Irreversible Photobleaching of the Solution of Fluorescent Dyes. I. Kinetic Studies on the Primary Process

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(Received July 19, 1954)

Introduction

Although our information about the photochemical behaviour of excited dye molecules has been much increased through many investigations on the photosensitized reactions induced by fluorescent dyes, there are rather few cases in which the primary processes of the reactions are definitely established.

In addition, few investigations have been made for the settlement of the problem whether the excited state of dye participating in the reaction is singlet or triplet, though the problem has become quite important since the confirmation of the triplet state as phosphorescent state by Lewis *et al*[1].

Now, the following three types of primary process have been presented for the photosensitized oxidation in general[2]:

1) Energy transference from an excited dye molecule to the molecular oxygen.
2) Direct combination of oxygen with an excited dye molecule.
3) Univalent oxidation or reduction process.

The latter two seem at present to be most favorable for many cases.

For example, the second scheme has recently been applied by Schenck[3] to photosensitized oxidation of various organic substances, while the third scheme has long been claimed by Weiss, Franck, Livingston and others[4] for many reactions.

It seems to be quite essential to decide, on the one hand, whether the excited dye molecule (or ion) produced in the solution primarily interacts with the molecular oxygen or alternatively with solvent molecules, and, on the other hand, to make clear whether the excited state of dye in question is singlet or triplet. The objective of the present series of investigations is to elucidate these questions through kinetic studies on the irreversible photobleaching of dyes by oxygen.

Now, there exist rather few investigations published in this field, among which the work of Blum and Spealman[5] seems to be most prominent. They studied the photobleaching of the aqueous eosine solution under the atmospheric oxygen and proposed such a scheme as is abbreviated in the following way:

1) $D^* + O_2 \longrightarrow D + O_2^*$
2) $O_2^* + H_2O \longrightarrow HO + HO_2$
3) Oxidation reaction induced by HO_2, formation of H_2O_2, etc.

The experimental facts which led them to the above conclusion were mainly the recognition of the induction period and the detection of hydrogen peroxide.

It must, however, be noted that they used a relatively concentrated solution of dye in which the interaction of excited dye molecules and those of the ground state is so great[6] that it is liable to make the features of the reaction rather complicated. The employment of more dilute solutions seems to be demanded and from such a view point the present authors have investigated the photobleaching of eosine, erythrosine and uranine in the aqueous solutions or in the mixed solutions of water and ethyl alcohol, the concentration of dyes being chosen as 10^{-5} mol./1. in most cases.

Though the definite answers to the above questions have not yet been attained, some remarkable results have been obtained and from them certain deductions have been derived, which we believe to be valuable for the further attack of the problem. The present paper reports the essential points of the experimental results and some discussions about them; the details of the experimental procedure and full data obtained will be published in the *J. Inst. Polytech. Osaka City University*.

Experimental

A. Procedure.—The following method was employed to measure the quantity of bleaching

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[1] G. N. Lewis and M. Kasha, *J. Am. Chem. Soc.*, 66, 2100 (1944), etc.

[2] For example, see Th. Förster, *Z. Elektrochem.*, 56, 716 (1952).

[3] G. O. Schenck, *Naturwiss.*, 40, 205, 229 (1953).

[4] For example, J. Weiss, *J. Soc. Dyers and Colourists*, 65, 719 (1949).

[5] H. F. Blum and C. R. Spealman, *J. Phys. Chem.*, 37, 1123 (1933).

[6] N. Mataga and M. Koizumi, This Bulletin, 28, 51 (1955).

successively for one sample. The solution was put into A (diameter 25 mm., thickness 16 mm.) of the reaction cell, Fig. 1, and was irradiated in the thermostat for a certain time with the light of constant intensity. The cell was taken out of the bath occasionally, and after the sample was poured into part B (inner diameter 15 mm., length 25–30 mm.), the quantity of bleaching was measured colorimetrically by use of RCA 931A photomultiplier.

As a light source a tungsten projection lamp (100v.–500w.) was employed and the range of wave length was adequately chosen by means of Matsuda color filters. The intensity was controlled with blackened metallic nets.

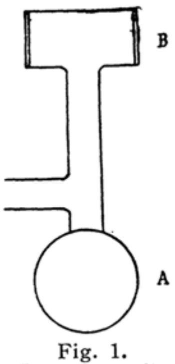

Fig. 1. Reaction cell

B. Results.—*1. Features of the photobleaching under atmospheric pressure.*—The degrees of photobleaching in the aqueous solutions of eosine, erythrosine and uranine, the concentrations of which were all 10^{-5} mol./1., were measured at first under the atmospheric pressure. The concentration-time relations, Fig. 2, which show only the results for eosine, are all simple and monotonous without any slight indication of the induction phenomenon which was observed by Blum and Spealman. The percentage depicted along each line concerns the relative intensity of the light source. The results for erythrosine and uranine are similar to those of eosine.

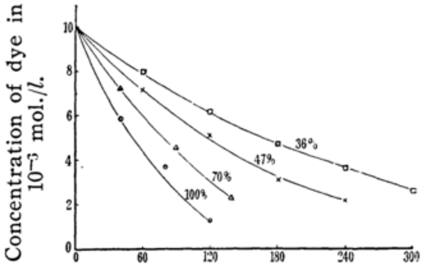

Fig. 2. Photobleaching of eosine in aqueous solution under various relative light intensities from 100 to 36% at 30°C.

As regards the formation of hydrogen peroxide reported by Blum and Spealman, it could not be detected by the iodometry in the nitrogen atmosphere, by $TiCl_3$-method and also by the polarographic method. The possibility of the intermediate formation of hydrogen peroxide in a very small quantity below the sensitivities of the above methods was scrutinized by the test of the addition of sodium pyruvate or sodium thiosulfate which ought to decrease the rate, if this be the case. The result was that the addition of the reagents each in several millimol./1. did not affect the rate at all. Thus one can deny even the slight formation of hydrogen peroxide in the present conditions.

The difference of the present result as compared with that of Blum and Spealman may plausibly be due to a different concentration of dye. In fact we could detect hydrogen peroxide by iodmetry when the concentration of 2×10^{-4} mol./1. of dye was employed.

As for the reaction products, we have not yet undertaken the analysis, and only the absorption spectra after irradiation were examined. Similar changes were observed for erythrosine, eosine and uranine; thus the main adsorption in the visible region and the neighboring one declined monotonously with the irradiation while a slight increase of extinction was observed in the region of 360–440 mμ.

2. Rate formula.—In view of the relatively simple form of the bleaching curves, the following simplest rate formula was tested at first.

If the rate of reaction only depends on and is proportional to the quantity of absorbed light, then

$$-\frac{dc}{dt}=kI_0(1-e^{-\alpha cd}), \tag{1}$$

where c is the concentration of dye, α the average molar absorption coefficient, d the thickness of the solution, I_0 the intensity of light, and k is the overall rate constant. Integrating eq. (1) one gets

$$\ln(1-e^{-\alpha c\,d})-\ln(1-e^{-\alpha cd})+\alpha d(c_0-c)=\alpha dkI_0t \tag{2}$$

where c_0 is the initial concentration of dye. The intensity of light was measured by a thermopile previously calibrated with the standard lamp and was expressed as a number of photons per unit time. For example, I_0 was 10^{16} photons/sec. cm.^{2} when 90 v. was applied on the lamp. α was also determined with the same thermopile. The values of α were 6.0×10^4, 5.6×10^4 and 4.2×10^4 for eosine, erythrosine and uranine, respectively. When the rate of bleaching is expressed as a number of dye molecules decomposed per sec., then the rate constant k obtained from eq. (2) is nothing but a quantum yield of the reaction.

Now it was proved that the eq. (2) can express the experimental results quite well from the beginning up to 70–80% bleaching, when the dye concentration lies in the region from 5×10^{-6} to 1×10^{-4} mol./1. It must be noted, however that in the case of 1×10^{-4} mol./1. the deviation is somewhat large, suggesting the non-applicability of eq. (2) in more concentrated regions. The value of k was not affected by the intensity of light, which was varied from 100% (full light) down to 25%. Some of the results are tabulated in Table I.

TABLE I
RATE CONSTANTS* (QUANTUM YIELDS) AT 30°C

Relative intensity (%)	$k_1\times10^4$	$k_2\times10^4$	$k_3\times10^4$	
	Eosine			
100	3.1	2.9	3.3	
	2.9	2.9	3.2	
70	2.9	3.1	3.9	
	2.7	2.7	3.2	
47	2.4	2.6	3.0	
	2.8	2.8	2.9	
36	2.6	2.6	2.8	
	2.7	2.5	—	±0.2
	Erythrosine			
78	4.9	4.9	5.2	
	4.7	5.0	5.0	
54	5.1	5.3	5.6	
	5.1	5.1	5.3	
36	4.1	4.4	4.9	
	4.5	5.2	5.4	
28	4.7	5.2	5.7	
	4.4	4.8	5.2	±0.3
	Uranine			
100	1.4	1.3	1.2	
	1.4	1.4	1.5	
70	1.2	1.2	1.4	
	1.4	1.3	1.3	
47	1.1	1.2	1.3	
	1.2	1.2	1.2	
36	0.9	1.0	1.2	
	1.5	1.2	1.3	±0.2

*k_1, k_2 and k_3 are calculated from time required for 25, 50 and 75% bleaching, respectively

3. *The effect of the quantity of the dissolved oxygen.*—Eq. (2) can express the experimental results equally well, when the dye solution of 10^{-5} mol./1. is completely evacuated and a certain amount of oxygen is added to the sample. The values of k are plotted against the concentrations of the dissolved oxygen in Fig. 3. Here the concentration of the dissolved oxygen was provisionally calculated from the quantity of added oxygen on the basis of the solubility data for pure water.

It is evident from this figure that in the cases of eosine and erythrosine, the rate rapidly declines when the concentration of dissolved oxygen is decreased below ca. $1\sim2\times10^{-5}$ mol./1., while above this critical concentration the rate remains constant and the k-value under atmospheric pressure coincides with it quite satisfactorily, showing no effect of the coexistent nitrogen on the rate. In the case of uranine, k increases gradually with the increase of the oxygen concentration. For eosine, the effect of the concentration of dissolved oxygen was particularly examined at three different temperatures. As shown in Fig.4, the critical concentration of the dissolved oxygen at which k reaches its maximum value, slightly increases when the temperature is raised.

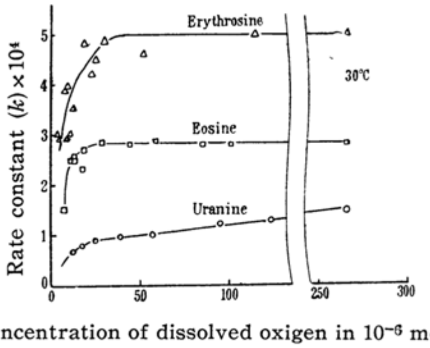

Fig. 3. Influence of the concentration of oxygen on the rate constants.

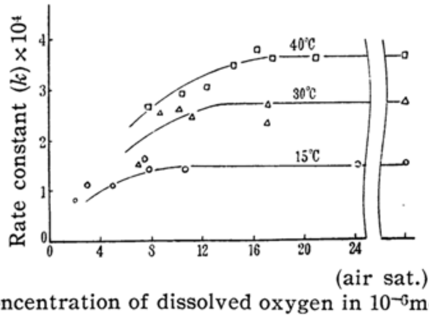

Fig. 4. Influence of temperature on the oxygen effect.

4. *The effect of temperature on the rate.*—The rate of photobleaching of the aqueous solution of uranine, eosine and erythrosine under atmospheric pressure was measured at several temperatures between 13 and 40°C. The k-values obtained are tabulated in Table II.

TABLE II
AVERAGE RATE CONSTANTS AT VARIOUS TEMPERATURES $k\times10^4$

	40	34	30	27	22	20	13°C.
Eosine	3.6	2.9	—	2.5	2.0	1.8	—
Erythrosine	5.6	—	5.2	—	—	3.7	2.9
Uranine	1.8	—	1.3	—	—	1.1	1.0

These results can approximately be expressed by the following Arrhenius equations:

eosine: $k=5.3\ \exp(-6000/RT)$ (3)
erythrosine: $k=2\ \exp(-5000/RT)$ (4)
uranine: $k=0.3\ \exp(-4000/RT)$ (5)

It may be necessary to remember here that for eosine and erythrosine, the k-value under atmospheric pressure is constant, irrespctive of the quantity of dissolved oxygen, hence eqs. (3) and (4) express the direct influence of the temperature on the reaction rate itself, while for uranine, the k-value in the region of atmospheric oxygen is still affected by the quantity of oxygen and so the effect of temperature on the solubility of oxygen participates in eq. (5).

5. The photobleaching of eosine in the water-ethyl alcohol mixtures.—With the hope of seeing how the water molecule takes part in the reaction, the effect of addition of ethyl alcohol to the aqueous eosine solution was examined. At first, the pure alcoholic solution of eosine of 10^{-5} mol./1. was irradiated under atmospheric pressure and it was found that not even slight photobleaching occurred even after two or three hours of irradiation by the light of the strongest intensity employed. On the contrary, when the dissolved oxygen was eliminated, a quite rapid photobleaching did take place. As to this unexpected and astonishing result, investigation is now going on. When a small quantity of alcohol was added to the aqueous eosine solution dissolving atmospheric oxygen, the rate of photobleaching decreased quite remarkably. The rate formula (2) still holds in this case at least up to 30 vol. % alcohol at which the rate is quite small.

The values of k are plotted against the concentration of alcohol in Fig. 5. The experiments were done at 20, 27, 34 and 40°C., but in this figure only two curves at 27 and 40°C. are given.

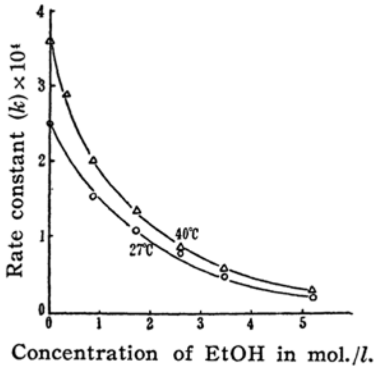

Fig. 5. Effect of EtOH concentration on the rate constant.

Now, it was found that the following formula can express the relation between k and the composition of the solvent quite satisfactorily in the low concentration region of alcohol (see the section of discussion).

$$\frac{1}{k}=\frac{1}{k^\circ}+K\frac{[\mathrm{EtOH}]}{[\mathrm{H_2O}]}, \tag{6}$$

were k° is the rate constant of the pure aqueous solution, K a certain constant experimentally determined, and [EtOH] and $[H_2O]$ are the molar concentrations of alcohol and water, respectively.

As is evident from eq. (6), $1/k$ when plotted against $[EtOH]/[H_2O]$ ought to be linear. In fact, the relations between $1/k$ and $[EtOH]/[H_2O]$ at various temperatures are just as shown in Fig. 6. This figure shows that at least up to 15–20 vol. % the formula (6) holds, but near 20 vol. % of alcohol, marked breaks appear at all temperatures, which lie at the lower value of $[EtOH]/[H_2O]$, as the temperature is higher.

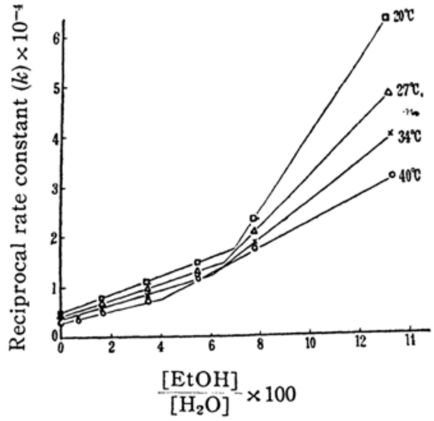

Fig. 6. Relation between $1/k$ and $[EtOH]/[H_2O]$ at several temperatures.

As to these discontinuous breaks, one may plausibly attribute them to the specific nature of the alcohol-water system. Because, it is well known that the partial molar volume of alcohol in this system, for example, begins to deviate from the linear relation at about the same composition[7].

Moreover, we measured the absorption spectra and the relative intensities of fluorescence of eosine in these solutions and found that both the displacements of the absorption maximum and the relative intensity as poltted against [EtOH] are almost linear up to this region, but from here upward the deviation comes to be notable.

In view of these results, it is quite natural that the applicability of the eq. (6) is limited up to 15–20 vol. %, and one may say

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[7] G. N. Lewis and M. Randall, "Thermodynamics", p. 40.

that the results shown in Fig. 6 confirm the relation (6) rather definitely.

The values of k and K at various concentrations of alcohol and at several temperatures are tabulated in Table III.

TABLE III

k- AND K-VALUES AT VARIOUS CONDITIONS

[EtOH] vol. %	$k\times10^4$				[EtOH]/[H_2O] $\times100$
	40°C.	34°C.	27°C.	20°C.	
0	3.6	2.9	2.5	1.9	0
2	2.9	2.7	—	—	0.64
5	2.0	1.8	1.5	1.3	1.65
10	1.4	1.2	1.1	0.9_0	3.47
15	0.9_5	0.8_0	0.7_7	0.6_7	5.52
20	0.5_7	0.5_4	0.4_8	0.4_3	7.82
30	0.3_2	0.2_6	0.2_1	0.1_6	13.4
$K\times10^{-5}$…	1.33	1.45	1.62	1.73	

Applying the Arrhenius equation to $1/K$ one gets

$$1/K=41\times10^{-5}\cdot\exp(-2500/RT) \qquad (7)$$

Combining eqs. (3), (6) and (7), $1/k$ at various temperatures and at various concentrations of alcohol the result can be expressed as

$$\frac{1}{k}=\frac{1}{5.3\exp(-6000/RT)}+\frac{1}{41\times10^{-5}\cdot\exp(-2500/RT)}\cdot\frac{[\mathrm{EtOH}]}{[\mathrm{H_2O}]} \qquad (8)$$

The coincidence of the calculated values of k with the observed ones is quite satisfactory at least up to 15 vol. % of alcohol.

Discussion

From the fact that the stationary state is reached at once just with the commencement of irradiation and that the rate is proportional to the quantity of absorbed photons, it can safely be concluded that the reaction proceeds as it is of the first order in respect to the excited dye, so far as the rate determining processes are concerned. The mechanism presented by Blum and Spealman which consists of the attack of hydrogen peroxide upon excited dye, the former being produced by the interaction of water with excited dye, is definitely denied in the present case on the ground of the above kinetic results as well as from non-existence of hydrogen peroxide.

Now, since the presence of oxygen and water is indispensable for the occurrence of the reaction the first attack on the excited dye molecule must be made either by oxygen or water, and as to the excited state of dye which participates in the reaction, on the other hand, two alternatives are considered, singlet and triplet states. Hence the following four schemes are conceivable, if one assumes that the reaction proceeds successively and further if one neglects the possibility of direct transference of energy from excited dye to oxygen, which is improbable from the above results as well as from our knowledge of the photosensitized reactions in general.

I. First attack by O_2.

a) Singlet state:

$$\mathrm{D}\xrightarrow{h\nu}\mathrm{D}^*\xrightarrow{\mathrm{O_2}}\mathrm{D}^*\cdots\mathrm{O_2}\xrightarrow{\mathrm{H_2O}}\cdots\mathrm{Prod.}$$

b) Triplet state:

$$\mathrm{D}\xrightarrow{h\nu}\mathrm{D}^*\rightarrow\mathrm{D}^t\xrightarrow{\mathrm{O_2}}\mathrm{D}^t\cdots\mathrm{O_2}\rightarrow\cdots\mathrm{Prod.}$$

II. First attack by H_2O.

a) Singlet state:

$$\mathrm{D}\xrightarrow{h\nu}\mathrm{D}^*\xrightarrow{\mathrm{H_2O}}\mathrm{D}^*\cdots\mathrm{H}\cdots\mathrm{OH}\xrightarrow{\mathrm{O_2}}\cdots\mathrm{Prod.}$$

b) Triplet state:

$$\mathrm{D}\xrightarrow{h\nu}\mathrm{D}^*\rightarrow\mathrm{D}^t\xrightarrow{\mathrm{H_2O}}\mathrm{D}^t\cdots\mathrm{H}\cdots\mathrm{OH}\xrightarrow{\mathrm{O_2}}\cdots\mathrm{Prod.}$$

It must be noted here, that all the formulas of the intermediates involved in the above schemes are only conventional without any specification of their real structures and, that D^t is to be taken as a representative of any metastable states of dye taking part in the reaction which comes from singlet excited state D^* directly produced by irradiation.

Let us consider in succession, which of the above four possible schenes conforms best with the experimental result, particularly on the basis of rate formula (section A) and the oxygen effect (section B).

(A)

Ia) First Attack of Oxygen on Singlet State of Dye

The rate constant in water-alcohol mixture for the reaction scheme, Ia, can be expressed as follows:

$$k_{Ia}=\frac{k_{O_2}}{k_e+k_{qi}+h_1[\mathrm{H_2O}]+k_2[\mathrm{EtOH}]+k_{o2}}\times\frac{k'_1[\mathrm{H_2O}]}{k'_{-1}+k'_1[\mathrm{H_2O}]+k'_2[\mathrm{EtOH}]} \qquad (9)$$

where

k_e: specific rate of fluorescence,

k_{qi}: specific rate of inner quenching of fluorescence,

k_1, k_2: rate constants of the dissipation of excitation energy by the interaction with water and alcohol,

k_{O_2}: rate of the reaction between oxygen and D^* expressed as a constant, since it does not depend on oxygen pressure under ordinary condition.

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* Deactivation of $D^*\cdots O_2$ by water is omitted for the sake of simplicity, since the ultimate result is not essentially affected.

* The ultimate formula is not essentially altered even if the deactivation of $D^*\cdots H_2O$ by oxygen is taken into account not only in this case but also in all other cases in II.

k'_{-1}: rate constant for the spontaneous deactivation of the intermediate $D^*\cdots O_2$,

k'_1: rate constant for the reaction between $D^*\cdots O_2$ and water,*

k'_2: rate constant for the deactivation of $D^*\cdots O_2$ by alcohol.

Since the intensity of fluorescence should decrease just to the same degree as that which is $D^*\cdots O_2$ produced, k_{o2} ought to be equal to the rate of quenching of fluorescence due to oxygen. In fact, however, there is no quenching action of oxygen observed and so the magnitude of k_{o2} must be 1/1000 or less of the denominator of the first factor if one provisionally assumes that the quantum yield of fluorescence is about 10% and that the sensitivity of the intensity measurement of fluorescence is about 1/100.

Thus the first factor of eq. (9) should be a very small value, which will plausibly be put approximately constant in the small concentration region of alcohol.

Putting the first factor as φ_{O_2} and making the probable assumption that k'_{-1} is much smaller than $k'_1[H_2O]$, one gets the following rate formula, which has just the same form as Eq. (6).

$$\frac{1}{k_{Ia}}=\frac{1}{\varphi_{o2}}\left\{\frac{k'_1[H_2O]+k'_{-1}}{k'_1[H_2O]}+\frac{k'_2[EtOH]}{k'_1[H_2O]}\right\}$$

$$\fallingdotseq\frac{1}{k^\circ_{Ia}}+\frac{1}{\varphi_{o2}}\frac{k'_2[EtOH]}{k'_1[H_2O]},$$

where k°_{Ia} is the overall rate constant in the pure aqueous solution. Then the constant K in eq. (6) is given as follows:

$$K=\frac{1}{\varphi_{o2}}\frac{k'_2}{k'_1}$$

Using the K-values in Table III and assuming the upper limit of φ_{O_2} as 10^{-3}, one can estimate that k'_2/k'_1 must be about 100 or so. Thus when $D^*\cdots O_2$ is deactivated by alcohol about 100 times more frequently than it reacts with water, then this scheme conforms quite well with the experimental results. **(deduction a)**

Ib) First Attack of Oxygen on Triplet State of Dye

Introducing the transition probability $\varphi_{S\to T}$ for $D^*\to D^t$, one can write the overall rate constant k_{Ib} as follows:

$$k_{Ib}=\varphi_{S\to T}\times\frac{k_{o2}}{k_{qt}+k_1[H_2O]+k_2[EtOH]+k_{o2}}$$

$$\times\frac{k'_1[H_2O]}{k'_{-1}+k'_1[H_2O]+k'_2[EtOH]}$$

where the constants in the second factor all refer to the triplet state.

If the product of the first and second factors is quite small and is approximately constant irrespective of alcohol concentration, that is, when $k_1\fallingdotseq k_2$ or when $k_{O_2}\gg k_{qt}+k_1[H_2O]+k_2[EtOH]$, then this scheme can give the experimental rate formula just in the same way as in the case of Ia, and the *deduction* (*a*) can be applied for this case too.

At this point one must note that there is another scheme conceivable in which the first attack on dye is by oxygen; it is the one in which the overall rate is determined by the rate with which oxygen attacks the hydrated dye ion of excited state. This mechanism, however, can be rejected quite conclusively in the following way.

Using the similar notations as above, one gets

$$k_{Ia}=k^\circ_{Ia}\frac{[D^*\cdot H_2O]}{[D^*\cdot EtOH]+[D^*H_2O]}$$

where $[D^*\ H_2O]$ and $[D^*\ EtOH]$ are the concentrations of the excited dye ions associated with water and alcohol. They may be put, in the low concentration region of alcohol, as $k'_1[H_2O]$ and $k'_2[EtOH]$, hence the following formula corresponding to eq. (6) can be derived.

$$\frac{1}{k_{Ia}}=\frac{1}{k^\circ_{Ia}}+K\frac{[EtOH]}{[H_2O]},\tag{11}$$

where
$$K=\frac{1}{k^\circ_{Ia}}\frac{k'_2}{k'_1}.\tag{12}$$

But the calculated values of k'_2/k'_1, which should be interpreted as the ratio of tendency of alcoholation to that of hydration, proved to be very unreasonable since they are too large and, moreover, increase with the rise of temperature (33 (20°C.), 40 (27°C.), 41(34°C.), 47 (40°C.)).

Thus it may be concluded that if the dye ion is first attacked by oxygen, then the reaction must be successive and the intermediate $D^*\cdots O_2$ or $D^t\cdots O_2$ must be deactivated by alcohol very much more frequently than it reacts with water, the ratio of the rate constants for both the reactions being about 100 or so (**deduction a**).

IIa) First Attack of Water on Singlet State of Dye

If all the colliding water molecules which quench the fluorescence produce the intermediates of the reaction, then the overall rate constant should be given as follows:

$$k_{IIa}=\frac{k_1[H_2O]}{k_e+k_{qt}+k_1[H_2O]+k_2[EtOH]}$$

$$\times\frac{k'_{o2}}{k'_{-2}+k'_1[H_2O]+k'_2[EtOH]+k'_{O_2}}\tag{13}$$

where all the rate constants involved are analogous to those in the case Ia. The magnitude of the first factor should be moderate since the quenching action of water is not expected to be so little. Hence the second factor must be very small in order to interpret the quantum yield experimentally obtained. Since the first factor can not be expected to be much affected by the composition of the solvent, the remarkable decrease of the rate caused by the added alcohol must be attributed mainly to the second factor and so the relation $k'_2[EtOH]>k_{-2}+k'_1[H_2O]$ must hold. If one takes these relations into account, the present scheme can never lead to the rate formula experimentally obtained.

But in the case when the process producing the intermediate of the reaction is only one of the various quenching processes caused by water molecule simultaneously occurring, there is a possibility, though quite improbable, that the present scheme conforms with the experiment. Thus if the following conditions are fulfilled,

1) the ratio of the intermediate-producing pro-

cess to the whole quenching processes due to water, is moderately small,

2) the denominator of the first factor can be considered approximately constant irrespective of the concentration of alcohol,

3) $k'_1[H_2O] \gg k'_{-2}+k'_{o2}$, and

4) $k'_2 \gg k'_1$,

then eq. (13) can be written approximately

$$k_{IIa}=\frac{k_1[H_2O]}{\text{const.}}\times\frac{k'_{o2}}{k'_1[H_2O]+k'_2[EtOH]}$$

which has the same form as eq. (6).

The scheme is quite improbable in view of the above conditions, but if it supposedly holds, then the most noteworthy condition to be fulfilled is that the intermediate produced from the excited dye and water, must be deactivated by alcohol at a much faster rate than those of the competing processes. **(deduction b)**

IIb) First Attack of Water on Triplet State of Dye

Formally it is only necessary to add a factor $\varphi_{S\rightarrow T}$ to the above case (IIa).

In case the triplet state loses its energy spontaneously or it interacts with water or alcohol, forming the intermediate of the bleaching reaction in the second case, while deprived of its energy in the last case, then the rate formula will be

$$k_{IIb}=\varphi_{S\rightarrow T}\times\frac{k_1[H_2O]}{k_{qt}+k_1[H_2O]+k_2[EtOH]}\times\frac{k'_{o2}}{k'_{-2}+k'_1[H_2O]+k'_2[EtOH]+k'_{o_2}} \qquad (14)$$

Now, if the product of the first and second factors is moderately small and if in addition, conditions analogous to those related to *deduction b* hold, then the above rate formula is approximated by the experimental one (6) just as in the case of the above speciallized case of IIa.

There is another possibility, however, that the above eq. (14) may lead to eq. (6).

Thus if in eq. (14)

1) the product of the first and second factors is very small,

2) k_{qt} is negligible compared with $k_1[H_2O]$ or $k_2[EtOH]$ in the second fator, and

3) $k'_1[H_2O]$ and $k'_2[EtOH]$ are far less than the other k'-terms in the denominator of the third factor,

then

$$k_{IIb}\fallingdotseq\varphi_{S\rightarrow T}\times\frac{k_1[H_2O]}{k_1[H_2O]+k_2[EtOH]}\times\frac{k'_{O_2}}{k_{-2}'+k'_{O_2}}.$$

Hence

$$\frac{1}{k_{IIb}}\fallingdotseq\frac{1}{\varphi_{S\rightarrow T}}\cdot\frac{k_{-2}'+k'_{o2}}{k'_{o2}}+\frac{1}{\varphi_{S\rightarrow T}}\cdot\frac{k_{-2}'+k'_{O_2}}{k'_{O_2}}\cdot\frac{k_2[EtOH]}{k_1[H_2O]}.*$$

Thus in this case the triplet state of dye must be deactivated by alcohol far more frequently than it interacts with water producing the reaction intermediate in order to interpret a large effect of the added alcohol. **(deduction c)**

In case the interaction of the triplet state and water only partially produces the intermediate for bleaching reaction, the discussion is essentially analogous to that above. Thus *deduction c* is applicable when most of the dye molecules in the triplet state attacked by water lose their energies in vain producing very little of the reaction-intermediate, while *deduction b* must hold in the opposite situation.

Among these three favorable cases mentioned above, the one corresponding to *deduction b* is quite improbable in view of many unreasonable conditions; for example the condition that the deactivation of water-dye intermediate by alcohol takes place more frequently than other competitive processes, seems quite improbable from the usual chemical reasoning. The case Ia or Ib corresponding to *deduction a* is most probable, but it is impossible to deny conclusively scheme IIb corresponding to *deduction c* on the basis of the above kinetic results. On the contrary there are some facts which seem to be favorable for the latter scheme. Thus in view of the fact that eosine in pure alcohol is photochemically bleached quite easily *in vacuo*, there is no doubt that the interaction of excited dye with alcohol can be of such a nature as to cause a genuine chemical reaction, and this fact appears to conform better to the scheme IIb.

(B)

As to the question whether oxygen attacks on singlet or triplet state of dye, one may judge the matter to some extent on the basis of the observed oxygen pressure effect on the rate. Thus the fact that the rate becomes independent of the oxygen pressure above a certain critical point requires that in this region the intermediate molecular species to be attacked by oxygen, in whatever scheme considered in section (A), must at least have enough chances of the attack during its life.

The number of collisions between oxygen and the intermediate in question during its life at the above critical pressure can easily be estimated if one assumes that the molecules of oxygen are distributed at random in the solution without any specific interaction with dye ion, and, that oxygen must reach the position of dye ion by diffusion.

Now in the case of eosine at 20°C., the rate becomes constant at ca. 10^{-5} mol./l. of the dissolved oxygen when calculated on the assumption that the solubility of oxygen in the dilute dye solution is the same as that in pure water. It follows that the mean distance of neighboring oxygen and dye ion is about 10^{-6} cm., and the time interval required for oxygen molecule to move this distance is roughly estimated to be about 10^{-7}–10^{-8} sec. on the basis of the usual formula for diffusion process and by use of the diffusion constant of oxygen in the aqueous solution, which is 2×10^{-5} cm.^{2}/sec. at 20°C.[8] The time interval thus obtained is quite great compared with the lifetime of singlet state of eosine, which is about

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[8] "International Critical Table", Vol. V, p. 64.

1.9×10^{-9} sec.[9] or so. Of course the above estimation is approximate in nature but it will be correct at least in the order of magnitude, hence one can safely conclude that the state of dye attacked by oxygen is not the singlet excited state directly produced by light absorption, as long as the assumption of random distribution of oxygen in the solution is correct. In other words, the life of the intermediate to be attacked by oxygen must be at least 10^{-6} sec. or longer for the experimentally observed oxygen saturation effect to be interpreted. It must, however, be added that Evans[10] has recently reported that certain organic substances can form molecular complexes with oxygen, which produce new absorption spectra. In case of eosine we examined the effect of the coexistence of oxygen upon the absorption spectra but could not find such a phenomenon.

Therefore it seems permissible to deny scheme Ia in which singlet excited state is attacked by oxygen if the possibility is neglected that a molecular complex, which can not be detected spectroscopically, is formed between oxygen and dye. The decisive conclusion may perhaps be obtained from the effect of dye on the solubility of oxygen in water.

Summary and General Conclusion

The irreversible photobleaching of eosine in the aqueous solution and in alcohol-water mixtures was studied and it was found that the rate of photobleaching depends only on and is proportional to the quantity of absorbed light when the dye concentration is 10^{-5} mol./1.

The overall unimolecular rate constant k or the quantum yield, when the quantity of dissolved oxygen is varied, does not change over a wide region but begins to decrease below a certain critical point.

The values of k in water-alcohol mixtures were found to be expressed by the following equation in the low concentration region of alcohol.

$$\frac{1}{k}=\frac{1}{k^\circ}+K\frac{[\mathrm{EtOH}]}{[\mathrm{H_2O}]}, \qquad (6)$$

where k° is the rate constant of the pure aqueous solution and K is a certain constant.

The possible schemes were given particularly on the bases of the rate formula (6) and of the effect of the quantity of dissolved oxygen.

It is most probable that the irreversible photobleaching of eosine by oxygen is a successive reaction as represented by the following scheme

Ib) $\mathrm{D}\xrightarrow{h\nu}\mathrm{D}^*\rightarrow\mathrm{D}^t\xrightarrow{O_2}\mathrm{D}^t\cdots\mathrm{O}_2\xrightarrow{H_2O}\cdots$Product.

In this case, the probability with which $\mathrm{D}^t\cdots\mathrm{O}_2$ is deactivated by alcohol is about 100 times greater than that of $\mathrm{D}^t\cdots\mathrm{O}_2$ being attacked by water.

There is some possibility that the reaction may proceed in the following way.

IIb) $\mathrm{D}\xrightarrow{h\nu}\mathrm{D}^*\rightarrow\mathrm{D}^t\xrightarrow{H_2O}\mathrm{D}^t\cdots\mathrm{H_2O}\xrightarrow{O_2}\cdots$Product

Two cases corresponding to this scheme are possible. When a moderate fraction of D^t contributes to the reaction, the intermediate $\mathrm{D}^t\cdots\mathrm{H_2O}$ must be deactivated by alcohol far more frequently than it reacts with oxygen, while when a very small fraction of D^t participates in the reaction, the probability with which D^t is deactivated by alcohol is much larger than that of the reaction with water. The former case is rather improbable, while, the latter one seems to conform to a newly discovered fact that eosine in alcoholic solution is photobleached quite easily under evacuated conditions.

The kinetic studies of the vacuum-state-photobleaching of eosine in alcohol, the comparison of the effects of various alcohols upon the rate of oxygen-water-bleaching and the measurement of the rate of the possible vacuum-bleaching in various alcohols, all these investigations now in progress will give a more definite conclusion.

The cost of the present research has been partly defrayed from the Scientific Research Grant from the Ministry of Education.

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