of the unquenched eosin ion and of the diffusion radii used in its calculation.

The Influence of Potassium Iodide on the Quenching Constant.—The influence of iodide concentration on the quenching constant was studied in isoionic strength solutions of 0.25 and 0.50 at 25° in water using potassium nitrate to keep the ionic strength constant. The results are given in Table III. The data may be expressed analytically in each case by the equations

$$k_{\mathrm{Q}} = 0.80(\mathrm{I}^-) + 3.96 \quad \mu = 0.25 \tag{7}$$

$$k_{\mathrm{Q}} = 1.83(\mathrm{I}^-) + 4.29 \quad \mu = 0.50 \tag{8}$$

These equations are in agreement with predictions of Rollefson and Boaz[13] requiring a linear relationship between k_{Q} and (I^-). The influence of ionic strength on the rate at which iodide concentration influences the quenching constant is quite marked. Doubling the ionic strength causes $\sim$130% increase in the slope of a k_{Q} *vs.* (I^-) plot. The increase in rate could be due to the reduction of repulsion between dye and quencher ion caused by the screening effect of the additional potassium ion atmosphere about the negative ions. These results show clearly that when the effect of quencher concentrations in ionic systems is being investigated, control must be maintained over the ionic strength, otherwise the ionic strength effect will be superimposed on the quencher concentration effect.

TABLE III

THE EFFECT OF IODIDE ION CONCENTRATION ON THE QUENCHING CONSTANT IN ISOIONIC SOLUTIONS AT 25° FOR 10^{-4} *M* EOSIN SOLUTIONS

μ = 0.25		μ = 0.50	
KI, mole/l.	k_{Q}	KI, mole/l.	k_{Q}
0.05	4.00	0.05	4.38
.10	4.07	.20	4.64
.15	4.05	.30	4.83
.20	4.11	.40	5.02
.25	4.12		

The Influence of Viscosity on Quenching as a Function of the Dielectric Constant.—Quenching constants and viscosities were obtained in isodielectric mixtures with dielectric constant values of 75, 65 and 55 for 5×10^{-5} *M* eosin solutions quenched with 0.10 *M* potassium iodide ($\mu = 0.10$ throughout) at 25°. It would have been preferable to obtain k_{Q} values over an ionic strength range at varying concentrations of eosin in each of the mixtures and then correct for salt and reabsorption effects. However, the increasing lower values of the quenching constants as the viscosity was increased made extrapolations unreliable. The data given in Table IV are for the concentrations specified above.

The molar encounter rate, k_{D}, in a diffusion controlled process is given[3] by equation 9

$$k_{\mathrm{D}} = \frac{4\pi \bar{D} N L e^{\kappa L}}{1000\,(e^{L/R} - 1)} \tag{9}$$

where κ and L are defined by $\sqrt{8\pi\epsilon^2 N\mu/1000\,DkT}$ and $2\epsilon^2/DkT$, respectively. In the above equations, $\bar{D}$ = diffusion coefficient, N = Avogadro's number, ϵ = electronic charge, D = dielectric constant, k = Boltzman's constant, T = absolute temperature, μ = ionic strength and R = reaction distance. If the Stokes–Einstein expression for $\bar{D}$, namely

$$\bar{D} = \frac{kT}{6\pi\eta}\left(\frac{1}{r_{\mathrm{E}^{-2}}} + \frac{1}{r_{\mathrm{I}^-}}\right) \tag{10}$$

is combined with equation 9, then on substitution of the appropriate constants one obtains

$$k_{\mathrm{D}} = \frac{3.32 \times 10^{12}\ TLe^{\kappa L}}{\eta(e^{L/R} - 1)} \tag{11}$$

where η is the viscosity. The values of the diffusion radii for the eosin ion[14] and the iodide ion[15] were taken as 7.0×10^{-8} and 2.2×10^{-8} cm., respectively.

The theoretical quenching constant in terms of the encounter frequency is defined by

$$k_{\mathrm{Q(theoretical)}} = \tau_0 k_{\mathrm{D}} = \frac{6.31 \times 10^3 TLe^{\kappa L}}{\eta(e^{L/R} - 1)} \tag{12}$$

where τ_0 is the average life of the unquenched eosin ion and was taken as 1.9×10^{-9} second.[16] The application of equation 12 at $\mu = 0.10$ and at 25° in the various mixed solvents yields the values of $k_{\mathrm{Q(theoretical)}}$ listed in Table IV. The values for the ratio of $k_{\mathrm{Q(theoretical)}}/k_{\mathrm{Q(exp.)}}$ are listed in the fifth column of Table IV. These ratio values indicate the number of encounters required in the various solvent mixtures in order for a quenching action to occur.

Since the data listed in Table IV were obtained in solutions at a constant ionic strength of 0.10, then on consideration of equation 12 and the definitions of κ and L it is apparent that $k_{\mathrm{Q(theoretical)}}$ should be dependent on viscosity, dielectric constant and temperature. If one assumes that $k_{\mathrm{Q(exp.)}}$ can also be expressed by a similar equation as far as the variables are concerned, then on the basis of certain approximations one can derive equation 13, namely

$$k_{\mathrm{Q(exp.)}} = (A'D^{1/2}/T^{3/2})/\eta \tag{13}$$

Inspection of equation 13 shows that if a plot of k_{Q} *versus* $D^{1/2}/\eta$ is made at constant temperature in isodielectric media, the slope would be constant.

---

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

[14] A consideration of the data of Marenesco (Table III, ref. 5) for the eosin molecule and of Hodges and La Mer[5] for the uranin ion shows that a highly probable value for the diffusion radius of the eosin ion is 7.0×10^{-8} cm. This value was used in calculating encounter frequencies in all solvents.

[15] L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1945, p. 350, gives 2.16 Å. as the radius of the iodide ion as obtained from crystallographic data. H. Ulich, *Trans. Faraday Soc.*, **23**, 388 (1927), concludes from transport data that the iodide ion is not solvated in water. W. M. Latimer, K. S. Pitzer and C. M. Slansky, *J. Chem. Phys.*, **7**, 108 (1939), conclude that the effective radii of negative ions in solution will be but slightly larger than ordinary crystal radii and estimate that the radius of the iodide ion in water is 2.26 Å. In light of the above conclusions, we selected 2.2 Å. as the diffusion radius of the iodide ion.

[16] E. Gaviola, *Z. Physik*, **42**, 853 (1927). Gaviola's data for eosin lead to τ_0 values of 1.9×10^{-9}, 3.4×10^{-9}, 5.1×10^{-9} and 7.8×10^{-9} second in water, methanol, ethanol and glycerol, respectively; *i.e.*, increasing τ_0 values with increasing viscosity. Since τ_0 values for eosin in the mixtures used in this investigation are lacking, we will assume that because the observed viscosities are, in general, of the same order of magnitude as that of water, the most acceptable value to use for τ_0 is 1.9×10^{-9} second. This will lead to a slight error in some solutions.

TABLE IV

THE INFLUENCE OF VISCOSITY ON THE QUENCHING CONSTANTS AT 25° IN ISODIELECTRIC MIXTURES

Eosin = 5×10^{-5} *M*; KI = 0.10 *M*; μ = 0.10.

Solvent–water mixtures	η, poise	k_Q, (exp.)	k_Q (theor.) (Calcd. by eq. 12)	$k_{Q(\text{theo})}/k_{Q(\text{exp})}$
		D = 75		
Sucrose	0.0140	1.93	24.75	13
Glycerol	.0119	2.52	29.10	12
Glycol	.0115	2.72	30.10	11
Ethanol	.0110	2.93	31.50	11
Methanol	.0108	2.95	32.10	11
Acetone	.0107	3.03	32.38	11
		D = 65		
Sucrose	0.1008	0.62	4.34	7
Glycerol	.0414	1.06	10.48	10
Glycol	.0237	1.60	18.32	11
Ethanol	.0189	1.83	22.96	13
Methanol	.0151	2.21	28.74	13
Acetone	.0129	1.95	33.65	17
		D = 55		
Glycerol	0.2121	0.37	2.88	8
Glycol	.0625	.75	9.77	13
Ethanol	.0237	.79	25.8	32
Methanol	.0154	1.43	39.7	38
Acetone	.0135	1.82	45.2	25

Furthermore, if plots are made of data obtained in isodielectric media of different dielectric values, the slopes would become progressively greater as the dielectric constant is increased. Figure 3 illustrates such plots in isodielectric media having dielectric values of 75, 65 and 55. It is apparent on inspection of Fig. 3 that the above predictions are fulfilled in the isodielectric media with the dielectric values of 75 and 65. However, acetone–water with the dielectric value of 65 and ethanol–water, methanol–water and acetone–water with the dielectric value of 55 are displaced from the regular curves. Each of these three organic solvents has a maximum in the viscosity *versus* solvent–water composition curves and an analysis of the data shows that at the dielectric values where discontinuity has occurred the three solvent–water mixtures have compositions either on the organic solvent-richer side of the maximum or are within 7% of the composition required at the maximum. The viscosities of aqueous solutions of sucrose, glycerol and ethylene glycol, on the other hand, show no maxima and the k_Q's increase regularly with increasing solvent concentration. Thus specific solvent influences are operative in these media. Specific solvent effects in methanol–water mixtures have been previously recognized[5] and were credited to the essentially different nature of methanol-rich mixtures and water-rich mixtures of the same microscopic viscosity. Our results support such a conclusion.

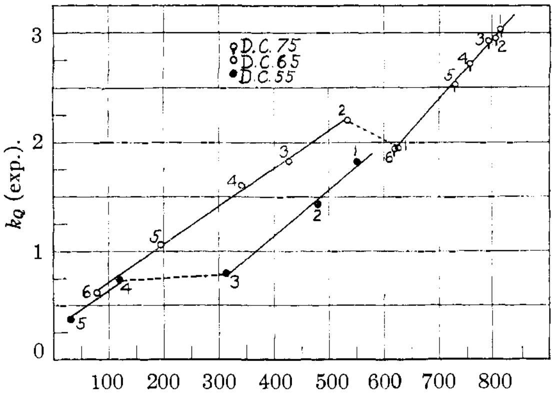


Fig. 3.—Effect of viscosity and dielectric constant on the quenching constant in isodielectric mixtures at constant ionic strength and at 25°: 1, 2, 3, 4, 5 represent aqueous solutions of acetone, methanol, ethanol, ethylene glycol, glycerol and sucrose, respectively.

Dioxane–water solutions gave totally unexpected behavior. The fluorescent intensities of eosin in unquenched dioxane–water solutions were not significantly different from those obtained in unquenched solutions using other solvents and were constant under continuous illumination. The intensities of eosin in quenched dioxane–water solutions, however, were quite low and increased steadily with time. Such behavior suggests either the formation of a new fluorescent compound or the loss of the quencher. Consequently, successive determinations of quenching constants in the same solution gave progressively lower values. Determination of quenching constants by extrapolation to zero time gave values 10 to 30 times as high as those determined in the other solvents.

The Effect of Temperature on Quenching.—Quenching constants and viscosities were obtained in isodielectric mixtures of glycerol–water (a combination which does not show a maximum in the viscosity–composition curve) with dielectric constant values of 65 and 55 for 5×10^{-5} *M* eosin solutions quenched with 0.10 *M* potassium iodide (μ = 0.10) over the temperature range from 25 to 45°. The results are presented in Table V along with other pertinent data. An examination of the data in the fourth column of Table V shows that a positive temperature coefficient is obtained in isodielectric media.

According to equation 13, a plot of k_Q *versus* $D^{1/2}T^{3/2}/\eta$ should be linear. Such a plot is illustrated by Fig. 4. It is evident that linearity is obtained.

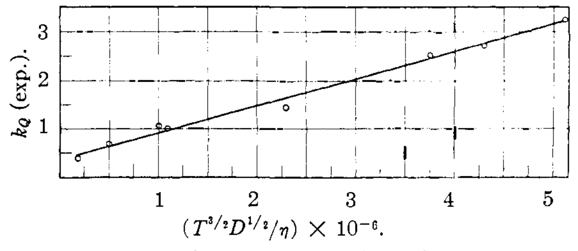


Fig. 4.—Effect of temperature, dielectric constant and viscosity on the quenching constant in glycerol–water solutions.

The theoretical quenching constants as defined by equation 12 are listed in the fifth column of Table V. The number of encounters required under the given conditions in order for a quenching action to occur are listed in the sixth column of Table V.

TABLE V

INFLUENCE OF TEMPERATURE ON THE QUENCHING CONSTANTS IN GLYCEROL–WATER MEDIA

Eosin = 5×10^{-5} *M;* KI = 0.10 *M;* μ = 0.10.

Temp., °C.	Dielectric constant	η, poise	k_Q, (exp.)	k_Q (theor.) (Calcd. by eq. 12)	$1/p = k_{Q(\text{theoretical})}/k_{Q(\text{exp})}$
25	78.5 (water)	0.00895	3.24[a]	36.31	11.21
	75	.0119	2.25[a]	29.10	11.55
	65	.0414	1.06	10.48	9.90
	55	.2121	0.37	2.88	7.79
35	65	.0190	1.44[a]	22.28	15.46
	55	.0814	0.68[a]	7.16	10.42
45	65	.0106	2.69	38.90	14.47
	55	.0368	1.01[a]	14.63	14.48

[a] Check runs were made in these cases only. Reproducibility was between 1 and 2%.

An equation for the calculation of the difference between chemical energy of activation and the diffusional energy of activation has been developed by Williamson and La Mer,[4] namely

$$\log\left(\frac{1}{p} - 1\right) = \log\frac{C}{P} + \frac{(E_a - E_d)}{2.3R_gT} \qquad (14)$$

where p is the probability of reaction per encounter, C and P are orientation or entropy factors for diffusion and reaction, respectively, R_g is the gas constant and E_a and E_d are the chemical and diffusional energies of activation, respectively. A plot of $\log\left(\frac{1}{p} - 1\right)$ 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 of $E_a = E_d$. The intercept will be a measure of the relative magnitude of the steric factors of diffusion and reaction. Using the values of $1/p$ listed in Table V, plots of $\log((1/p) - 1)$ *versus* $1/T$ are shown in Fig. 5 for the isodielectric mixtures with dielectric constant values of 65 and 55. In the case of the D 55 mixtures, the slope is negative over the whole temperature range, indicating that the existing energy of activation is less than the existing energy of diffusion. The value of $(E_a - E_d) = -6500$ cal., and the intercept $\log (C/P) = 5.59$, from which $C/P = 3.9 \times 10^5$. Since C is approximately unity,[4] P becomes 2.56×10^{-6}.

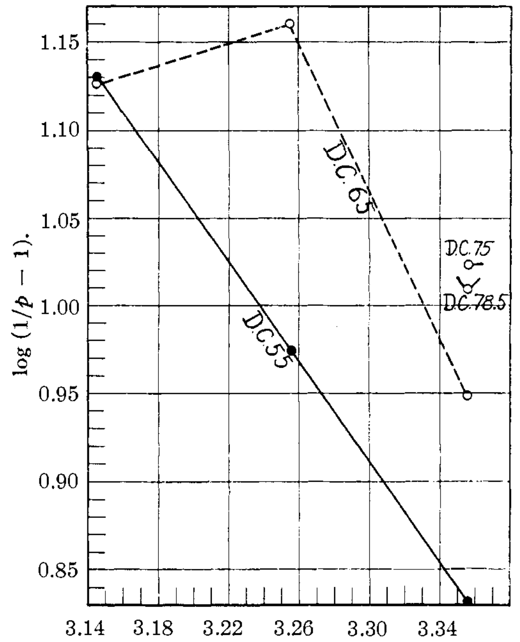


Fig. 5.—The probability of quenching per encounter as a function of the temperature.

In the case of the D 65 mixtures, the slope changes sign over the temperature range investigated. This change in sign can be interpreted to mean that in those mixtures there is a change in the relative importance of the two energy of activation terms over the temperature range investigated.

COLLEGE PARK, MARYLAND

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1567]

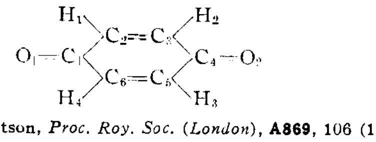
The Structure of Vaporized *p*-Benzoquinone

BY STANLEY M. SWINGLE[1]

RECEIVED OCTOBER 26, 1953

An electron diffraction investigation of the structure of vaporized *p*-benzoquinone has led to results consistent with those for related compounds, but differing from the values reported for the crystalline material. On the assumption of symmetry D_{2h} and C–H = 1.08 Å., the results are C=C = 1.32 ± 0.04 Å., C–C = 1.49 ± 0.04 Å., C=O = 1.23 ± 0.04 Å., and $\angle C_2C_1C_6$ = 116 ± 3°.

A length of 1.47 Å. for the conjugated C–C bond in glyoxal and dimethylglyoxal has been reported by LuValle and Schomaker,[2] indicating that resonance gives these bonds about 20% double bond character. The C=O bond length (1.20 Å.) was about the same as that in the unconjugated formaldehyde[3a] and acetaldehyde.[3b] It would be expected that the results of a similar resonance in *p*-benzoquinone would be apparent in its C–C bond and that the C—C=O bond angle would be nearly the same as in glyoxal and dimethylglyoxal (123°). However, Robertson[4] reported for crystalline *p*-benzoquinone the symmetrical, planar structure I with $C_1{=}O_1$ =



(I)

---

[1] Deceased October 5, 1953.

[2] J. E. LuValle and V. Schomaker, THIS JOURNAL, **61**, 3520 (1939).

[3] (a) D. P. Stevenson, J. E. LuValle and V. Schomaker, *ibid.*, **61**, 2508 (1939); (b) D. P. Stevenson, H. D. Burnham and V. Schomaker, *ibid.*, **61**, 2922 (1939).

[4] J. M. Robertson, *Proc. Roy. Soc.* (*London*), **A869**, 106 (1935).