Photoreduction of Methylene Blue by the Visible Light in the Aqueous Solution Containing Certain Kinds of Inorganic Salts. III. Kinetic Treatment of the Reaction

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It has been established in the previous papers[1] that upon irradiation of the aqueous solution of methylene blue containing various kinds of salts by visible light, the formation of leuco dye takes place accompanied by demethylation and the irreversible decomposition of dye; and in particular its rate is quite high in the presence of phosphate, sodium hydroxide and borate. Leuco formation and demethylation proceed as simultaneous reactions and in most cases the rate of the former is higher than that of the latter, which can be suppressed almost completely under suitable conditions.

Now it is very remarkable that the leuco-formation can occur without any reducing agents in the ordinary sense, and how this occurs still awaits further studies and is a very important problem to be solved. In the previous paper two possibilities were suggested for the primary process, i. e., the reaction of the excited dye and that of the ground state on one hand and the reaction between the excited dye and hydroxide ion on the other. Of course the problem must be attacked from various standpoints, and the present paper is chiefly concerned with the investigation from the reaction kinetics.

Kinetic Treatment

The present reaction as a whole is too complicated to be treated exactly, but it is desirable to get an approximate rate formula which can reproduce the experimental results, as a means for studying further the reaction scheme. Now the most reliable data supplied by the experiments are the decline of the main absorption band of the dye. But as already stated, the formation of leuco dye is accompanied with demethylation which causes the shift of the maximum toward the shorter wavelength. Therefore, by applying the minimum ratio method of Blaisdell[2] to the absorption spectra of the irradiated solution, the one due to the remaining methylene blue was evaluated and its analysis was undertaken. The decrease in dye concentration obtained from the above treatment is, of course the sum due to leuco-formation, demethylation and irreversible decomposition, but in view of the fact that these reactions take place with about the same ratio for a particular system during the most part of one run, the rate derived therefrom may well correspond to that of leuco-formation. It is necessary however, to pay due attention to this point when the rates for different systems are compared; for example, in case of phosphate the rate of demethylation is of about the same order of that of leuco-formation, while in case of sodium hydroxide the former is quite a good deal smaller than the latter.

In Fig. 1 plots of $\varepsilon_{\max}$ against time are given for the typical results so far obtained, in which $\varepsilon_{\max}$ pertains to the maximum of the absorption spectra of methylene blue after

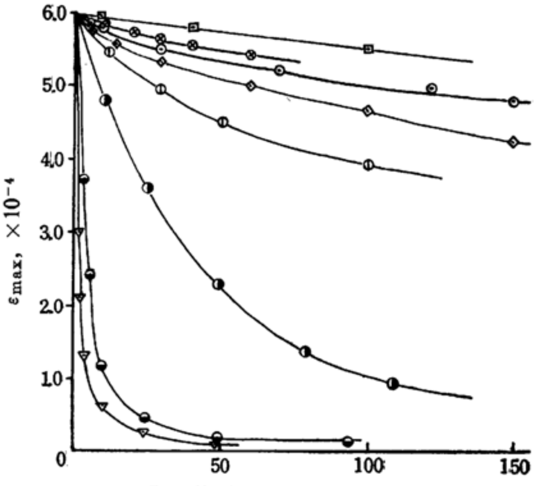

Fig. 1. Photobleaching of methylene blue in deaerated solutions containing various inorganic compounds.

▣ HCl(1×10^{-3} M), ◑ NaOH(1×10^{-3} M), ⦶ Na_2SO_4, ⊗ NaCl, ⊙ NaI, ◈ Without any salt, ◐ Borate buffer, ▽ Phosphate buffer

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[1] Y. Usui, H. Obata and M. Koizumi, This Bulletin, 34, 1049 (1961); H. Obata, ibid., 34, 1057 (1961).

[2] B. E. Blaisdell, *J. Soc. Dyers. & Colorists*, 65, 619 (1949).

Blaisdell's treatment*. It is seen that broadly speaking, they are classified into three groups on the grounds of the magnitude of bleaching. The first group with a very small rate contains sodium sulfate, sodium iodide, sodium chloride and the pure aqueous solution. The second group with a medium rate contains only borate buffer, and the third one with the largest rate are phosphate buffer and sodium hydroxide solution. As stated below the first and the second groups have the rate formula which is approximately of the first order in respect to dye while for the third group the second order rate formula fits the experimental data quite satisfactorily. Hence the first and second group may be lumped together. In the following the rate for borate buffer and that for phosphate buffer will be separately discussed, each as a representative of the two groups.

Borate Buffer Solution.—Approximately, the rate is represented as of the first order with respect to dye, but as the reaction proceeds it becomes progressively smaller than that calculated from the simple first order rate formula. The cause for this retardation may supposedly be due to the interference of a certain reaction product; for example, such a product as hydrogen peroxide and this was really confirmed by the following experiment. Thus into the solution of methylene blue of which 80～90% had previously been photobleached, a suitable quantity of dye was newly dissolved in vacuo to make the concentration about equal to that of the previous one. Using such a solution the photoreaction was once more repeated and its rate was compared with that of the previous experiment.

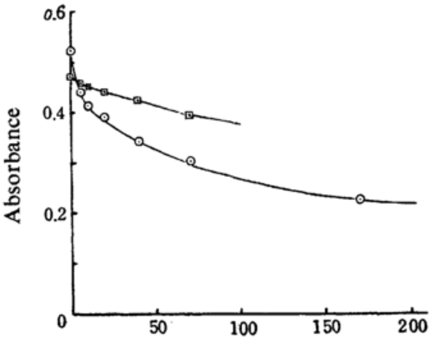

Fig. 2. Retardation by reaction product(s) in borate buffer at 30°C.

(⊙: 1st run, ▣: 2nd run)

As shown in Fig. 2, the rate in the second run is much smaller than that in the first one. On the basis of this result we now propose the following scheme as a tentative one.

$$\begin{tikzcd}
\mathrm{MB} \arrow[r, "h\nu"] & \mathrm{MB}^* \arrow[r] \arrow[d] & \mathrm{MB}^t \arrow[r, dotted, "a"] \arrow[d] & \mathrm{X} \arrow[r, "k_1"] \arrow[d, "k_d"] \arrow[dr, "+\mathrm{P}", "k_2"'] & \mathrm{P} \\
 & \mathrm{MB} & \mathrm{MB} & \mathrm{MB} & \mathrm{MB}
\end{tikzcd} \qquad \text{(A)}$$

In this scheme it is assumed that the dye molecule goes via the triplet state to a certain intermediate X, and a is a fraction of excited dye which attains to X. X then is assumed to react in three different ways, i. e., the product-forming reaction of the (apparent) first order (rate constant; k_1) the usual deactivation process (rate constant; k_d) and a process of particular deactivation caused by the attack of some reaction product (rate; $k_2[\mathrm{P}][\mathrm{X}]$). In fact it is entirely unknown how the reaction product may interfere with the reaction, but here the above scheme may conventionally be adopted rather as a trial one, partially because of the simplicity of the formula obtained therefrom.

If the reaction proceeds in a steady state according to the above scheme, the rate formula may be expressed as follows;

$$-\frac{\mathrm{d}[\mathrm{MB}]}{\mathrm{d}t}=k_1[\mathrm{X}]=\frac{ak_1C}{k_1+k_d+k_2(C_0-C)} \qquad (1)$$

in which C_0 is the initial concentration of dye. In the absence of the retarding effect of the product, this equation is reduced to the following,

$$-\frac{\mathrm{d}[\mathrm{MB}]}{\mathrm{d}t}=\frac{ak_1C}{k_1+k_d}$$

Here it is to be added that the absorption of light is assumed to be approximately proportional to the dye concentration C.

Below it will be shown that the above trial formula is satisfactory to some extent.

Integration of 1 leads to

$$\frac{\ln C_0-\ln C}{C_0-C}=\frac{k_2}{k_1+k_d+k_2C_0}+\frac{ak_1}{k_1+k_d+k_2C_0}\,\frac{t}{C_0-C} \qquad (2)$$

This equation requires a linear relation between $(\ln C_0-\ln C)/(C_0-C)$ and $t/(C_0-C)$, which in fact is well satisfied by most of the experimental results within the experimental error. This is shown in Fig. 3.

It is also evident from the figure that Eq. 2 does not hold in the case of phosphate buffer. The values for intercept, $S=k_2/(k_1+k_d+k_2C_0)$, inclination, $\alpha=ak_1/(k_1+k_d+k_2C_0)$ and the ratio of the latter to the former, $ak_1/k_2(=\alpha/S)$,

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* Since the optical density of the solution during the course of the reaction is relatively small, the inner filter effect of the demethylated dye need not be taken into account.

TABLE I. RESULTS OF KINETIC TREATMENT

	Condition of soln, M	pH	Concn. of dye*, M	$S\times10^{-5}$	$\alpha\times10^{4}$	α/S, mol. 1^{-1}. $\sec^{-1}$
a)	Na_2SO_4 ; 1.0×10^{-2}	6.10	1.2×10^{-5}	0.968	1.63	1.7×10^{-9}
b)	NaI ; 1.0×10^{-2}	6.10	1.2×10^{-5}	0.640	0.32	5.0×10^{-10}
c)	NaCl ; 1.0×10^{-2}	6.18	2.0×10^{-5}	0.676	0.69	1.0×10^{-9}
d)	Plain aq. soln.	6.22	1.6×10^{-5}	0.566	0.71	1.3×10^{-9}
e)	Na-Borate ; 7.8×10^{-3} HCl ; 1.6×10^{-2}	7.50	4.0×10^{-6}	6.96	17.7	2.5×10^{-9}
f)	Na-Borate ; 7.8×10^{-3} HCl ; 1.6×10^{-2}	7.50	8.0×10^{-6}	2.46	4.84	2.0×10^{-9}
g)	Na-Borate ; 7.8×10^{-3} HCl ; 1.6×10^{-2}	7.50	1.2×10^{-5}	1.34	3.10	2.3×10^{-9}
h)	Na-Borate ; 7.8×10^{-3} HCl ; 1.6×10^{-2}	7.55	1.6×10^{-5}	1.16	3.68	3.2×10^{-9}
i)	Na-Borate ; 7.8×10^{-3} HCl ; 0	9.15	1.2×10^{-5}	0.103	54.3	5.3×10^{-7}
j)	Na-Borate ; 7.8×10^{-3} HCl ; 1.2×10^{-2}	8.30	1.2×10^{-5}	0.103	10.8	1.1×10^{-8}
k)	Na-Barate ; 7.8×10^{-3} HCl ; 1.4×10^{-2}	7.85	1.2×10^{-5}	0.108	4.97	4.6×10^{-9}
l)	Na-Borate ; 7.8×10^{-3} HCl ; 1.7×10^{-2} NaCl ; 1.0×10^{-1}	7.61	1.2×10^{-5}	1.01	9.30	9.3×10^{-9}

* Analytical concentration of the initial solution.

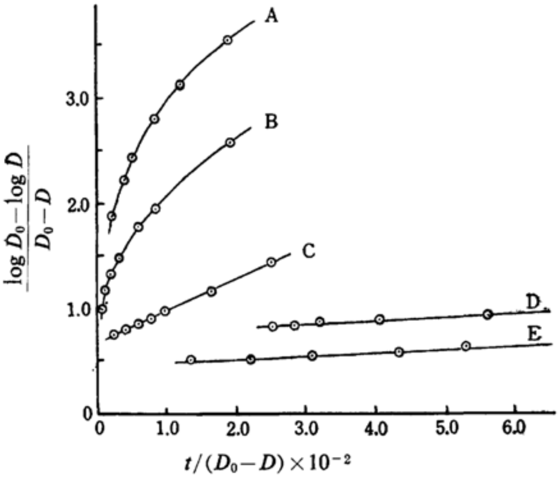


Fig. 3. Application of Eq. 2 for various salt solutions.
A. Phosphate buffer
B. NaOH aq. soln.
C. Borate buffer
D. Na_2SO_4 aq. soln.
E. aq. soln.

are summarised in Table I except for phosphate and sodium hydroxide for which Eq. 2 does not hold.

For the further support of Eq. 2, the effect of the initial concentration on the rate was examined in case of borate buffer. From Eq. 2 the reciprocal of the intercept ($=1/S$) should satisfy the following equation,

$$\frac{1}{S}=\frac{k_1+k_d}{k_2}+C_0 \tag{3}$$

hence the plot of $1/S$ against C_0 should be a straight line of slope 1. The slope experimentally obtained is about 0.91 as shown in Fig. 4.

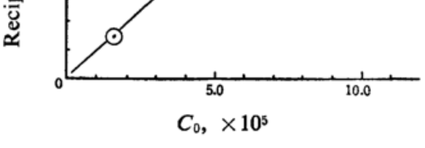


Fig. 4. Plot of $1/S$ against initial concn. C_0.

Further, the reciprocal of inclination of Eq. 2 being represented as follows,

$$\frac{1}{\alpha}=\frac{k_1+k_d}{ak_1}+\frac{k_2}{ak_1}C_0 \tag{4}$$

the plot of $1/\alpha$ against C_0 should be linear with the inclination of k_2/ak_1. This was in fact satisfied by the experimental data, the value obtained being 4.4×10^8 (mol^{-1} l. sec.)

which agrees approximately with the mean value of (e)—(h) in Table I ($S/\alpha=4.0\times10^8$).

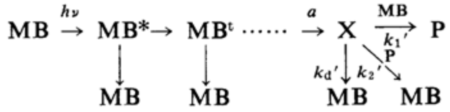
Thus the reliability of Eq. 2 was substantiated to some extent, and so the effect of pH on the rate, which was examined in the region of pH, 7.40∼9.15 keeping the concentration of borate ion constant, was analyzed by Eq. 2. The values of $\log\alpha/S(\alpha/S=ak_1/k_2)$ for the various pH values are shown in Table II.

TABLE II

pH	$\log[OH^-]$	$\log\alpha/S$
9.15	−4.85	−7.28(−7.69)
8.30	−5.70	−7.97
7.85	−6.15	−8.32
7.55	−6.45	−8.63
7.40	−6.60	−8.48

It is apparent that the value of α/S increases approximately proportionally to the concentration of $[OH^-]$.

Phosphate Buffer Solution. — It is assumed that the essential difference against the case of borate lies only in the bimolecular reaction of X and MB, which leads to the production of leuco dye, all the other steps being quite similar as in the previous one. Thus



$$\begin{tikzcd}
MB \arrow[r, "h\nu"] & MB^* \arrow[r] \arrow[d] & MB^t \cdots\cdots \arrow[r, "a"] \arrow[d] & X \arrow[r, "MB", "k_1'"'] \arrow[d, "k_d'"'] \arrow[dr, "P", "k_2'"'] & P \\
 & MB & MB & MB & MB
\end{tikzcd}$$

whence the rate is expressed as follows,

$$-\frac{d[MB]}{dt}=\frac{2ak_1'C^2}{k_1'C+k_d'+k_2'(C_0-C)}$$
$$=\frac{2ak_1'C^2}{k_d'+(k_1'-k_2')C+k_2'C_0} \qquad (5)$$

In view of the fact that the experimental data can quite satisfactorily be reproduced by the second order rate formula, it may be permissible to put $k_1'=k_2'$. Then,

$$-\frac{d[MB]}{dt}=\frac{2ak_1'C^2}{k_d'+k_2'C_0}=2kC^2 \qquad (6)$$

where

$$k=\frac{ak_1'}{k_d'+k_2'C_0}$$

Some examples for the relation $1/C\sim t$ are shown in Fig. 5. Most of the experimental results satisfy the linear relation. (Table III).

When the initial concentration is varied, the following relation should hold between the reciprocal of k and C_0

$$\frac{1}{k}=\frac{k_d'}{ak_1'}+\frac{k_2'}{ak_1'}C_0 \qquad (7)$$

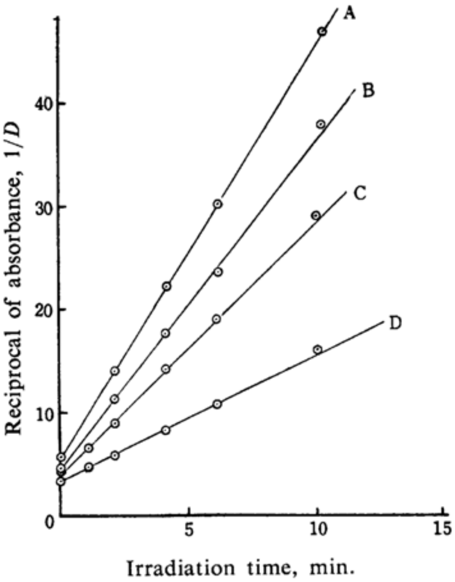


Fig. 5. Plot of the reciprocal of absorbance (D) against the irradiation time at constant pH.

Initial concn. of dye; A…… 4×10^{-6} M
B…… 8×10^{-6} M
C……1.2×10^{-5} M
D……1.6×10^{-5} M

TABLE III. KINETIC DATA AND CONDITION OF THE SOLUTION

(Concn. of total phosphate$=6.7\times10^{-2}$ M)

Concn. of dye, M	pH	k, mol^{-1} l. sec^{-1}
4×10^{-6}	7.56	2.00×10^3
8×10^{-6}	7.58	1.09×10^3
1.2×10^{-5}	7.54	1.03×10^3
1.6×10^{-5}	7.58	0.67×10^3
1.2×10^{-5}	6.94	0.94×10^3
1.2×10^{-5}	7.71	1.16×10^3
1.2×10^{-5}	8.07	1.30×10^3
1.2×10^{-5}	8.81	1.48×10^3

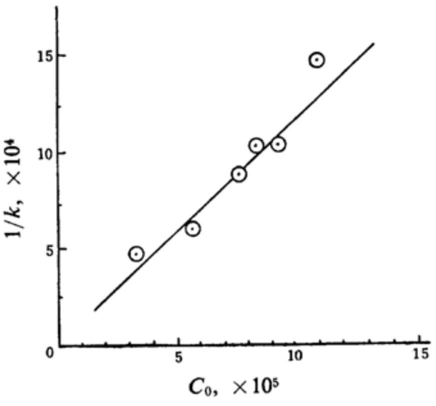


Fig. 6. Plot of $1/k$ against initial concn. C_0 for Eq. 7.

It was confirmed that the experimental results approximately satisfy the above relation. This is shown in Fig. 6.

The effect of pH on the rate was examined and the results are shown in Table IV.

TABLE IV

pH	log[OH⁻]	log *k*
8.81	−5.19	3.17
8.07	−5.93	3.11
7.71	−6.29	3.06
7.52	−6.48	3.02
6.94	−7.06	2.97

In contradistinction to the case of borate buffer, the rate is scarcely affected by the variation of pH.

The Effect of Some Added Substances

The Behavior when Ferric Chloride and *o*-Phenanthroline are Added. — In the previous paper it was reported that when these substances are added to the borate buffer solution of methylene blue and irradiated, the complex between Fe^{2+} and *o*-phenanthroline is formed, the quantity of which attains to more than ten times that of the dye. Thus, it was concluded from the above result, that no impurity contained in dye participates essentially in the reaction. In addition to this conclution, the investigation of this system is important with regard to the quantitative relation between the leuco-formation and the irreversible photodecomposition. So further description will be given below.

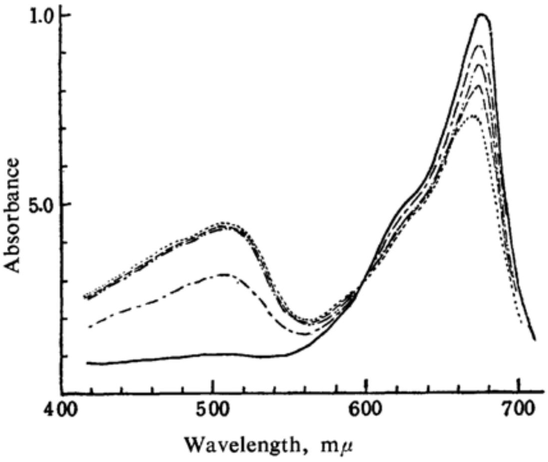


Fig. 7. The spectral change of the mixture of methylene blue-borate buffer, *o*-phenanthroline and ferric chloride solution when irradiated.

—— initial
-·-·- 2 min.-irradiation
-··-·· 5 min.-irradiation
— — 20 min.-irradiation
······ 50 min.-irradiation

A typical result is shown in Fig. 7, in which the absorption band of 510 mμ is due to trisphenanthroline iron(II) complex. In this experiment the concentration of the complex produced by 2 min. irradiation is about fourteen times that of the decrease in dye concentration. This decrease is due to the irreversible bleaching, the solution not being decolorized by aeration. Thus it may be judged that if the formation of leuco dye takes place only by the accompanying irreversible decomposition of dye, then one molecule of dye decomposed ought to be able to produce more than ten molecules of leuco dye. This makes the above view highly improbable.

It is to be added that when the irradiation is continued after all the Fe^{3+} has been consumed to produce trisphenanthroline iron(II) complex, the bleached dye partially recovers its color upon the introduction of air. Therefore it is certain that as long as Fe^{3+} exists the produced leuco dye reduces Fe^{3+}, but after the disappearance of Fe^{3+} the existence of the reduced dye persists.

It is regrettable that no such experiment can be performed for the phosphate solution on account of the small solubility of Fe^{3+} salt.

The Effect of the Addition of Pyruvate. — It was reported in the previous paper that a small quantity of hydrogen peroxide was detected in case of borate buffer but not in phosphate buffer. Now it is well known that in general hydrogen peroxide is rapidly decomposed by the addition of pyruvate, and it is interesting to investigate the effect of the addition of pyruvate upon the rate and to compare the two cases when the phosphate or borate buffer is used. The concentrations of pyruvate (C_{py}) added to the borate buffer are 1.2×10^{-4}, 2.2×10^{-4}, and 2.4×10^{-4} M. The results are shown in Fig. 8.

It is apparent from the figure that the slope becomes steeper with the increase of the concentration of pyruvate. Further, when α/S-values for the two cases, one with pyruvate and the other without it but otherwise in the similar conditions are compared, the value for the former is definitely larger; thus from Fig. 8 for $C_{py}=2.2\times10^{-4}$ M, $\alpha/S=7.57\times10^{-9}$ and for $C_{py}=0$, $\alpha/S=2.97\times10^{-9}$ (mol. l^{-1} sec^{-1}). The above results are well comprehended if the k_2-value in the second term on the right side of Eq. 2 gets smaller with the addition of pyruvate. Although the possibility for other steps to be affected might not be neglected, it seems most plausible that the

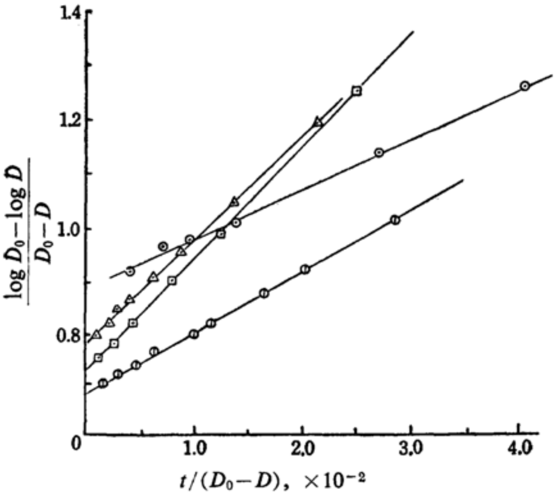


Fig. 8. The effect of the addition of pyruvate on Eq. 2 (D……optical density).

⊙ [Pyr]=0
⦶ [Pyr]=1.2×10^{-4} M
△ [Pyr]=2.2×10^{-4} M
⊡ [Pyr]=2.4×10^{-4} M

addition of pyruvate chiefly affects the k_2-value in view of the result obtained for phosphate buffer (described below).

When ten times as much pyruvate as the concentration of methylene blue was added to phosphate buffer, the rate was also represented as of the second order with respect to dye and the k value of Eq. 6 was evaluated to be 5.3×10^2 (mol^{-1} l. sec^{-1}). This is slightly smaller than 6.2×10^2, which is the k-value in the absence of pyruvate but otherwise having similar conditions. It is evident that the addition of pyruvate to the phosphate buffer scarcely affects the rate.

Thus the effect of pyruvate-addition is quite different in the case of phosphate and borate, and this is just what is expected from the results already obtained.

The Effect of the Addition of Quinone. — It was reported in the previous paper that in the case of phosphate buffer, neither hydrogen peroxide nor oxygen was detected. But it is premature to deny the formation of oxygen only on the basis of this analysis, because the produced oxygen may be consumed in the irreversible photodecomposition of dye. In order to detect the oxygen formed, it seems rather essential to make the experiment under conditions such that the oxygen which might be produced, be accumulated so rapidly that it is scarcely consumed in the photooxidation of dye.

With this view in mind and from the analogy of the Hill reaction in photosynthesis, the addition of quinone was tested. Thus if the quinone does not attack the excited dye but only oxidizes the leuco dye produced as in the case of the Hill reaction, the oxygen is expected to be accumulated.

Preliminary tests demonstrated, however, that the addition of quinone, the quantity being up to several times (in mole) that of the dye induces a quite complicated spectral change of methylene blue when the evacuated solution is irradiated. In addition, spectra of methylene blue in the above solution were found to change even in the dark though the rate was much slower.

Thus there is no doubt that the direct photoreaction does take place between dye and quinone. This reaction seems to be interesting from a different point of view, but it is clear that quinone is too reactive to be used for the above object. Hence further studies have been abandoned. Other kinds of quinones or other types of oxidants more mild in character should be searched.

Quantum Yield

In both buffer solutions, the quantum yield (γ) for the initial stage was evaluated by using the inclination of the curve of optical density against time, and the number of photons (I_{abs}) absorbed by the solution, both in the initial stage. The latter quantity was measured with the combination of the thermopile (Moll and Burger standard thermopile) and White single potentiometer (Leeds and Northrup).

The following table shows the results obtained for borate and phosphate buffer solutions.

TABLE V. QUANTUM YIELD

Condition	I_{abs} (photons. sec^{-1} cc^{-1})	$\Delta D/\Delta t$ (D, min^{-1})	γ
a) Phosphate	7.2×10^{15}	0.089	9.2×10^{-4}
buffer	6.9×10^{16}	0.029	7.1×10^{-4}
b) Borate	3.5×10^{15}	0.006	3.0×10^{-4}
buffer	8.7×10^{15}	0.010	2.2×10^{-4}

Discussion

It was found in the previous paper that the general feature of the reaction is somewhat different in borate and phosphate buffer. Now in the present paper additional differences were found from the kinetic standpoint. Thus the rate in borate buffer is approximately of the first order in dye while that of the phosphate buffer, is of the second order, and moreover the pH effect is quite different in two cases. Therefore, two cases will be separately discussed, anticipating the essential difference in mechanism.

In case of borate the rate being of the first order in dye, the interaction of two dye molecules of which one acts as an electron-donor and the other as an acceptor can safely be neglected. The result of the experiment pertaining to the formation of trisphenanthroline iron(II) chloride also denies such an interaction. Thus one cannot but consider that in this case the oxidation of water is taking place under some cooperative action of the salt. In fact, as reported in the previous paper, a small quantity of hydrogen peroxide was detected.

As regards the photofission of water by visible light, Yamafuji reported a long time ago[3] that hydrogen peroxide was produced when the solution of eosine or chlorophyll was irradiated by the solar light. Their experiments, however, were criticized by Rabinowitch[4] and at present their results seem to be judged to be doubtful.

Indeed the decomposition of water by the visible light by virtue of the following scheme

$$H_2O \rightarrow H+OH$$

or

$$HO^- \rightarrow e+OH \qquad (6)$$

must be almost impossible from the energetic reason, except for the case in which the absorption of more than two photons takes part in one act. However, if the reaction in the following molecular mechanism occurs,

$$D^+ + 2OH^- \rightarrow DH + HO^-_2 \qquad (7)$$

the free enthalpy change is not so great. It is ca. $\Delta G^\circ_{298} = 49.65$ kcal./mol. so that with an efficient use of the energy of photon absorbed, ca. 43.0 kcal., only 6.6 kcal. needs to be provided for the reaction 7 to occur. If borate ion can afford a certain amount of free enthalpy in some way, then the quantum yield experimentally obtained would be attained by the process 7.

In connection with this it is tempting to recall that borate is an electron deficient substance and to suppose that some of the borate ions may be in a certain energy-rich state combining with hydroxide ion. Alternatively there might be a possibility that a certain species of dye such as $(D\cdots OH)H^+$ which Lewis[5] proposed, takes part in the reaction to make it energetically feasible. The result that the rate is approximately of the first order in $[OH^-]$ in spite of the second order for the stoichiometric equation, might be related with this.

Next the case of phosphate will be discussed. In this case neither hydrogen peroxide nor oxygen was detected and the reaction proceeds in the second order with regards to dye. These facts seem at first strongly to suggest that the initial process is the interaction of the excited dye with the ground-state-dye molecule, the electron transfer taking place between them according to

$$D^* + D \rightarrow D^- + D^+ \qquad (8)$$

and resulting in the leuco-formation from one dye molecule and the irreversible decomposition of another. But judging from the result that 70∼80% of the bleached dye is recolorized by the introduction of air, it seems necessary that one molecule of dye decomposed ought to be able to reduce about three dye molecules and that the reducing power of the substances produced by dye-decomposition should be much larger; otherwise the quantity of reduced dye would be much less. Hence the above scheme would not be accepted unless it is confirmed experimentally that the above requirements are really satisfied. At the present stage it seems rather improbable that the leuco-formation only occurs through 8 though it may contribute to some extent.

On the other hand, the reaction

$$2D^+ + 2OH^- \rightarrow 2DH + O_2 \qquad (9)$$

seems not entirely impossible from the energetic view point. Thus its ΔG°_{298} is 55.93 kcal. and taking the energy of photon, 43.0 kcal. into account, the free enthalpy is evaluated to be about 6.4 kcal. for one dye molecule. Should the phosphate ion be able to provied energy in some way, reaction 9 would be realized just as reaction 7.

The role of phosphate ion must be clarified irrespective of wether the reaction goes via 8 or 9.

The photoreduction which has been confirmed in this series of investigation would be expected to occur in other thiazine dyes and this has really been ascertained in the case of thionine. Since the demethylation need not be taken into account for thionine, the quantitative study would be more feasible particularly as regards the interrelation between leuco-formation and irreversible decomposition. These are now in course of study.

Summary

Kinetic treatment was undertaken for the photobleaching of the aqueous solution of methylene blue containing various inorganic salts.

If a certain kind of inhibiting action due to

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3) K. Yamafuji et al., *Biochem. Z.*, **301**, 404 (1939).

4) E. I. Rabinowitch, "Photosynthesis and Related Processes", Vol. I, Interscience Publishers, Inc., New York (1956), p. 78.

5) G. N. Lewis et al., *J. Am. Chem. Soc.*, **65**, 1150 (1943).

some reaction product(s) is taken into consideration, the rate for borate buffer is satisfactorily reproduced as the first order of excited dye, while for phosphate buffer it is second order with regard to dye. It was further found from this analysis that the rate of the essential step (or steps) of the reaction for the case of borate is approximately proportional to the concentration of OH^-, while for the case of phosphate it is almost independent of pH.

The quantity of irreversible decomposition of dye in case of borate buffer was estimated to be very small against that of the reversible bleaching, the former being a few tenths of the latter from the experimental results obtained when ferric chloride and *o*-phenanthroline were added to the borate buffer solution.

Judging from the results hitherto obtained, it is most plausible that the photoreduction of methylene blue in case of borate takes place through the decomposition of water. But in case of phosphate, there still remains some possibility that the reaction commences by the interaction of excited dye and ground state dye, electron transfer taking place between them.

The quantum yield of the bleaching reaction was measured and found to be $(2\sim3)\times10^{-4}$ for borate buffer and $(7\sim9)\times10^{-4}$ for phosphate buffer.

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