[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Quenching of Fluorescence in Solution. Effect of the Structure of the Quencher on the Efficiency of the Reaction[1,2]

BY JOHN C. ROWELL[3] AND VICTOR K. LAMER

This investigation was carried out in an attempt to elucidate the mechanism of the quenching of fluorescence in solution by studying the effect of the structure of the quencher on the efficiency of the reaction. It has been shown that the value of p, the fraction of encounters between the excited dye and quencher which result in quenching, furnishes a measure of the efficiency of the reaction. The method of evaluating p has been discussed. Molar rate constants for the quenching of the fluorescence of uranin by a number of selected nitrogen compounds have been determined, free of all known secondary effects, and values of p have been calculated. For the reactions studied the more efficient quenchers possessed non-localized π electrons as a result of resonance. Alterations in the structure of the quenchers which enhanced or inhibited this resonance, respectively, increased or decreased the quenching efficiency. The experimental results are in good qualitative agreement with the ideas of Weiss, that the quenching reaction involves an electron-transfer.

Introduction

The high degree of specificity exhibited by the quenching of fluorescence in solution is rooted in the mechanism of the reaction, which is not yet understood.[4,5] Therefore, a study of the effect of the structure of the reactants on the quenching efficiency should yield useful information concerning the mechanism. From the more recent work on the kinetics of the reaction,[6] it is apparent that the determination of the quenching efficiency involves a number of factors which were not realized in the earlier investigations. The purpose of this work is to obtain as nearly as possible a true measure of the quenching efficiency for the reaction between uranin and a number of selected quenchers and to interpret the results from a consideration of theories of the quenching process.

From the previous papers in this series[6,7,8] expressions are obtained

$$k = pk_{\text{D}} \qquad (1)$$

and

$$k_{\text{D}} = \frac{2kTN}{3000\eta a} \times \frac{(r_{\text{Q}} + r_{\text{D}})^3}{r_{\text{Q}} r_{\text{D}}} \text{ liters mole}^{-1} \text{ sec.}^{-1} \qquad (2)$$

where k in (1) is the molar rate constant of the quenching reaction and p is the probability of reaction per encounter between the reactants. The value of p furnishes a good criterion of the efficiency of quenching and may be arrived at by evaluating k and k_{D}.

Experimental

Reaction.—It would be desirable to study a number of dyes and quenchers. However, the use of different dyes would necessitate a knowledge of τ_0 for each dye, and the determination of structural effects in such complex molecules would be even more difficult. Consequently it was felt that a greater amount of information could be obtained by using different quenchers and one dye. The divalent ion of uranin was chosen as the fluorescent compound because τ_0 is known (0.45×10^{-8} second)[9] and the absorption and fluorescence spectra have been measured.[10] Also its stability in solution and the validity of the Stern–Volmer equation as applied to the quenching of its fluorescence have been established.[6,11] The selection of the quenchers was made with the purpose of avoiding as much as possible any secondary effects. The use of organic compounds made available a series of related compounds whose structure could be varied to give widely different electronic and molecular configurations. The quenching reaction between aniline and uranin has been studied and found to be free of the salt and electrostatic effects since the aniline is uncharged in alkaline solution.[7] A preliminary survey of the properties of a number of other nitrogen compounds indicated that they possessed the requisite solubility and stability and would be suitable for use as quenchers.

Uranin.—Solutions of uranin were prepared from Eastman Kodak Co. grade fluorescein. The molar extinction coefficient at the absorption peak (λ = 492 mμ) was determined to be 79.0×10^3 as compared with 78.2×10^3 and 76.9×10^3 in the literature.[10,12]

Triethylamine, N-Dimethylaniline, *o*-Toluidine, N-Dimethyl-*o*-toluidine, Benzylamine, N-Dimethylbenzylamine, *n*-Propylamine.—Eastman Kodak Co. grade. All liquid quenchers were prepared for use by distillation from zinc dust, at reduced pressure, in an atmosphere of nitrogen. The middle fraction of each distillate was collected as a clear colorless liquid. Some of these compounds darken rapidly on standing and are not very soluble in water. Both the stability and solubility were increased by converting them into solutions of their hydrochloride salts. In this form they could be kept for several days without exhibiting noticeable formation of color or alteration of molar quenching rate constant.

Acetanilide.—Eastman Kodak Co. grade; recrystallized once from water; m.p. 113–114°.

Ammonia.—Alkaline solution of Baker C.P. ammonium chloride.

Aniline.—Paragon Testing Laboratories aniline was redistilled from zinc dust, b.p. 183.5–184°. Eimer and Amend aniline hydrochloride, C.P., was recrystallized from ethanol. Aniline from both of these sources gave the same quenching rate constant.

***p*-Toluidine.**—Eastman Kodak Co. grade; recrystallized twice from ethanol with decolorizing carbon; m.p. 43–44°.

Pyridine.—Baker and Adamson reagent grade.

***p*-Chloroaniline.**—Monsanto grade: distilled at atmospheric pressure. The middle fraction was collected as a colorless crystalline solid; m.p. 57–58°; b.p. 230°.

***p*-Anisidine.**—Technical grade; distilled at atmospheric pressure. The middle fraction was collected as a colorless crystalline solid, m.p. 69–70°; b.p. 240°.

Methyl Alcohol.—Eimer and Amend reagent grade (99.5%).

4-Aminopyridine.—A sample with m.p. 155–156° was furnished by Prof. W. E. Doering of Columbia University.

Mesidine.—Mesidine was prepared by catalytic reduction of nitromesitylene: 156 g. of nitromesitylene was dissolved

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(2) Dissertation submitted by John Charles Rowell in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

(3) E. I. du Pont de Nemours & Co., Chemical Department, Wilmington, Del.

(4) G. K. Rollefson and R. W. Stoughton, THIS JOURNAL, **63**, 1517 (1941).

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

(6) J. Q. Umberger and V. K. LaMer, THIS JOURNAL, **67**, 1099 (1945).

(7) B. Williamson and V. K. LaMer, *ibid.*, **70**, 717 (1948).

(8) K. C. Hodges and V. K. LaMer, *ibid.*, **70**, 722 (1948).

(9) F. Perrin, *Ann. phys.*, [10S] **17**, 283 (1932).

(10) V. L. Lewschin, *Z. Physik*, **72**, 368 (1931).

(11) G. K. Rollefson and H. W. Dodgen, *J. Chem. Phys.*, **12**, 107 (1944).

(12) W. R. Orndorff, R. C. Gibbs and V. Shapiro, THIS JOURNAL, **50**, 819 (1931).

in 80 ml. of 95% ethyl alcohol; 0.300 g. of PtO_2 was added as catalyst. The same amount of catalyst in 15 ml. of alcohol was added twice more during the course of the reaction. Upon completion of the hydrogenation the alcohol was evaporated under reduced pressure. To remove unreacted nitromesitylene the remaining dark liquid was dissolved in dilute HCl and extracted with ether. The acid solution was then made alkaline and the mesidine was recovered by ether extraction. Distillation from zinc dust of the liquid obtained from the ether solution yielded 117 g. of colorless mesidine; b.p. 229–230°.

N-Dimethylmesidine.—This compound was prepared from mesidine according to the method described by Ingham and Hampson.[15] From 25 g. of mesidine was obtained, following a distillation from zinc dust, 14 g. of colorless product; b.p. 214–215°.

Measurement of Quenching Rate Constants.—The details of procedure and equipment were similar to those already described.[6] All measurements were carried out at 25°. Since some of the quenchers were subject to decomposition on standing in alkaline solution, the dye solutions containing quenchers were not made alkaline until just before the measurements were made. In most cases the hydroxide concentration was 0.01 *M*. When the quenchers were strong bases, higher hydroxide concentrations were used to ensure the presence of the free base. The fluorescence intensity of uranin solutions whose hydroxide concentration was varied over the range used in the measurements was found to be constant, indicating that there was no change in the molecular species of the fluorescent compound. Some of the quenched solutions contained appreciable concentrations of sodium chloride as a result of neutralizing the hydrochloride salts. The effect of these amounts of chloride on the fluorescence of uranin was found to be negligible. The concentration of uranin employed was 10^{-5} *M* and less because at such low concentrations self-quenching and dimerization are negligible.

Reabsorption Correction.—Due to the overlap of absorption and fluorescence spectra, the true value of the quenching rate constant is obtained by extrapolation to zero concentration of dye. For concentrations below 10^{-5} *M* log *k* is a linear function of the uranin concentration. Therefore, the extrapolation may be accomplished by determining the slope of a plot of log *k* *vs.* uranin concentration. According to theory[6] the slope should depend on the dye and the geometry of the cell and be independent of the quencher so that, once determined, it should be applicable to all quenchers with any one dye. Quenching rate constants were measured for N-dimethylaniline and N-dimethyl-*o*-toluidine at uranin concentrations between 10^{-5} *M* and 2×10^{-6} *M*. The results are given in Table I and plotted in Fig. 1. Within the experimental error the value of the slope was the same for both compounds. The dependence of the slope on cell arrangement was also observed. Following the above measurements, adjustments were made on the apparatus which changed the path of the beam of exciting light with respect to the cuvettes. Another set of measurements was carried out using *o*-toluidine as the quencher, and the value of the slope was found to have changed from 9600 to 7500. Consequently, measurements were repeated with N-dimethylaniline and N-dimethyl-*o*-toluidine at 10^{-5} *M* uranin. When extrapolated to zero dye concentration the results agreed with the previous extrapolated values. On this basis it was considered justifiable to use the latter value of the slope to correct for the reabsorption effect in all subsequent measurements.

TABLE I

Quencher	Uranin concn. moles/l.	$k \times 10^{-8}$ l. mole^{-1} sec.$^{-1}$	log *k*	Slope
N-Dimethylaniline 0.007 *M*	10^{-5}	72.3	9.858	
	8×10^{-6}	68.9	9.839	
	6×10^{-6}	65.6	9.817	
	4×10^{-6}	63.8	9.805	
	2×10^{-6}	60.0	9.778	
	0	(57.8)	(9.761)	9700
N-Dimethyl-*o*-toluidine 0.004 *M*	10^{-5}	63.8	9.804	
	8×10^{-6}	61.6	9.789	
	6×10^{-6}	58.7	9.769	
	4×10^{-6}	56.5	9.752	
	2×10^{-6}	53.4	9.727	
	0	(51.4)	(9.711)	9500
Cell position altered				
o-Toluidine 0.004 *M*	10^{-5}	61.1	9.786	
	8×10^{-6}	58.2	9.765	
	6×10^{-6}	56.7	9.753	
	4×10^{-6}	54.9	9.740	
	2×10^{-6}	52.9	9.724	
	0	(51.1)	(9.709)	7500
N-Dimethylaniline 0.007 *M*	10^{-5}	69.4	9.841	
	0	(58.2)	(9.766)	7500
N-Dimethyl-*o*-toluidine 0.004 *M*	10^{-5}	60.5	9.782	
	0	(50.9)	(9.707)	7500

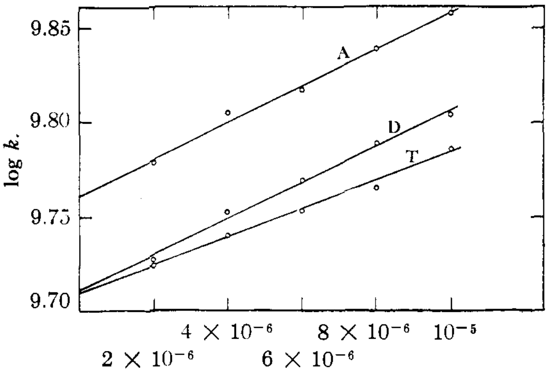

Fig. 1.—Quenching rate constant as a function of the concentration of uranin: A, N-dimethylaniline, slope = 9700; D, N-dimethyl-*o*-toluidine, slope = 9500; T, *o*-toluidine, slope = 7500.

Concentration of Quencher.—It was observed[7] in the quenching of uranin by aniline that k_Q increased with increasing concentration of aniline, but at concentrations near 0.01 *M* the effect is quite small and vanishes on extrapolation to zero concentration. Therefore, for all the quenchers used, measurements were made at different concentrations of quencher. The more efficient quenchers were used in concentrations of 0.01 *M* and less. For the less efficient quenchers, higher concentrations had to be used, some as high as 0.1 *M*. Only in very few cases was any significant effect observed. In these instances the effect was negligible at the lower concentrations of quencher. In Table II are summarized the rate constants for the quenching of uranin by the selected quenchers.

Discussion of Results

Quenching Efficiencies.—Quenching efficiencies were obtained by equation (1), using values of k_D' calculated from equation (2). Although the

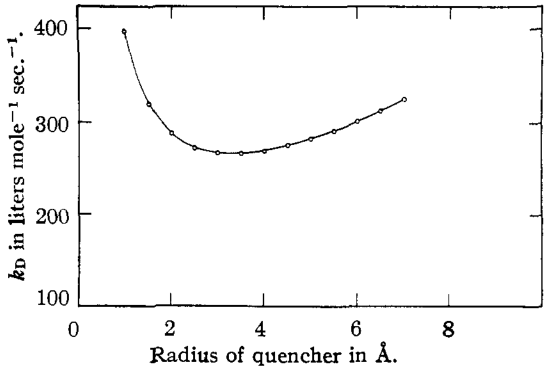

Fig. 2.—Calculated k_D as a function of the radius of the quencher for reaction with uranin.

TABLE II

Quencher	Concn. of quencher in mole per liter	Molar quenching rate constant $\times 10^{-8}$	p
Triethylamine	0.01	11	0.040
	.02	11	
	.04	12	
n-Propylamine	.03	0.1	.0004
	.07	0.1	
Ammonia	.05	0.1	.0004
Benzylamine	.02	0.9	.003
	.04	0.9	
N-Dimethylbenzylamine	.01	5.3	.019
	.02	5.3	
	.04	5.1	
Aniline	.01	49.1	.180
N-Dimethylaniline	.0028	57.8	.213
	.0049	58.0	
	.007	57.9	
o-Toluidine	.004	51.4	.189
	.01	51.1	
	.02	52.2	
N-Dimethyl-o-toluidine	.004	50.8	.187
p-Toluidine	.005	55.0	.203
	.01	55.4	
Mesidine	.004	65.1	.241
	.01	65.8	
	.02	72.5	
p-Chloroaniline	.002	59.8	.216
	.004	57.3	
	.01	58.9	
p-Anisidine	.002	69.4	.255
	.004	68.9	
	.01	69.6	
Acetanilide	.01	3.1	.011
	.02	2.9	
	.04	2.9	
Anilinium ion[a]	.025	1.6	
	.05	1.7	
Pyridine	.04	0.7	.002
	.10	0.4	
4-Aminopyridine	.01	27.8	.102
	.02	27.8	
66% by weight methanol solution			
Aniline	0.01	31.4	
Mesidine	.01	34.7	
N-Dimethylmesidine	.005	36.4	
	.01	36.9	

[a] Measured in 0.002 M perchloric acid where the fluorescent species is fluorescein and not uranin. Since τ_0 for fluorescein is not known, the k_Q is reported here.

radii of most of the quenchers are unknown, the radii of uranin and aniline have been determined from diffusion measurements and found to be 6.6 and 2.5 Å., respectively.[8] The value of a is assumed to be equal to the cube root of the volume per molecule of the solvent and is 3.1 Å. for water. Calculations of k_D for aqueous solutions at 25° were made for quencher radii from 1.0 to 7.0 Å. The results are plotted in Fig. 2. The curve exhibits a minimum at a quencher radius of 3.5 Å. A relatively flat portion was found at radii near that of aniline. Between 2.5 Å., the radius of aniline and 5.0 Å., double that radius, the variation of the k_D from that of aniline was −2% and +4%. With the possible exception of ammonia and n-propylamine, all of the quenchers used were of molecular dimensions nearly the same or slightly larger than those of aniline and would be expected to exhibit solution radii within the above limits. Therefore, the k_D calculated for the reaction between aniline and uranin was used to obtain the quenching efficiencies of all the other reactions. The value of k_D for the methanol solution (a = 3.6 Å. by interpolation between values for water and methanol) was calculated to be 190, using the data and method described earlier.[8] The results are given in Table II. In comparing the efficiencies for the various compounds, the assumptions made concerning the effect of the different quenchers on k_D must be borne in mind. As a result, the small differences in efficiency found in some instances cannot be considered significant.

Structural Effects and Mechanism.—For the quenching of uranin by aniline the interaction may be considered as follows. In the collisions the usual van der Waals forces exist. Since both of the molecules are polar and polarizable, the contribution of the dipole and induced dipole forces should be relatively large. However, the direct conversion of the electronic excitation energy of the dye to kinetic energy of the quencher as a result of collision is not to be expected.[13] A strong interaction with the excited electronic system, such as would result from the formation of a complex, is necessary. For these reactants bond formation would seem to be most likely to occur by means of electron-transfer or a coördinate link. Compounds like aniline are known to be capable of losing an electron rather easily and the nitrogen atom in amines has an unshared pair of electrons which can form a coördinate link.

The experimental results clearly show that the basicity of the nitrogen atom (*i.e.*, the availability of the unshared pair of electrons for bond formation) is not important in the quenching reaction. The stronger bases such as ammonia, n-propylamine and benzylamine are very inefficient quenchers. According to Weiss' idea[14] of electron-transfer in the quenching reaction, quenchers which lose an electron more easily should be more effective in quenching. Such compounds are those in which π electrons come from electron-repelling groups like the amine group which are capable of resonance by means of the unshared pair of electrons. The fact that aniline and its alkyl derivatives are weak bases is attributed to this resonance in which there is a shift of the unshared pair of electrons on the nitrogen atom toward the benzene ring. With the exception of N-dimethylmesidine the efficiency of the quenchers reflected a marked dependence on such resonance. Although dipole moment measurements indicate[15] that formation of the N-dimethyl derivative reduces the resonance of mesidine, little change in the quenching efficiency occurred. Methanol was employed in the solvent to increase the solubility of the mesidines. As an added check on the steric effect in N-dimethyl-

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[13] J. Franck and H. Levi, *Z. physik. Chem.*, **B27**, 409 (1935).

[14] J. Weiss, *Trans. Faraday Soc.*, **35**, 48 (1939).

[15] C. E. Ingham and G. C. Hampson, *J. Chem. Soc.*, 981 (1939).

mesidine, its absorption curve was measured as well as that of mesidine, and the curves are shown in Fig. 3. Although they show that the difference between the ground and first excited electronic states has been increased, the actual ionization potential of the molecule may have been little affected, which would explain the results of the quenching measurement.

The anilinium ion was employed as an example of tying up the electron pair on the nitrogen atom in a valence bond. However, it is not possible to use the anilinium ion with uranin since the ion exists in acid solution and uranin in alkaline. As an approximation to the desired reaction, the acid form of fluorescein may be used in place of uranin. The average life of the acid form of fluorescein is not known, so only k_Q can be determined. A basis for relating the two reactions can be found in quenching by iodide ion. For the reaction between iodide and uranin the k_Q is 3.7 as compared to 21.4 for the reaction with the acid form of fluorescein.[6] This increase could be accounted for by a longer average life of H_2Fl and the absence of electrostatic repulsion. Therefore, with fluorescein aniline might be expected to give a value for k_Q larger than 22 which is found for uranin. The fact that the k_Q for anilinium ion has a value of only 1.7 is an indication that the inhibition of the resonance is the important effect.

A striking analogy is found between the structural effects in the quenching reaction and in the formation of molecular compounds. Aniline and similar amines form molecular compounds with aromatic nitro compounds. These molecular compounds are ionic and are formed by electron-transfer from the amine to the nitro compound.[16] In general, the reaction is rapid and has a low energy of activation. The ability of the amines to undergo the reaction depends upon their total conjugated system and not upon the nitrogen atom itself. For example, aniline forms a molecular compound with nitrobenzene, but N-ethylbenzylamine does not. The equilibrium constants for these reactions in solution are considerably less than one.

There is a possibility that the dye is destroyed as a result of the quenching reaction. Under the conditions which are employed in the measurement of quenching rates, the exciting light intensities are sufficient to excite all of the fluorescent molecules in times of the order of a few minutes. When very short exposures are used in the measurements an irreversible reaction in which the dye was destroyed would probably not be detected. However, solutions of uranin containing iodide ion and a few of the quenchers used in this work have been allowed to stand in diffuse daylight for a number of hours. In two instances this treatment produced an increase in the quenching effect which was attributed to absorption of the exciting light by products of the decomposition of the quenchers in the alkaline solutions. In the other cases, no significant effect was observed, indicating that the dye was not being irreversibly removed from the solution at a rate comparable to quenching.

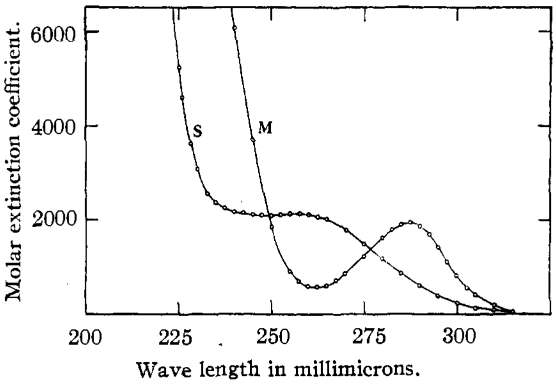

Fig. 3.—Absorption curves of mesidine and N-dimethylmesidine in 66% by weight methanol and 0.005 M NaOH: M, mesidine; S, N-dimethylmesidine.

Therefore, if a reduced or bleached form of the dye is produced in the reaction, it is reconverted to its original form at such a rate that its equilibrium concentration is not detectable by absorption measurements. Also, the fraction of the dye molecules which are excited at any instant is so small ($\sim 1/10^6$) that the presence of any reaction complex such as ($D^- + Q^+$) cannot be observed spectroscopically.

Although it is difficult to interpret some of the results in the literature because of the varying conditions under which the measurements were made and a lack of detailed data, there would seem to be an additional indication of the importance of electron-transfer forces in quenching. Stoughton and Rollefson[4] have classified a number of quenchers and dyes in two groups. The first group of dyes includes fluorescein and quinine sulfate and is quenched by ions such as the halides which are, in general, easily oxidized. On the other hand, the second group is quenched by ions like bromate and iodate, which are oxidizing agents. The mere existence of these two groups is strongly suggestive of electron-transfer in the reactions. According to Weiss' original postulate, the transfer may take place from the excited dye to the quencher when the quencher has a suitable electron affinity (*i.e.*, an empty level close to that of the excited electron).

From the foregoing discussion it may be concluded that the experimental results reported here, and other evidence, although not offering real positive support, are consistent with the idea of electron-transfer in the quenching reaction. At the present, too little is known of the actual energies of the electronic states of the reactants for any quantitative treatment to be carried out. In addition, it would seem likely that an orientation effect exists, since the structures of the reactants are quite complex and it is known that only the condensed ring part of the uranin molecule is involved in fluoresence.

It was thought that in this work a limiting value of the quenching efficiency might be found, but such was not the case. If it could be established that, for a particular reaction, every encounter resulted in quenching, a detailed investigation of the diffusion theory would be possible.

Acknowledgments.—The authors wish to ex-

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(16) J. Weiss, *J. Chem. Soc.*, 245 (1942).

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[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, POLYMER CORPORATION LIMITED]

The Cumene Hydroperoxide–Iron(II) Reaction in the Absence of Oxygen[1]

BY J. W. L. FORDHAM AND H. LEVERNE WILLIAMS

In dilute aqueous solutions of low *p*H and in the absence of monomer the ratio of iron(II) oxidized to cumene hydroperoxide consumed is less than one to one. In the presence of acrylonitrile experimental data indicate that iron(II) disappears only by the bimolecular reaction involving iron(II) and cumene hydroperoxide. In the temperature range from 0 to 25° the rate constant of this primary reaction is given by $k_1 = 1.07 \times 10^{10} e^{-12,000/RT}$ l. mole^{-1} sec.$^{-1}$. The amount of reaction between the hydroperoxide and the tri-α,α′-bipyridyl complex of iron(II) is negligible initially at least. The presence of dihydroxyacetone results in a slight lowering of the ratio of iron(II) oxidized to hydroperoxide consumed. In 0.1 *N* NaOH there is a rapid reaction between the hydroperoxide and the monomer, acrylonitrile.

Introduction

This paper is the fourth of a series[2,3,4] on a study of cumene hydroperoxide (CHP) as a polymerization initiator. In the third paper it was reported that the rate constant (k_1) of the primary radical producing reaction in the temperature range from 0 to 25° is given by $k_1 = 3.9 \times 10^9 e^{-11,100/RT}$ l. mole^{-1} sec.$^{-1}$. In those experiments an excess of oxygen was present, and to simplify the algebraic equations two assumptions were made. The first assumption was that the contribution to the total rate of disappearance of iron(II) made by the reactions between iron(II) and the hydroperoxides formed during the reaction is negligible during the initial stages because of the low concentrations of those hydroperoxides initially. The other assumption was that the primary rate constant is considerably smaller than those of certain subsequent reactions. However the slight dependence of the rate constant on the initial concentrations of CHP and iron(II) indicated that any value obtained from experiments conducted in an oxygen atmosphere might be too large. Furthermore there was the added inducement of measuring the rate constant in the presence of a monomer and in a nitrogen atmosphere. This report contains that work together with an account of the effect on the reaction of α,α′-bipyridyl and dihydroxyacetone (DHA), representative of two types of compounds used in low temperature polymerization recipes.

Theoretical

If the RO· radical, formed by the primary reaction

$$\mathrm{Fe^{++} + RO_2H \xrightarrow[k_1]{} Fe^{+++} + OH^- + RO\cdot}$$

where R represents the cumyl radical, $C_6H_5C(CH_3)_2$, reacts solely with a monomer molecule to initiate polymerization, the iron(II) should disappear by a second order reaction. Then eq. 1 should be valid.

$$\ln\,(1 + (a - b)/[\mathrm{Fe^{++}}]) = (a - b)k_1t + \ln a/b \quad (1)$$

where $a = [\mathrm{RO_2H}]_0$, initial CHP concentration and $b = [\mathrm{Fe^{++}}]_0$, initial iron(II) ion concentration. When $a < b$ and $t \rightarrow \infty$

$$(b - a) = [\mathrm{Fe^{++}}]_\infty \quad (2)$$

where $[\mathrm{Fe^{++}}]_\infty = [\mathrm{Fe^{++}}]$ at infinite time. Equation 2 was used to determine $(b - a)$ rather than the difference between the initial concentration of iron(II) and hydroperoxide because an initial reaction involving traces of oxygen, very difficult to remove experimentally, results in a decrease in the value of $(b - a)$ for the subsequent oxygen-free reaction.

When the complexing agent α,α′-bipyridyl (bip) is added to the system, the following equilibria are assumed to be valid.

$$\mathrm{Fe^{++} + bip \rightleftarrows Fe(bip)^{++}} \qquad K_1$$

$$\mathrm{Fe(bip)^{++} + bip \rightleftarrows Fe(bip)_2{}^{++}} \qquad K_2$$

$$\mathrm{Fe(bip)_2{}^{++} + bip \rightleftarrows Fe(bip)_3{}^{++}} \qquad K_3$$

$$\mathrm{(bip)H^+ \rightleftarrows bip + H^+} \qquad K_a$$

From these equilibria it can be derived that

$$[\mathrm{Fe(bip)^{++}}]/[\mathrm{Fe^{++}}] = K_1[\mathrm{bip}] = K_1([\mathrm{bip}]_T - [\mathrm{bip}]_c)\,\frac{K_a}{K_a + [\mathrm{H^+}]} \quad (3)$$

where $[\mathrm{bip}]_T$ = total bipyridyl concn. and $[\mathrm{bip}]_c$ = combined bipyridyl concn. If the ratio of $[\mathrm{Fe(bip)_2{}^{++}}]/[\mathrm{Fe^{++}}]$ is negligible compared to the ratio $[\mathrm{Fe(bip)^{++}}]/[\mathrm{Fe^{++}}]$

$$[\mathrm{Fe^{++}}] + [\mathrm{Fe(bip)^{++}}] = [\mathrm{Fe^{++}}]_T - [\mathrm{Fe(bip)_3{}^{++}}] \quad (4)$$

where $[\mathrm{Fe^{++}}]_T$ = total iron(II) concn. Equation 3 may be solved by trial and error substitution, and from eqs. 3 and 4 the concentration of uncomplexed iron(II), $[\mathrm{Fe^{++}}]$, may be determined.

Results

In 0.1 *N* sulfuric acid with $a = 4 \times 10^{-5}$ *M* and $b = 10 \times 10^{-5}$ *M* the stoichiometric ratio of iron(II) oxidized to CHP consumed in the absence of monomer at 25° was 0.85 to 1. Under these conditions the iron(II) does not disappear by a second order reaction. Under similar conditions in 0.1 *N* NaOH the ratio was 1.90 to 1, and the rate was much too rapid to measure even at this low concentration.

When acrylonitrile (AcN) is present under similar conditions in the acidic medium, the experimental

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[1] Presented before the Division of Inorganic and Physical Chemistry, American Chemical Society, Chicago, September, 1950.

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