Photoreduction of Methylene Blue and Thionine in Ethanol

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Studies of the photoreduction of xanthene dyes in alcohol were started by Imamura and Koizumi,[1] and the mechanism has been clarified to some extent since then. However, as for the similar photoreduction of thiazine dyes, there seem to have been no kinetic studies undertaken with the aim of elucidating its mechanism, although a few reports, rather qualitative, have been made, including those of Kutsaya and others.[2]

There are two reasons why the author has started the present investigation. One is to add to our knowledge concerning the photochemical behavior of various dyes is alcohol and, in particular, to compare thiazine dyes with xanthene dyes and acridine, which have been already investigated.[3] The other is to examine the peculiar behavior of the thiazine dyes, which are known to undergo photoreduction in an aqueous solution in the absence of

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[1] M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955).

[2] B. F. Kutsaya and Ya. B. Dain., *Ukrain. Khim. Zhur.*, **17**, 820 (1951).

[3] S. Kato, S. Minagawa and M. Koizumi, This Bulletin, **34**, 1026 (1961); S. Niizuma and M. Koizumi, ibid., **36**, 1629 (1963).

a reducing agent.[4] It has been found, in sharp contrast to the behavior of xanthene dyes and acridine, that this reaction is second order in respect to the dye. The mechanism is discussed on the basis of the hypothesis proposed by Koizumi et al.[5]

Experimental

Materials. — *Methylene Blue (MB).* — Grübler's reagent was purified according to the procedure described before.[4] The molar absorbance at the 655 mμ peak was 8.8×10^4 for an ethanol solution.

Thionine (Th).—Merck's reagent was recrystallized twice from purified ethanol and dried under a vacuum. The molar absorbance in an ethanol solution was 7.12×10^4 at λ_{max} (605 mμ).

Ethanol.—Wako Pure Chemical Industry's G. R. grade ethanol was used. After removing traces of aldehyde, water, etc., by the ordinary method, it was fractionally distilled three times. Such a sample, however, did not show, spectroscopically, any noticeable difference compared with the twice-fractionally distilled sample; the ultraviolet spectra of these two samples were essentially identical, thus assuring their purity.*

Acetaldehyde.—To obtain an absolute aldehyde, a commercial 80% acetaldehyde aqueous solution (Junsei Chemical Co.) was submitted several times to distillation from a dry-ice methanol to a liquid air bath under a high vacuum.

Procedure.—The alcoholic solution of methylene blue was degassed in vacuo four or five times, and then irradiated with a 500 W.-85 V. tungsten projection lamp at 25.0±0.1°C. In the case of methylene blue, the light shorter than 620 mμ was eliminated by means of a Walz WR-2 cut-off filter. For thionine, a Matzuda SO56 filter was used to cut off the light shorter than 560 mμ. The reaction was followed by measuring the change in optical density at the absorption peak by the use of an interference filter. The absorption spectra were measured on a Hitachi EPU-2A or EPS auto-recording spectrophotometer. The reaction cell was a quartz spectrophotometer cell ($1\times1\times4\,\mathrm{cm}^3$), the upper part of which was connected to an ampule by a quartz-telex-SB-grade tube.

Results

Some General Features of the Reaction.—*Photobleached Product.* — Figure 1 shows the spectral change of an irradiated solution of methylene blue and thionine in the ultraviolet and visible region. The introduction of the air into the bleached sample restored the original absorption spectra almost completely in both cases. This is considered to be strong evidence that the dye was photoreduced to its leuco form. Further evidence was obtained from a comparison of the absorption spectrum of the photobleached product with that of the chemically prepared leuco dye. The latter compound was prepared by reducing methylene blue with Pd–H_2, and its spectrum was measured in a hydrogen atmosphere. Table I gives a comparison of the two spectra.

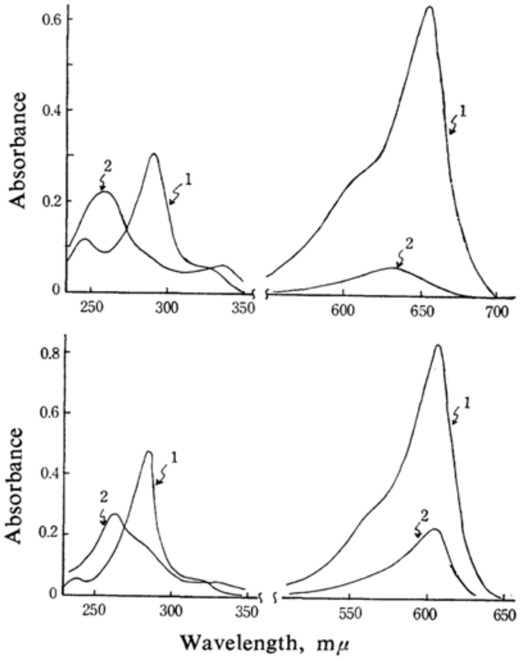

Fig. 1. The absorption spectra of the irradiated ethanol solution of methylene blue (upper) and thionine (lower) in vacuo.

$[\mathrm{MB}]_{\mathrm{init}}=8.0\times10^{-6}$ M
$[\mathrm{Th}]_{\mathrm{init}}=1.15\times10^{-5}$ M
1 Before irradiation
2 After irradiation

TABLE I

	Chemically prepared leuco methylene blue		Photobleached product	
Absorption peak	261(s)*	315(w)	261(s)	320(?)
ε_{max}	3.0×10^3		3.2×10^3	
% of recovery by air-introduction	100		90—100	

* s, strong; w, weak

From the agreement of the position and intensity of the absorption and from the fact that the response is quite similar to the admittance of oxygen or hydrogen peroxide, there is scarcely any doubt that the photobleached product in question consists mainly

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4) Y. Usui, H. Obata and M. Koizumi, ibid., **34**, 1048, 1651 (1961).

5) M. Koizumi, H. Obata and S. Hayashi, ibid., **37**, 108 (1964).

* It appeared that the purity or the age of ethanol had a very delicate effect on the reaction rate, even when the solvent was optically pure. In the present investigation, such an obstacle was avoided by using alcohol from the same source.

of leuco dye. Furthermore, aldehyde was detected among the reaction products by gas chromatography. Thus, the overall reaction can safely be written as follows:

$$D^{+*}+C_2H_5OH \rightarrow DH+CH_3CHO+H^+ .$$

The Influence of the Addition of Acetaldehyde.—Since acetaldehyde is produced by the reaction and since a trace of it might have been present as a contaminant in the ethanol used, its effect on the rate of the reaction was examined. It was found that the rate of bleaching increased about six times when 1.16 M acetaldehyde was added to the solution (Fig. 2). On the basis of this experiment, it can safely be said that the small quantity of acetaldehyde produced during the reaction does not affect the rate appreciably. As Fig. 2 shows, the original dye is reformed when the bleached sample is kept in the dark. This is probably due to the gradual oxidation of the leuco dye by acetaldehyde.

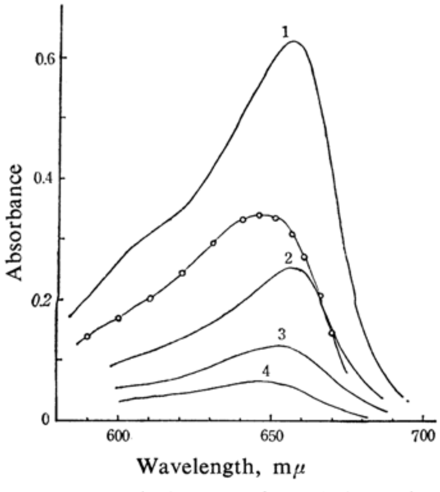

Fig. 2. Spectral changes of methylene blue in deaerated ethanol containing acetaldehyde (1.16 M).

1 Before irradiation
2 2 min-irradiation
3 5 min-irradiation
4 10 min-irradiation
-○- After keeping for 14 hr. in the dark

Kinetic Studies. — *Rate Formula.* — Although the photoreduction of xanthene dyes and acridine, as has already been reported,[1,3)] proceeds at a rate simply proportional to the absorption of light, the same can not necessarily be expected with thiazine dyes. In view of the peculiar behavior of thiazine dyes, namely, that they are photoreduced without the addition of any reducing agent, and further, in view of the fact that this reaction in the case of methylene blue and toluidine blue in a phosphate buffer proceeds as a simple second order reaction with regard to the dye concentration,[5)] it is necessary in particular to compare the applicability of the first order and the second order rate formula.* As Fig. 3 shows, the second order rate formula is far more satisfactory than the first order one. Although this result by itself appears quite extraordinary, it is not really so if one recalls that the general scheme of the photoreduction of such dyes proposed by Koizumi et al., i. e., the scheme that electron transfer occurs between the excited and the ground state dye molecules, is supported by the flash experiment to some extent.[6)] Next, the effect of various factors upon the second order rate constant k (rate$=kC^2$) was examined.

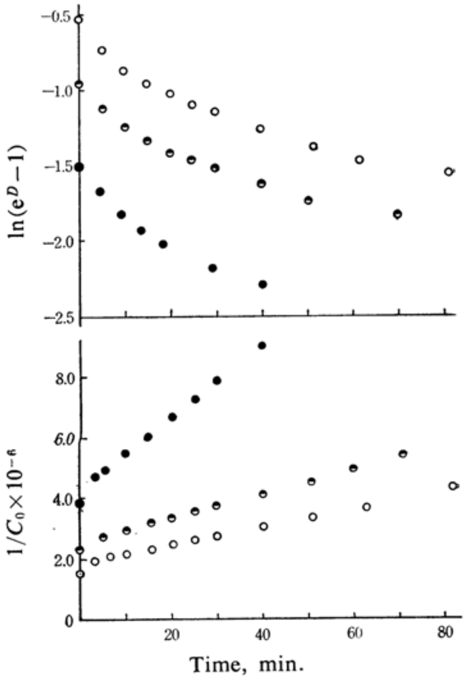

Fig. 3. Comparison of the following two rate formules; second order in dye concentration (lower), and proportional to the absorption of light (upper).

○ $C_0=6.2\times10^{-6}$ M
◒ $C_0=4.5\times10^{-6}$ M
● $C_0=2.7\times10^{-6}$ M

The Effect of the Light Intensity. — The intensity of the incident light was varied by means of neutral filters by a factor of about ten. The plot of k against the relative intensity, as may be seen from Fig. 4, gives a good straight line. This implies that the

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* The absorption of light is approximately proportional to the dye concentration, since the concentration is low.

6) S. Kato, M. Morita and M. Koizumi, This Bulletin, 37, 117 (1964).

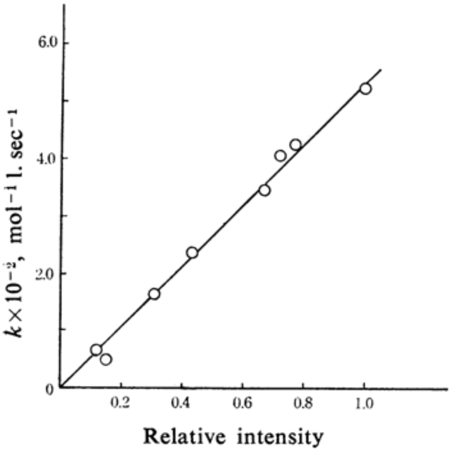

Fig. 4. Intensity effect on the overall rate constant for the second order reaction. ([MB]$=10^{-5}$ M)

net rate is essentially controlled by the interaction of the excited dye and the ground state dye, because the overall rate is of the second order with regard to the dye concentration.

Quantum Yield.—The quantum yield (Φ) for the initial stage was evaluated from the inclination of the plot of the dye concentration against the time, together with the number of photons absorbed by the solution in a unit time (I_{ab}).

The latter quantity was measured with a combination of a Moll and Burger thermopile and a Shimadzu K2 potentiometer. For 5.95×10^{-6} M methylene blue, I_{ab} was measured as 4.8×10^{18} photons l^{-1} sec^{-1}, and the value of Φ was found to be $1-3\times10^{-3}$ (at 25°C). For 8.5×10^{-6} M thionine, I_{ab} was 3.6×10^{18} photons l^{-1} sec^{-1} and Φ was 1.1×10^{-3} at 25°C.

Further Studies of the Basis of the Second Order Rate Formula.—As has been stated above, the photobleaching of methylene blue in ethanol was accompanied with some degree of demethylation, which caused a slight shift of the absorption maximum toward a shorter wavelength. Although the minimum ratio method was employed in order to exclude the absorption of the demethylated dye, this procedure made the kinetic treatment less exact. In the case of thionine, on the other hand, such a shift does not occur and the kinetic treatment is expected to be simpler and more accurate.

Therefore, a more detailed investigation was carried out, using thionine as well as methylene blue. First, the effect of the initial dye concentration on the rate was examined quantitatively, and second, the effect on the rate of the addition of the leuco dye was investigated quantitatively in order to establish the reaction mechanism. Lastly, the photobleaching in a mixed solvent of water and ethanol was studied.

The Effect of the Initial Dye Concentration.—The initial dye concentration, C_0, was varied in the region 10^{-6}—10^{-5} M for both methylene blue and thionine. It was found that the overall second order rate constant, k, decreases with the increase in C_0 (Table II).

Figures 5a, 5b and 5c give plots of $1/k$ or $1/\Phi$* against C_0. The latter plot gives a good straight line; this leads to the following equation:

$$1/k=\alpha C_0(C_0+\beta) \quad (1)$$

in which α and β are constants. The kinetic

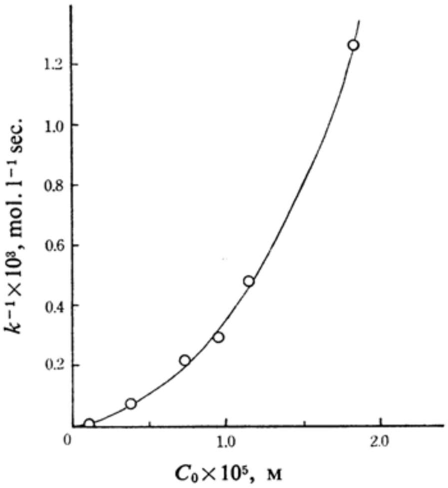

Fig. 5a. Dependence of k^{-1} on the initial concentration (C_0) of thionine.

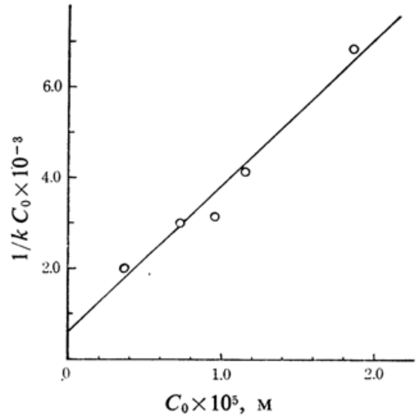

Fig. 5b. Dependence of $(kC_0)^{-1}$ on the initial concentration of thionine.
(Slope, $S_k=3.36\times10^8$, intercept, $I_k=4.5\times10^2$)

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* When C_0 is small enough for the initial stage, Φ is proportional to kC_0.

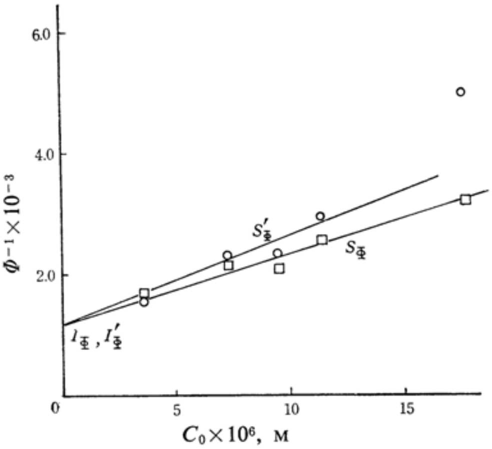

Fig. 5c. Dependence of Φ^{-1} on the initial concentration of thionine.
-○- : the plot evaluated by the approximation $I_{ab}\approx I_0\tilde{\varepsilon}C_0$
-□- : the plot evaluated by $I_{ab}=I_0(1-e^{-\tilde{\alpha}C_0})$

TABLE II. THE INITIAL CONCENTRATION EFFECT ON THE SECOND ORDER RATE CONSTANT

Thionine

C_0 M	k mol^{-1} l. sec^{-1}	Φ
1.14×10^{-6}	1.24×10^4	(0.02)
3.80×10^{-6}	1.38×10^2	6.8×10^{-4}
7.30×10^{-6}	4.64×10	4.7×10^{-4}
9.56×10^{-6}	3.42×10	4.5×10^{-4}
1.15×10^{-5}	2.12×10	3.4×10^{-4}
1.84×10^{-5}	0.79×10	2.0×10^{-4}

Methylene blue

C_0 M	k mol^{-1} l. sec^{-1}	Φ
2.39×10^{-6}	1.29×10^3	4.6×10^{-3}
3.44×10^{-6}	1.40×10^3	7.2×10^{-3}
4.95×10^{-6}	2.16×10^2	1.6×10^{-3}
6.02×10^{-6}	1.71×10^2	1.5×10^{-3}
9.59×10^{-6}	1.53×10^2	2.2×10^{-3}
1.04×10^{-5}	4.81×10	7.5×10^{-4}
$1.9\ \times10^{-5}$	1.76×10	5.0×10^{-4}

results so far obtained are quite similar to those for methylene blue and toluidine blue in the phosphate buffer solution.

The Retarding Effect of the Leuco Dye.—As has already been stated, acetaldehyde, which is a reaction product, was found to have no significant influence on the rate. The effect of the leuco dye on the rate is more difficult to examine,* since the leuco dye is very sensitive to oxygen and is easily oxidized to the original form.

The procedure employed was as follows. The leuco dye, which was synthesized by reducing thionine with Pd–H_2 in alcohol in the dark, was added to the deaerated solution of thionine in vacuo. Using this solution, the experiment was performed and the results were compared with those for an ordinary solution with no leuco dye added. The comparisons are summarized in Table III, in which k_L and k_0 are, respectively, the second order rate constants for the solutions with or without the leuco dye.

It is evident that the addition of the leuco dye causes a marked reduction in the rate.

The Photoreduction of Thionine in a Mixed Solvent of Ethanol and Water.—The absorption maximum for thionine in an aqueous solution differs from that in ethanol by only 5 mμ. Furthermore, the dye exists solely in the monomeric form when the concentration of ethanol is more than 25% (v/v). These features are thought to make the results easier to interpret.

Once again, the second order rate formula could be applied satisfactorily to all the results, except for a small deviation in the initial stage. Table IV gives the maximum wavelength, its molar absorbance, and the overall second order rate constant (k) and the quantum yield (Φ) for various compositions ($C_0=8.0\times10^{-6}$ M).

The values of the quantum yield are plotted against the molar fraction of ethanol (χ_R) in Fig. 6. Evidently, there is a good linear relation between them. Thus, experimentally,

$$\Phi = \Phi^0{}_{H_2O} + (\Phi^0{}_R - \Phi^0{}_{H_2O})\chi_R$$

where $\Phi^0{}_{H_2O}$ and $\Phi^0{}_R$ are the quantum yields in the pure aqueous solution and in an ethanol solution respectively.

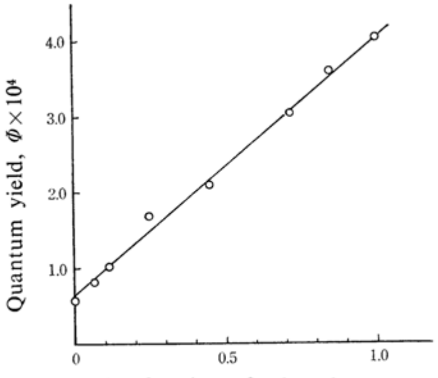

Fig. 6. The effect of water on the quantum yield for a mixed solvent.
$[Th]_0=1\times10^{-5}$ M

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* When the deaerated ethanol solution of leucothionine and thionine was kept in the dark for a few days, a queer phenomenon was observed; the peak of the absorption shifted from 605 mμ to 635 mμ, and the absorption in the wavelength region above 500 mμ increased. This phenomenon becomes more pronounced when the solution is illuminated.

TABLE III. THE RETARDING EFFECT OF LEUCO THIONINE

Exp. No.	[Th]	[LTh]	k_L $\mathrm{mol^{-1}\,l.\,sec^{-1}}$	k_0 $\mathrm{mol^{-1}\,l.\,sec^{-1}}$	k_L/k_0
i	6.88×10^{-6}	4.48×10^{-6}	59.6	52.6	(1.12)
ii	8.06×10^{-6}	8.77×10^{-6}	33.7	39.6	0.86
iii	8.80×10^{-6}	2.43×10^{-5}	17.8	32.2	0.55
iv	1.10×10^{-5}	3.80×10^{-4}	0.65	21.2	0.03

TABLE IV. KINETIC DATA IN THE MIXED SOLVENT

χ_R		λ_{max} mμ	$\varepsilon_{max}\times10^{-4}$	k $\mathrm{l.\,mol^{-1}\,sec^{-1}}$	Φ $(kC_0/I_0\tilde{\varepsilon})$
0	(100)*	600	3.98	2.5	4.3×10^{-5}
0.077	(80)	602	4.99	5.9	8.0×10^{-5}
0.125	(70)	602	5.06	7.4	10.0×10^{-5}
0.247	(50)	605	5.59	12.2	15.5×10^{-5}
0.430	(29.9)	605	5.90	17.4	21.5×10^{-5}
0.740	(9.95)	605	6.58	25.1	30.5×10^{-5}
0.846	(5.0)	605	6.76	29.0	36.4×10^{-5}
1.000	(0)	605	7.38	34.1	40.3×10^{-5}

* Volume percentage of water

From the above results, it is reasonable to assume that similar mechanisms prevail throughout the whole range of composition.

A Flash Experiment on the Ethanol Solution of Methylene Blue. — Flash experiments were performed only to compare the transients in alcohol with those in the phosphate buffer solution. Figure 7 shows the transient spectra in the degassed ethanol solution at various delay times. The present results resemble those for the aqueous solution⁶⁾ so closely that there is scarcely any doubt that the strong band at ~420 mμ and the weak shoulder at ~520 mμ correspond, respectively, to a half-reduced form and a half-oxidized form. Although it is believed that the triplet state absorption superposes at an early stage, the above two absoptions decay at about the same rate in the latter stage, suggesting that most of the half-reduced and the half-oxidized states revert to the original two dye molecules by the backward reaction.

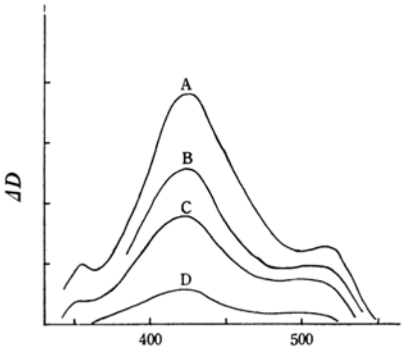

Fig. 7. The absorption spectra of the transient species in ethanol.
$[\mathrm{MB}]_0=1\times10^{-5}$ M, ΔD is the difference in the blackness of two plates.

A : After 20 μsec.
B : After 100 μsec.
C : After 200 μsec.
D : After 400 μsec.

All the results obtained thus support the view that the mechanism of the photoreduction in an alcoholic solution is quite similar to that in an aqueous solution.

The Effect of Dissolved Oxygen.—The effect of the oxygen concentration on the rate of the irreversible decomposition of methylene blue in a plain aqueous solution was studied a few years ago.⁷⁾ It was found that the quantum yield of the irreversible decomposition of dye by oxygen increased with the rise in oxygen concentration but reached a constant maximum value above a certain oxygen concentration, suggesting that the reaction is competitive with the self-deactivation of the triplet state dye. A similar behavior had been observed for an aqueous solution of xanthene dyes.¹,⁸⁾ On the other hand, quite a different observation had been made of an alcoholic solution of eosine.⁹⁾ When a very small amount of oxygen was added to this solution, the photobleaching was inhibited almost completely, but after a certain induction period, the length of which was proportional to the amount of oxygen, the reaction commenced at almost the same rate as in the

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7) H. Obata, K. Kogasaka and M. Koizumi, This Bulletin, **32**, 125 (1958).

8) M. Imamura, *J. Inst. Polytech., Osaka City Univ.*, **5**, 85 (1956).

9) M. Imamura and M. Koizumi, This Bulletin, **29**, 899 (1956).

deoxygenated solution. This result was interpreted as being due to a very prompt attack of oxygen on the semiquinone of the dye.

In view of the bimolecular nature of the present reaction, it was thought interesting to investigate the oxygen effect on the photoreduction of an alcoholic solution of methylene blue. The concentration of dye was 8.0×10^{-6} M in all cases. This reaction, however, proved rather complicated. Thus, the rate of the photobleaching decreased with an increase in the oxygen concentration. However, in this case the bleaching was interpreted as consisting of the reversible photobleaching as well as the irreversible decomposition of dye, because the introduction of air after the reaction caused a partial recovery of the dye. Besides, when the partially bleached solution was kept in the dark, the color was restored a little, perhaps due to the oxidation of leuco dye by the existent oxygen. The reversible photobleaching could occur even in the air-saturated solution; its extent was estimated to reach about ten per cent of the total bleaching.

Another feature which complicated the reaction was that significant demethylation accompanied the decoloration. As Fig. 8 shows, the degree of demethylation increases gradually with the progress of bleaching. In spite of all these complicating factors, the general effect of oxygen can be seen from Fig. 9, which gives the rate of bleaching at an initial stage as a function of the concentration of oxygen. A notable fact is that a rather rapid fall of the rate caused by a small amount of oxygen is followed by a very gradual decrease in the range of oxygen concentration beyond that of the dye.

TABLE V. INITIAL RATES AT VARIOUS OXYGEN CONCENTRATIONS

O_2 Concn., M	$(\Delta C/\Delta t)_{t=0}$, M sec^{-1}
0	3.6×10^{-8}
8.6×10^{-6}	3.6×10^{-8}
1.7×10^{-5}	1.5×10^{-8}
2.6×10^{-5}	5.6×10^{-9}
4.7×10^{-5}	2.2×10^{-9}
9.2×10^{-5}	1.8×10^{-9}
1.2×10^{-4}	1.4×10^{-9}
1.7×10^{-4}	0.7×10^{-9}
$\sim2\times10^{-3}$ (air sat.)	0.5×10^{-9}

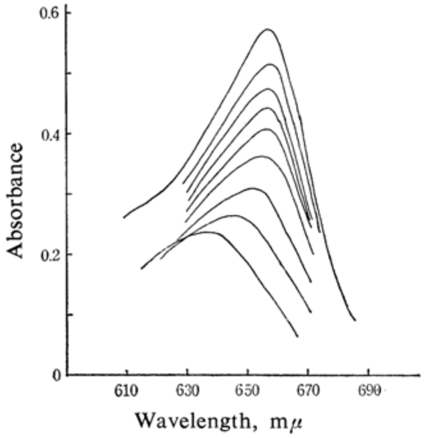


Fig. 8. An example of spectral changes in the oxygen pressure effect.
$[MB]_0=6.46\times10^{-6}$ M, $[O_2]=2.6\times10^{-5}$ M

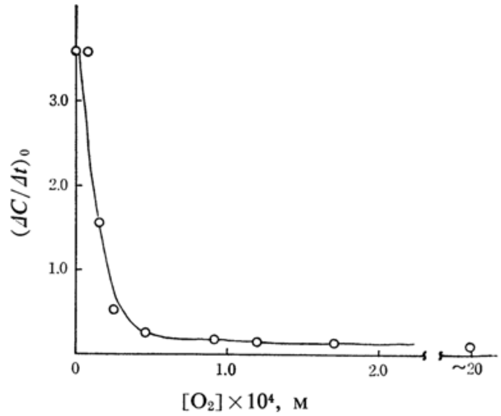
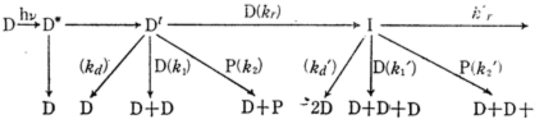


Fig. 9. Dependence of initial rate $(\Delta C/\Delta t)_0$ on oxygen concentration ($[MB]_0=8\times10^{-6}$ M).

Discussion

In an alcoholic solution, the kinetic result is quite similar to that obtained for methylene blue and toluidine blue in a phosphate buffer solution. Hence, one can feel confident in discussing the results on the basis of a similar scheme. The scheme involves an electron transfer between the triplet dye (D^t) and the ground state dye, which produces another transient species (I) consisting of a half-oxidized and a half-reduced dye. Further, both D^t and I are presumed to be deactivated by the ground state dye (D) and the resultant leuco dye (P), the processes being controlled by diffusion.

The above scheme can be written schematically as follows:



Scheme A

From the above scheme, one can easily derive the following equation by means of the steady state method:

$$-\frac{\mathrm{d}C}{\mathrm{d}t}=$$

$$\frac{\alpha k_r k_r' C^2}{\left\{\begin{matrix}\{k_d+k_1{}^s C+k_2(C_0-C)\}\times \\ \{k_d'+k_r'+k_1'C+k_2'(C_0-C)\}\end{matrix}\right\}} \qquad (2)$$

where α, the rate constant for the production of the triplet dye, can be expresse alternatively by $\alpha=\tilde{\varepsilon}\phi_{s-t}I_0$, and where $k_1{}^s$ is (k_1+k_r). Putting $k_1{}^{s(\prime)}=k_2'$ and $k_d(k_d'+k_r')\approx 0$, one gets:

$$-\frac{\mathrm{d}C}{\mathrm{d}t}=$$

$$\frac{\alpha k_r k_r' C^2}{(k_d+k_2C_0)(k_d'+k_r'+k_2'C_0)}\equiv kC^2 \qquad (2')$$

$$k=\frac{\alpha k_r k_r'}{\{(k_d k_2'+k_2 k_d'+k_2 k_r')+k_2 k_2' C_0\}C_0} \qquad (3)$$

This is just the same form as experimental relation 1. From relation 3:

$$\frac{1}{kC_0}=\frac{(k_d'k_2+k_2k_r'+k_2'k_d)}{\alpha k_r k_r'}+\frac{k_2k_2'C_0}{\alpha k_r k_r'} \qquad (4)$$

Alternatively, by multipling $I_0\tilde{\varepsilon}$*:

$$\frac{1}{\Phi}=\frac{k_d'k_2+k_2k_r'+k_2'k_d}{\phi_{s-t}\ k_r k_r'}+\frac{k_2k_2'C_0}{\phi_{s-t}k_r k_r'} \qquad (4')$$

The values of the slopes and the intercepts of the plots in Figs. 5b and 5c are given in Table VI. From these one may discuss the rate constant of each elementary reaction to some extent.

TABLE. VI. THE CONCENTRATION EFFECT OF DYE

	Methylene blue	Thionine
S_k (l. mol^{-1} sec.)	1.68×10^8	3.36×10^8
S'_Φ (l. mol^{-1})	$1.1\ \times10^8$	$2.3\ \times10^8$
S_Φ (l. mol^{-1})	$\sim1\times10^8$	$2.1\ \times10^8$
I_k (sec.)	~10	$4.5\ \times10^2$
I'_Φ, I_Φ	~6.7	$1.2\ \times10^3$
S_k/I_k	$\sim10^7$	$7.5\ \times10^5$
S_Φ/I_Φ	$(\sim10^7)$	$2\ \times10^5$

i) The slopes; S_k, S_Φ and S_Φ', and the intercepts, I_k, I_Φ and I_Φ', of each line in Figs. 5b and 5c refer to the same experimental data.

ii) For methylene blue, the values of the intercepts are too small to be treated quantitatively.

If one tentatively assumes $k_2\approx k_2'=10^{10}-10^9$ (l. mol^{-1} sec^{-1}), which is the value for a diffusion controlled process, then for thionine $k_d+k_d'+k_r'=1.3\times10^4$ (sec^{-1})** and $\phi_{s-t}k_r k_r'=5\times10^{11}$ (l. mol^{-1} sec^{-2}), and for methylene blue, $k_d+k_d'+k_r'\approx10^3$ (sec^{-1}) $\phi_{s-t}k_r k_r'\sim1\times10^{12}$ (l. mol^{-1} sec^{-2}). Inserting $\phi_{s-t}\approx10^{-1}$ as a plausible value, the $k_r k_r'$ values for thionine and methylene blue are, respectively, 5×10^{12} and 10^{13}. Since the k_r' values for thionine and methylene blue are, respectively, less than 10^4 and $\sim10^3$, the lowest limits of the k_r value for the two dyes are, respectively, 10^9 and $\sim10^{10}$. These data conform well with the fact that in the flash experiment a rather large quantity of the intermediate I is produced and that its life is of the order of a millisecond. They also seem to be consistent with the results obtained in the photoreduction of thiazine dyes in the phosphate buffer solution.5) So far, Scheme A is quite satisfactory.

Next, the effect of leuco dye on the reaction rate will be discussed. According to Scheme A, the following rate formula should hold for the case in which a definite quantity of leuco dye is added beforehand:

$$-\frac{\mathrm{d}C}{\mathrm{d}t}=k_LC^2$$

$$=\frac{\alpha k_r k_r' C^2}{\left\{\begin{matrix}(k_dk_2'+k_d'k_2+k_2k'_r)\{[\mathrm{LTh}]_0+[\mathrm{Th}]_0\} \\ +k_2k_2'\{[\mathrm{LTh}]_0+[\mathrm{Th}]_0\}^2\end{matrix}\right\}}$$

For the solution to which no leuco dye is added:

$$-\frac{\mathrm{d}C}{\mathrm{d}t}=k_0C^2$$

$$=\frac{\alpha k_r k_r' C^2}{(k_d+k_2[\mathrm{Th}]_0)(k_d'+k_r'+k_2'[\mathrm{Th}]_0)}$$

where $[\mathrm{LTh}]_0$ and $[\mathrm{Th}]_0$ are, respectively, the initial concentrations of leuco thionine and thionine. Using the value $k_2k_2'/(k_2k_d'+k_2'k_d+k_2k_r')=7.5\times10^5$, k_L can easily be calculated from k_0. The values thus obtained are compared with the observed values in the following table:

Exp. No. in Table III	$(k_L/k_0)_{\mathrm{obs}}$	$(k_L/k_0)_{\mathrm{calcd}}$
ii	0.86	0.22
iii	0.55	0.10
iv	0.03	0.0009

Since the observed effect is much smaller than the calculated one, one can not say that the effect of the addition of the leuco dye is consistent with the proposed scheme. In connection with this discrepancy, it should be noted that a relation quite similar to that of

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* $\tilde{\alpha}$ is the average molecular absorption coefficient defined by the relation:

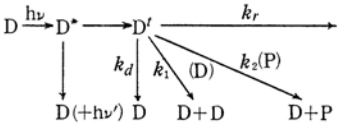
$$I_{\mathrm{ab}}=I_0\ (1-\mathrm{e}^{-\tilde{\alpha}cd})$$

and $\tilde{\alpha}$ is $2.303\,\tilde{\varepsilon}$.

From the measurement of I_0 and I_{ab} by means of a thermopile, the values of $\tilde{\varepsilon}$ were determined for methylene blue and thionine to be 3×10^4 and 2.1×10^4 respectively. In view of the magnitude of $\tilde{\alpha}$, one may safely use the approximation, $I_{\mathrm{ab}}=I_0\tilde{\varepsilon}C_0$, when C_0 is less than $\sim7\times10^{-6}$ M. The $I_0\tilde{\varepsilon}$ values used for MB and Th were 0.67 and 0.71 respectively.

** According to Parker, the k_d value of thionine in an aqueous acidic solution is 5×10^4 sec^{-1} (C. A. Parker, *J. Phys. Chem.*, **63**, 26 (1959)).

4 can be derived even when the reaction scheme is such that the primary process consists of the attack of an alcohol molecule on D^t. Thus, if one adopts the following scheme:



Scheme B

then:

$$-\frac{dC}{dt}=\frac{I_{ab}\phi_{s-t}k_r}{k_d+k_r+k_1C_0}$$

on the assumption that $k_1 \approx k_2$.

Hence,

$$\frac{1}{\Phi}=\frac{k_d+k_r}{\phi_{s-t}k_r}+\frac{k_1C_0}{k_r\phi_{s-t}} \qquad (6)$$

This is formally the same as 4′. In other words, the values for S_Φ and I_Φ experimentally obtained according to Scheme B are written as follows:

$$S_\Phi=k_1/k_r\phi_{s-t} \quad I_\Phi=(k_r+k_d)/k_r\phi_{s-t}$$

Inserting the proper values, one gets, for thionine, $k_1/(k_d+k_r)=2\times10^5$ and $k_r\phi_{s-t}=10^2$. If one assumes that ϕ_{s-t} for thionine is of the 10^{-1} order, k_r is $\sim10^3\ \mathrm{sec}^{-1}$.

The effect on k of the addition of leuco thionine should be as follows, according to Scheme B:

Exp. No. in Table III	$(k_L'/k_0)_{\mathrm{calcd}}$
ii	0.51
iii	0.42
iv	0.033

Thus, as far as the effect of leuco dye is concerned, Scheme B seems to be more satisfactory than A.

However, the value for k_r on the basis of Scheme B is unreasonable if one takes the oxygen effect into consideration (the result given in Fig. 9). The reason is as follows.

A rapid decrease in the bleaching caused by a small concentration of oxygen is interpreted by the competition of D^t+O_2 (mainly deactivation) and $D^t+D\rightarrow$ according to Scheme A, while only the latter process needs to be replaced by $D^t+\mathrm{alc}\rightarrow$ for Scheme B. In either case, it is very plausible to assume that the deactivation of D^t by oxygen is an encounter-rate-determining process. If so, the effect of oxygen should become appreciable at the oxygen concentration given by the following relation:

$$\text{for Scheme A} \qquad k_r[\mathrm{D}]\approx k_{\mathrm{diff}}[\mathrm{O_2}] \qquad (7)$$

$$\text{for Scheme B} \qquad k_r\approx k_{\mathrm{diff}}[\mathrm{O_2}] \qquad (8)$$

The k_r values in Eqs. 7 and 8 have the meanings given in Scheme diagrams A and B, and k_{diff} is the rate constant for the diffusion controlled process and is of the order of 10^9—10^{10} l. mol^{-1} sec^{-1}.

For Scheme B, the value of k_r, $\sim10^3\ \mathrm{sec}^{-1}$ requires that the concentration of oxygen in question be 10^{-6}—10^{-7} M. As Fig. 9 shows, however, the real concentration of oxygen necessary to compete with the reductive bleaching is more than $\sim10^{-5}$ M, a value much larger than the above value. In addition, the fact that a reversible photoreduction takes place to a measurable extent, even in an air-saturated solution, is quite difficult to understand when the above line of reasoning is followed.

For Scheme A, there is no such inconsistency if the value of k_r is large enough; this is plausible, as has already been shown.

Summing up all the results, one cannot decide conclusively whether Scheme A or Scheme B predominates. Yet, Scheme A seems more plausible on the basis of the various results so far obtained. The inconsistency between the second order rate formula and the effect of the leuco dye is the only question which needs to be further studied.

It is to be hoped that more extensive studies of the dye concentration effect on the rates for the degassed solution and the aerated solution will give a more definite conclusion.

Lastly, let us add a brief discussion of the results obtained for a mixed solvent. If the attack of the water molecule and the alcohol molecule on I (in the case of Scheme A) or on D^t (in the case of Scheme B) occurs according to the mass-action law, the rate would be given by:

$$\mathrm{Rate}=\mathrm{const.}\times\frac{k^r{}_{\mathrm{w}}[\mathrm{H_2O}]+k^r{}_{\mathrm{alc}}[\mathrm{C_2H_5OH}]}{k^s{}_{\mathrm{w}}[\mathrm{H_2O}]+k^s{}_{\mathrm{alc}}[\mathrm{C_2H_5OH}]}$$

where k^r and k^s refer, respectively, to the rate constant of the genuine reaction of I or D^t as the case may be, and the rate constant for the disappearance (the sum of the deactivation and the genuine reaction of I or D^t). If $k^s{}_{\mathrm{w}}=k^s{}_{\mathrm{alc}}$ holds approximately, then the expected rate formula would result.

Summary

The photochemical behavior of methylene blue and thionine in ethanol is quite different from that of eosine in the same solvent. They are compared from several viewpoints below.

From the preliminary flash experiment and the kinetic results by steady illumination, it has been concluded that interaction between

	Methylene blue	Eosine
% of leuco dye	About 100% leuco	Above 50% leuco
Quantum yield	$1-3\times10^{-3}$	5.6×10^{-4}
Air introduction	Complete recovery	No recovery
Intensity (I_0) effect	Proportional to I_0	Proportional to I_0
Rate formula	Second order in dye concentration	Proportional to the absorption of light
O_2 effect	Very complex oxidative and reductive bleaching simultaneously occur	The reaction is completely suppressed by a trace of O_2.

the ground state and the excited state dye is essential for photoreduction.

It is most probable that the overall scheme for the photoreduction of thiazine dyes in alcohol is similar to that in the case of the same dyes in a phosphate buffer solution.

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