Photochemical Reactions between Methylene Blue and Tri-, Di- and Monomethylamine. III. The Behavior of Methylene Blue in the Presence of Oxygen

By Hiroshi OBATA, Kenichi KOGASAKA and Masao KOIZUMI

(Received August 11, 1958)

In the preceding papers[1)], the photochemical reactions between methylene blue and three kinds of amine in vacuo were investigated, and the following scheme was proposed.

$$MB^+ + OH^- + Am \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Intermediate} \xrightarrow{k_3} MBH + AmO \qquad (1)$$

Thus, methylene blue when irradiated, reacts with hydroxyl ions and amine and produces a certain type of intermediate with a life time of a few minutes, a part of the intermediate then transforming into leucodye and amine oxide. Now according to a recent paper of Oster[2)], methylene blue is photobleached by various kinds of secondary and tertiary amine including ethylenediaminetetraacetic acid, and the reaction which is somewhat similar to ours, was interpreted to take place via the triplet state of dye ion on the ground that the fluorescence of methylene blue is not affected by the added amines. His conclusion will certainly apply to our case, since we have afterwards ascertained that the fluorescence of methylene blue in aqueous solution is practically the same as that of the solution used for the photochemical reaction, both in intensity and in structure.

The objective of the present paper is to elucidate the following three kinetic features which are considered to be important in relation to the above reaction.

(1) *The decision of a species which reacts with oxygen, when the aerobic solution of methylene blue containing amine is irradiated.*—In the studies of photosensitization by methylene blue, Weil[3)] observed that amine is oxidized but methylene blue does not practically bleach, and we have also ascertained this fact under our experimental conditions. As to the regeneration of methylene blue in such cases, it seems generally considered especially by biochemists, that the attack of oxygen on leucodye reproduces the original dye. But on the basis of our scheme 1, the possibility of oxygen attack on the intermediate can not be excluded a priori, especially in view of our result that cupric ions react with the intermediate very effectively. The problem must therefore be decided experimentally and for this purpose, a quantitative comparison of the rate of photobleaching in vacuo and that of oxygen absorption by the same solution was undertaken.

(2) *The reactivity of oxygen with a triplet state (T state) of methylene blue.*—Generally, triplet states of dye ions are easily attacked by oxygen[4)] as exemplified by the case of eosine studied by Imamura and Koizumi[5)]. In the photobleaching of the aqueous solution of eosine under aerobic condition, the reaction takes place chiefly via T state, but most part of the intermediate in the T state is deactivated by oxygen and only a very small fraction of it interacts with oxygen in such an effective way as to lead a bleaching reaction. On this view, the question is raised whether the T state of methylene blue, in the presence of amine, is not attacked altogether by oxygen or alternatively, although attacked by oxygen it is only deprived of its excitation energy, no genuine chemical reaction occurring. This problem is intimately connected with the first one. As a help for the elucidation of this problem, the photobleaching reaction of methylene blue in the aqueous

---

1) H. Obata and M. Koizumi, This Bulletin, **30**, 136, 142 (1957).

2) G. Oster and N. Wotherspoon, *J. Am. Chem. Soc.*, **79**, 4836 (1957).

3) L. Weil and J. Maher, *Arch. Biochem.*, **29**, 241 (1950).

4) e. g. G. O. Schenck, *Z. Elektrochem.*, **55**, 505 (1951); **56**, 855 (1952); **57**, 675 (1953); *Naturwiss.*, **40**, 205, 229 (1953).

5) M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955); **29**, 913 (1956).

solution involving no amine was investigated. It turned out that the rate is somewhat slower than that of eosine under similar conditions, and in relation to the above mentioned question, further investigations were undertaken to make clear of the effect of pH and oxygen pressure on the photobleaching rate.

3) *The behavior of T state of methylene blue in the presence of amine and oxygen.*— A quantitative comparison was made between the rate of photobleaching of methylene blue in vacuo by virtue of trimethylamine and that of oxygen absorption by the same system when irradiated, taking into account of the rate with which the aqueous solution of methylene blue is photobleached by oxygen. A conclusion is that T state of methylene blue is very efficiently deactivated by oxygen in the absence of amine, but such a process is practically suppressed by the presence of amine, oxygen only reacting with leucodye.

Experimental and Results

I. Measurement of the rate of oxygen absorption.—*a) Apparatus and Procedure.*—For our purpose, the rate of oxygen absorption must be compared with that of photobleaching under as much the same condition as possible. This requirement imposes some severe conditions on the apparatus, and as a result of examination of various methods from this viewpoint, it was decided to adopt Tödt's electrochemical method⁶⁾.

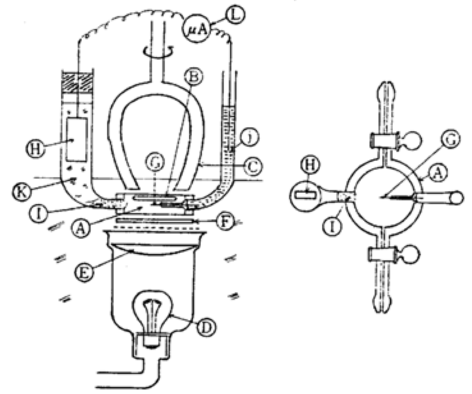


Fig. 1. Apparatus for the measurement of oxygen absorption

A	reaction vessel
B	magnetic stirrer
C	magnet
D	light source (automobile head-light lamp 35 w/35 w)
E	condensor lens to prepare parallel light
F	filter to cut off below 600 mμ
G	cathode (platinum wire)
H	anode (iron)
I	KCl-agar bridge
J	mercury
K	0.1 N KCl
L	microammeter or galvanometer

The apparatus constructed is shown in Fig. 1. In this figure, A is a reaction vessel with an internal diameter of 28 mm. and a depth of 10 mm., B is a magnetic stirrer, a magnet C being rotated by a phonomotor 80 r. p. m. The cell is irradiated by an automobile head-light lamp 35 w/35 w (D) placed below the cell, E is a condensor lens to prepare parallel light and F is a filter which cuts off below 600 mμ. The intensity of illuminating light is approximately the same as that of the preceding papers¹⁾. A cathode G., which is polarizable, is a platinum wire 0.3 mm. diameter and 4 mm. length and an anode H, which is unpolarizable, is a piece of iron 30 mm.×10 mm.×4 mm. Tödt's anode contains 2% carbon and 0.6% copper and according to him, the iron rich in carbon and copper generally gives a better stability. Our anode contains 0.18% carbon and 0.98% copper, the specimen being supplied from Osaka Special Steel Ltd. The anode is dipped in 0.1 N potassium (K) solution and is connected to a cell via a potassium chloride-agar bridge I. As shown by Tödt, the amount of current supplied by this cell is determined by the diffusion of oxygen in the neighborhood of platinum electrode, and is given by the following relation after the establishment of a steady state.

$$i = n\boldsymbol{F}Dqc/\delta$$

(n=4, $\boldsymbol{F}$: Faraday, D: diffusion constant, q: area of the cathode surface, c: oxygen concentration in the bulk solution, δ: thickness of diffusion layer).

In our experiment the speed of rotation of the magnet is slower than that in Tödt's experiment, hence the diffusion current is very dependent on the rotating speed and particular caution must be paid to keep constant the speed of rotation. Another thing to be added is that a platinum electrode needs to be dipped in hydrochloric acid to make its surface clean before use. Perhaps a platinum electrode, when exposed in the air, would have its surface covered with a thin film of oxide or the like, which would reduce the current.

b) Calibration.—Calibration between the diffusion current and the concentration of dissolved oxygen was made using the same solution for the absorption experiment, the composition being 2.0×10^{-5} M methylene blue, 0.1 N potassium chloride and pH 9.2 controlled by borate buffer. Firstly, the air mixed with a certain amount of nitrogen gas was passed through the solution immersed in a thermostat at 40°C, and the cell was filled quickly with this solution without any gaseous phase left behind. The current was measured in 30°C thermostat by a microammeter

---

6) F. Tödt, *Z. Elektrochem.*, **54**, 485 (1950); F. Tödt et al., *Biochem. Z.*, **323**, 192 (1952); **325**, 210 (1954); F. Tödt, D. Burk and O. Warburg, *Biochim. et Biophys. Acta*, **12**, 347 (1953).

in higher oxygen concentration or a galvanometer in lower oxygen concentration, while the concentration of oxygen was analyzed by Winkler's method⁷⁾ using the remaining solution. Winkler's method, however, when applied directly to the above solution did not give correct results owing to some difficulty in iodometry. Hence the dissolved oxygen was excluded by a flow of nitrogen gas and then redissolved in the mixed solution of manganese chloride and sodium hydroxide which was analyzed by the same method. Between the current and the concentration of oxygen, a good linear relationship holds, as shown in Fig. 2.

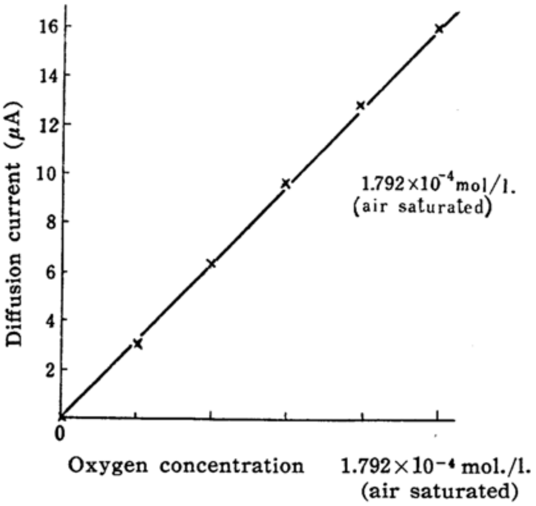


Fig. 2. Calibration curve between the diffusion current and the concentration of oxygen.

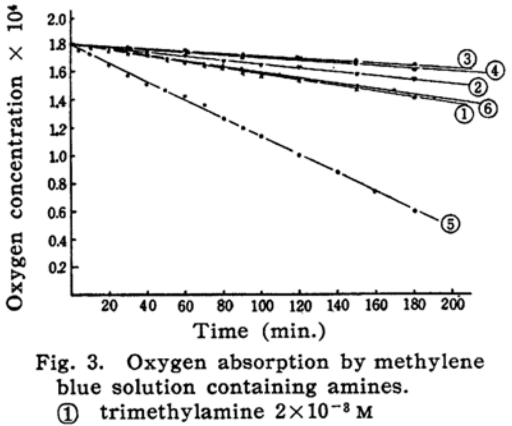


Fig. 3. Oxygen absorption by methylene blue solution containing amines.

① trimethylamine 2×10^{-3} M
② 〃 1.5×10^{-3} M
③ 〃 1.0 〃
④ 〃 0.6 〃
⑤ dimethylamine 4×10^{-2} M
⑥ monomethylamine 〃

c) Results. — The composition of the solutions employed were usually 2×10^{-5}M methylene blue, 0.1 N potassium chloride pH 9.2 controlled by borate buffer, the amount of trimethylamine being varied from 0.6×10^{-3}M to 2.0×10^{-3}M. In cases of dimethylamine and monomethylamine, the concentration was raised to 4.0×10^{-2}M. A few results obtained for the relation between the concentration of oxygen and time are shown in Fig. 3. As seen from the figure, a linearity holds in all cases. But for a later stage (below about 3×10^{-5}M of oxygen), not shown in the figure, an abnormally rapid decrease of oxygen is observed in cases of trimethyl and monomethylamine, but not in dimethylamine. The origin of the above phenomena is not known at the present stage. It may perhaps be related to the electrode process and different kinds of behavior may partly be due to the mechanism of each reaction in this region. For our present purpose, however, only those regions of the normal behavior were employed and the rate of oxygen absorption was evaluated from its inclination.

The results are summarized in Table I. In this table two kinds of calculated value were obtained as follows.

Calculation A. If one assumes that leucodye, when formed, reacts very rapidly with oxygen according to the following stoichiometric relation

$$\mathrm{MBH}+\frac{1}{2}\mathrm{O_2} \longrightarrow \mathrm{MB^+}+\mathrm{OH^-}{}^{*}$$

then, the rate of oxygen consumption will be given by

$$-\frac{d[\mathrm{O_2}]}{dt}=\frac{1}{2}\,\frac{k_1k_3}{k_1+k_2+k_3}[\mathrm{MB}]_0$$

where $[\mathrm{MB}]_0$ is the analytical concentration of methylene blue.

Figures given under the heading Cal. A are the values by the above formula.

Calculation B. If one assumes that as soon as the intermediate is produced, it is attacked by oxygen immediately according to the following stoichiometric relation,

$$(\mathrm{MB\cdots HO\cdots Am})+\frac{1}{2}\mathrm{O_2} \longrightarrow \mathrm{MB^+}+\mathrm{OH^-}+\mathrm{AmO}$$

then the rate of oxygen absorption will be given by

---

7) L. W. Winkler, *Ber.*, **21**, 2843 (1888); F.P. Treadwell and W. T. Hall, "Analytical Chemistry", vol 2. John Wiley of Sons, New York (1942) p. 704.

* In view of Weil's results that hydrogen peroxide was not detected by an ordinary method though perceptible by pyruvic acid method, the overall reaction in the main would be such as written above.

TABLE I

Methylene blue (MB) 2.0×10^{-5}M
pH=9.2, Temp. 30°C

[TA]	[TA]/[MB]	k_1	k_2	k_3	$-\frac{d[O_2]}{dt}$		
					Cal. A	Cal. B	obs.
2.0×10^{-3} M	100	0.109	0.055	0.039	2.07×10^{-7}	1.09×10^{-6}	1.98×10^{-7}
1.5 〃	75	0.057	0.028	0.026	1.373 〃	5.7×10^{-7}	1.42 〃
1.0 〃	50	0.052	0.044	0.027	1.122 〃	5.2 〃	0.9 〃
0.6 〃	30	0.039	0.025	0.024	1.018 〃	3.9 〃	0.96 〃
*1.5 〃	75	0.325	0.216	0.170	7.75 〃	3.25×10^{-6}	5.69 〃
**2.0 〃	100	0.202	0.127	0.071	3.06 〃	1.72 〃	2.73 〃

* pH=10.0
** pH=9.2, temp. 40°C and MB=1.7×10^{-5}

[DA]	[DA]/[MB]	k_1	k_2	k_3	Cal. A	Cal. B	obsd.
4.0×10^{-2}	2000	0.052	0.060	0.041	1.392×10^{-7}	5.2×10^{-7}	5.73×10^{-7}

[MA]	[MA]/[MB]	k_1	k_2	k_3	Cal. A	Cal. B	
4.0×10^{-2}	2000	0.052	0.049	0.035	1.334×10^{-7}	5.2×10^{-7}	2.0×10^{-7}

$$-\frac{d[O_2]}{dt}=\frac{1}{2}k_1[MB]_0$$

Figures given under the heading Cal. B are the values obtained by this formula. k_1, k_2 and k_3 are the constants evaluated from the analysis of the bleaching of the dye ion in vacuo in the same solution used for the oxygen absorption experiment.

II. The photobleaching of the aqueous solution of methylene blue by dint of dissolved oxygen.—*a*) *Apparatus and procedure.* — The experiment was done in a similar way to that described in Imamura and Koizumi's paper[5]. The concentration of dye was usually 1.6×10^{-5}M. The rate of photobleaching was calculated from the fall of the maximum absorbance. In certain cases the position of maximum absorption showed a slight shift toward a shorter wave length giving rise to some ambiguity to the analysis, although the initial rate generally adopted is quite reliable. The data can be analyzed by Imamura and Koizumi's method and the relation

$$\{\ln(1-e^{-\alpha c_0 d})+\alpha c_0 d)\}-\{\ln(1-e^{-\alpha c d})+\alpha c d\}$$
$$=k't \qquad (2)$$

can express the experimental results satisfactorily. Thus in almost all cases the plot of $\{\ln(1-e^{-D})+D\}$ (D, optical density) against t gives a straight line and from its inclination the value of k' is evaluated, k' is related with the quantum yield k by the following equation

$$k'=\alpha dI_0k \qquad (3)$$

in which α is not a genuine extinction coefficient but is to be considered rather a kind of experimental parameter. By an experiment employing a thermopile, k was determined for a certain solution and the proportionality constant between k and k' thus obtained was utilized for calculating k from k' for all the other solutions.

b) *Effect of oxygen pressure on the bleaching rate of the aqueous methylene blue solution.*—The aqueous solution of methylene blue was found to bleach gradually when irradiated, but if evacuated there occurs no bleaching perceptible. Thereupon, in order to examine the effect of oxygen concentration on the rate, the experiments were done with the solutions which had been previously brought to equilibrium with the air of various pressures. The concentration of the dissolved oxygen was calculated by use of Ostwald's absorption coefficient of oxygen in water neglecting the effect of a small quantity of dye. The results obtained are summarized in Table II and a plot of $1/k$ against the reciprocal of the oxygen concentration is given in Fig. 4, which shows a good straight line.

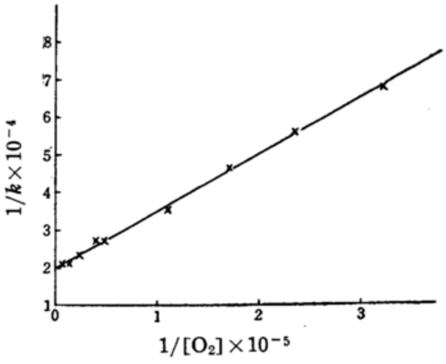


Fig. 4. Plot of $1/k$ against the reciprocal of the oxygen concentration.

TABLE II

Concentration of oxygen mole/l.	k'	k
2.5×10^{-4}	0.0240	4.63×10^{-5}
1.07×10^{-4}	0.0247	4.75×10^{-5}
4.95×10^{-5}	0.0239	4.61×10^{-5}
4.85×10^{-5}	0.0225	4.34×10^{-5}
2.38×10^{-5}	0.0174	3.36×10^{-5}
2.15×10^{-5}	0.0180	3.48×10^{-5}
9.1×10^{-6}	0.0143	2.76×10^{-5}
5.76×10^{-6}	0.0117	2.26×10^{-5}
4.25×10^{-6}	0.00912	1.76×10^{-5}
3.13×10^{-6}	0.00766	1.48×10^{-5}
1.16×10^{-6}	0.00288	5.55×10^{-6}

As the reaction goes via T state, k is to be related with the constants of various steps by the same formula already applied to the case of eosine.

$$k=\varphi_{S\to T}\frac{k^{I}_{D_2}[O_2]}{k^{IS}_{O_2}[O_2]+k^{I}_{H_2O}[H_2O]}\times\frac{k^{II}_{H_2O}}{k^{IIS}_{H_2O}}$$

$$\text{or}\quad \frac{1}{k}=\frac{1}{\varphi_{S\to T}}\frac{k^{IS}_{O_2}}{k^{I}_{O_2}}\frac{k^{IIS}_{H_2O}}{k^{II}_{H_2O}}+\frac{1}{\varphi_{S\to T}}\frac{k^{I}_{H_2O}[H_2O]}{k^{I}_{O_2}[O_2]}\frac{k^{IIS}_{H_2O}}{k^{II}_{H_2O}}$$

where $\varphi_{S\to T}$ means transition probability for the suffixes I, II refer respectively to the first and second step and the suffix S means the sum of all the reactions of the related species. Other symbols are evident. From the inclination and the intercept of the plot in Fig. 4,

$$\frac{1}{\varphi_{S\to T}}\times(k^{I}_{H_2O}[H_2O]/k^{I}_{O_2})(k^{IIS}_{H_2O}/k^{II}_{H_2O})=1.55\times10^{-1}\tag{4}$$

$$\frac{1}{\varphi_{S\to T}}\times(k^{IS}_{O_2}/k^{I}_{O_2})(k^{IIS}_{H_2O}/k^{II}_{H_2O})=2.0\times10^{4}$$

$$\text{or}\quad \varphi_{S\to T}(k^{I}_{O_2}/k^{IS}_{O_2})(k^{II}_{H_2O}/k^{IIS}_{H_2O})=5\times10^{-5}\tag{5}$$

From 4 and 5

$$k^{I}_{H_2O}[H_2O]/k^{IS}_{O_2}=7.75\times10^{-6}\tag{6}$$

and putting $[H_2O]=55.5$ M

$$k^{I}_{H_2O}/k^{IS}_{O_2}=1.4\times10^{-7}$$

For eosine the above quantities have already been evaluated as follows[8]

$$\varphi_{S\to T}(k^{I}_{O_2}/k^{IS}_{O_2})(k^{II}_{H_2O}/k^{IIS}_{H_2O})=2.8\times10^{-4}$$

$$k^{I}_{H_2O}/k^{IS}_{O_2}=1.3\times10^{-8}$$

Comparing the two sets of values, it may be said that the reactivity (deactivation + genuine reaction) of T state of methylene blue against oxygen is about 1/10 of that of eosine.

c) The effect of pH on the bleaching rate. —The pH effect on the photobleaching was examined only for the solution saturated with air. It is to be noted that absorption spectra of methylene blue change above and below a certain pH value and especially in the strong alkaline region, the dye seems to be quite unstable even in the dark. Hence the effect was examined only in the region where absorption spectra are not affected or affected little. In most experiments the pH values were controlled by the veronal-sodium acetate-hydrochloric acid buffer. All the data could be analyzed by 2, a linearity between $\{\ln(1-e^{-D})+D\}$ and t holding exactly except for a single case of pH 8.59 (the most alkaline solution).

The results are summarized in Table III and are shown in Fig. 5.

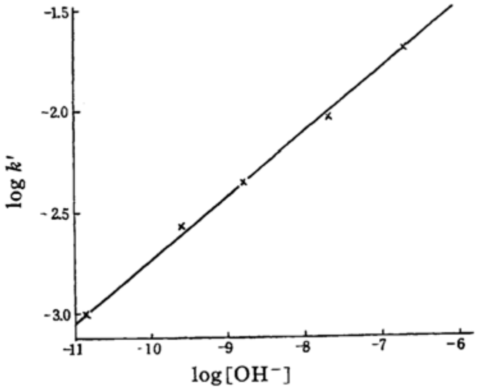


Fig. 5. Relation between $\log k'$ and $\log[OH^-]$.

TABLE III

buffered solution		non-buffered solution	
pH	k'		k'
3.35	0.001	10^{-3}N HCl	0.0019
4.40	0.0027	*pure water	0.024
5.20	0.0043	10^{-3}N NaOH	0.062
6.34	0.0086		
7.30	0.0196		
8.59	0.023		

* pH of the aqueous solution MB $1.6\times10^{-}$ M was measured by pH meter to be ca. 6.8.

From this figure, it can be said that k is approximately proportional to $[OH^-]^{1/3}$ for the buffered solution and the results for the non-buffered solution are somewhat higher at pH<7.0.

---

[8] M. Imamura, This Bulletin, **30**, 249 (1957).

Discussion

Firstly, the discussion will be given about a species which is attacked by oxygen in the amine added solution of methylene blue. The solution of methylene blue containing trimethylamine does not practically bleach in spite of the presence of oxygen and this fact conforms well with the result that the quantum yield of amine induced photobleaching (reversible photoreduction) in vacuo ($10^{-3}\sim10^{-2}$) is quite larger than that of the oxygen induced photobleaching (irreversible photoöxidation) of the aqueous methylene blue solution ($\sim4.6\times10^{-5}$)***. From the above results the possibility that oxygen attacks T state decomposing methylene blue molecules can be excluded in the amine added solution. And whether oxygen attacks intermediate or leucodye, the rate must be so fast that as soon as the intermediate (or the leucodye) is formed, the consumption of oxygen must immediately take place, because the rate of oxygen absorption is the zeroth order in oxygen concentration. However, there is one thing to be taken into consideration. That is the possibility of deactivation of T state by oxygen. From the results of the irreversible photoöxidation of the aqueous solution, although the value of quantum yield is quite small

$$\varphi_{S\to T}(k^{I}_{O_2}/k^{IS}_{O_2})(k^{II}_{H_2O}/k^{IIS}_{H_2O})=k=5\times10^{-5}$$

the rate constant $k^{IS}_{O_2}$ (for the whole process consisting of deactivation and genuine reaction) is quite larger than $k^{I}_{H_2O}[H_2O]$ ($k^{IS}_{O_2}/k^{I}_{H_2O}[H_2O]=1.3\times10^{5}$). Therefore, there is no reason to deny a priori the posibility of such a deactivation in the solution of methylene blue containing amine. But if this should occur in about the same order as in the aqueous methylene blue solution, the rate of oxygen consumption ought to be exceedingly smaller than the calculated value in Table II. The agreement between the calculated and the observed value in the order of magnitude definitely denies such a possibility, and for the case of trimethylamine, the close agreement between the observed and the calculated value (Cal. A) strongly supports the view that oxygen attacks only on leucodye and not on intermediate. Thus it may be concluded that the behavior of T state toward oxygen is essentially different in two solutions with and without amine. For the solution containing amine, such a view as that competitive reactions of T state with oxygen on the one hand and with amine and OH^- on the other take place, the latter greatly predominating over the former, can not be adopted. Rather, the interpretation that oxygen in the solution containing amine loses the power of deactivating T state or alternatively loses a chance of effective encounter with T state, seems to fit experimental results. This interpretation is further supported from the following argument about the different features of the kinetics already established in the reversible photoreduction and the irreversible photoöxidation. On the basis of a broad outline of the reaction scheme proposed in the previous paper¹⁾, let us asume tentatively a more detailed mechanism which of course must await further studies to confirm it. One plausible scheme would be such as the following

$$MB^{+}+h\nu\longrightarrow MB^{+*}\longrightarrow MB^{+t}$$

$$\begin{tikzcd}
MB^{+t} \arrow[r, "\overrightarrow{k}^{I}_{q}"] \arrow[d, shift right, "\overrightarrow{k}^{I}[OH^-]"'] & MB^{+} & \\
MB\cdots OH \arrow[u, shift right, "\overleftarrow{k}^{II}"'] \arrow[rr, "\overrightarrow{k}^{II}[Am]"] & & MB\cdots HO\cdots Am \arrow[d, "k_2"] \\
& & {}
\end{tikzcd}$$

$$\xrightarrow{k_3}\text{Product}$$

Applying the stationary state condition to T state and $MB\cdots OH$, k_1 in 1 can be expressed as follows in the above scheme

$$k_1\sim\frac{I_{ab}\varphi_{S\to T}\overrightarrow{k}^{I}\overrightarrow{k}^{II}[OH^-][Am]}{\overrightarrow{k}^{I}_{q}\overleftarrow{k}^{II}+\overrightarrow{k}^{I}[OH^-]\overrightarrow{k}^{II}[Am]+\overrightarrow{k}^{I}_{q}\overrightarrow{k}^{II}[Am]}$$

Although somewhat different relations may result from different mechanisms, the essential point would not be affected. It is that the relation experimentally found, $k_1\propto[OH^-][Am]$ requires a far larger magnitude of $\overrightarrow{k}^{I}_{q}\overleftarrow{k}^{II}$ term or the like compared with other terms involved in the denominator. Thus it is a natural expectation that the reaction of T state with OH^- and amine leading to an intermediate is slower than the deactivation or quenching process of T state by water molecules or itself.

The above situations are in sharp contrast with those of the solution without amine, in which T state is deactivated by

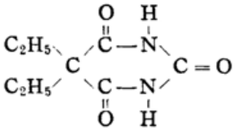
---

[***] To avoid confusion let us designate the two reactions as reversible photoreduction and irreversible photooxidation hereafter.

O_2 far more rapidly than water. Thus the different kinds of behavior in two cases are comprehensive only from the viewpoint mentioned above.

Lastly it is to be added that for the case of monomethylamine, the observed rate of oxygen absorption coincides approximately with the values calculated by A method while it lies near to the values calculated by B method for the case of dimethylamine. But in view of few data and their accuracy being not very good, further studies will be necessary for a decisive conclusion except the case of trimethylamine for which the statement that oxygen attacks leucodye may be beyond doubt.

Next the retarding effect of veronal upon the irreversible photoöxidation may be taken up.



A structural formula of veronal contains two secondary amino nitrogens, and in view of the results reported by Oster[2)], it is a natural expectation that another reaction competing with the irreversible photoöxidation takes place between T state of methylene blue and veronal molecule. To ascertain this conjecture, it was examined whether the evacuated buffered solution displays the photobleaching or not. The result as shown in Fig. 6, is that the photobleaching does really take place and moreover the introduction of oxygen into the bleached solution recovers a greater part of the lost color. Such a bleaching process does not occur in the evacuated dye solution containing 10^{-3}M sodium hydroxide.

Thus, there is no doubt that a reversible photoreduction such as the formation of leucodye is taking place in this case. The retardation of the irreversible photoöxidation caused by the addition of veronal, can be attributed to this competitive reductive reaction, the produced leucodye being recovered by the existent oxygen. Veronal can be said to have amino-like action although to a far lesser extent. It may be worthy of adding here that an enough caution must be paid when the buffer solution is used for such experiments as the present one, since the buffer solutions usually employed often involve such a N-containing compound like veronal.

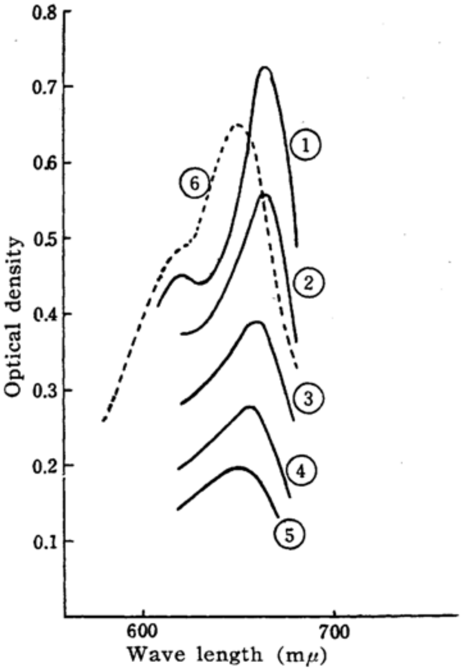


Fig. 6. Photobleaching of the methylene blue solution containing veronal in vacuo.

1 initial
2 after 10 minutes
3 after 20 minutes
4 after 40 minutes
5 after 60 minutes
6 after exposed to the air

Studies on the pH dependence of the rate certainly be essential for the elucidation of a detailed mechanism of the irreversible photoöxidation. But the results obtained in this paper are difficult to interpret because of the complexity introduced by the above circumstances and of the lack of experiments for examining the effect of oxygen concentration upon the rate in various pH solutions. A further more careful and extensive study will be necessary in order to make clear a detailed mechanism of the present reaction.

Summary

In connection with the reversible photoreduction of methylene blue in vacuo published already, the rate of the consumption of oxygen in the irradiated solution of methylene blue containing three kinds of amine was measured. In addition, the irreversible photoöxidation of the aqueous methylene blue solution was

studied, particular attention being paid to the effect of oxygen pressure and pH on the rate. The following conclusions were drawn from the above two series of experiments.

1) In the irradiated aqueous solution of methylene blue containing trimethylamine, oxygen does not affect the T state of dye and attacks only the leuco form, reproducing the dye molecule. Oxygen reacts almost instantaneously with the leucodye as soon as the former is produced.

2) In the aqueous solution of methylene blue (with no amine added) the reactivity of T state against oxygen is about one order less than that of eosine in the analogous condition. The deactivation of T state by oxygen in case of methylene blue is about 10^{-7} times greater than that by water.

3) Comparison of the above results 1 and 2 (taking into account of the result that in the evacuated state the reactivity of T state against OH^- and amine is much less than the deactivation by water) leads to the conclusion that the deactivation of T state by oxygen is almost completely suppressed by the addition of amine.

4) The rate of irreversible photoöxidation increases with the alkalinity of the solution. But in the buffer solution containing N-compound like veronal, a competitive reaction somewhat like that between methylene blue and amine occurs. It is pointed out that enough caution must be taken for such a possibility when the buffer solutions are employed.

A part of this research was done in the Institute of Polytechnics of Osaka City University. The cost of this research has been partially defrayed from the Scientific Research Grant from the Ministry of Education.

Department of Chemistry, Faculty of Science, Tohoku University
Katahira-cho, Sendai