Photoreduction of Methylene Blue by Visible Light in the Aqueous Solution Containing Certain Kinds of Inorganic Salts. I. General Features of the Reaction

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In connection with the study of photoreduction of methylene blue with aliphatic amines, we did some experiments on the irreversible photodecomposition of the dye in the aerated aqueous solution[1]. It was our plan at that time to investigate systematically, varying pH of the solution, about the effect of the oxygen concentration upon the rate of this irreversible photodecomposition. But it happens that we had found the reversible photobleaching to take place with an appreciable rate in the degassed solution containing certain kinds of inorganic salts commonly used for buffering. Here " reversible " means that the dye is recovered when the air is introduced into the solution and the colorless product was confirmed to be the leuco compound of the dye. This fact which we have found seems to be of foundamental importance for the chemistry of dye in general, since the buffered solution which was found to be effective in promoting this novel photoreduction, is usually used for the dye solution. The object of this paper is to report some reults of our experiments undertaken to clarify the general nature of this extraordinary photoreduction which proceeds without any addition of particular reducing agent.

Experimental

Sample. — *Methylene Blue.* — Grübler's dye was recrystallized from *n*-butyl alcohol three times and was dried in vacuum.

$$\varepsilon_{\lambda=665\,\mathrm{m}\mu}=6.0\times10^4$$

Inorganic Reagents.—Sodium borate, sodium monohydrogen phosphate, potassium dihydrogen phosphate, sodium sulfate, sodium chloride, sodium iodide, hydrochloric acid, sodium hydroxide. All of the salts were of special grade and they were recrystallized from the redistilled water 2 or 3 times. Hydrochloric acid of special grade was distilled twice at the azeotropic point. Sodium hydroxide of special grade was used without further purification.

Apparatus and Procedure. — Degassing of the solution was performed in a suitable ampule by repeating freezing and evacuation 5∼7 times. The solution then was poured into a reaction cell which is a spectrophotometer cell $1\times1\times4$ cm., connected to the evacuation apparatus via the tube fused to the upper part of the cell. The reaction was performed in a thermostat of 30 ± 0.5°C. The light source was a 500 W–75 V. projection lamp. It was used at 55.0 ± 0.5 V. and the intensity was checked by a photocell (PT 17 V 2) during the course of the reaction. At various time intervals, the cell was taken out and the absorption spectrum was measured by a Hitachi or a Kotaki spectrophotometer.

Results

1. The Irreversible Bleaching by Oxygen and the Reversible One in the Evacuated Solution.— The feature of the photobleaching of methylene blue in the borate buffer solution containing various amounts of oxygen was examined.

The composition of the solution was as follows; methylene blue 1.2×10^{-5} M, borate, 7.8×10^{-2} M, hydrochloric acid 1.4×10^{-1}M, pH$=7.62$.

In case of the dye solution saturated with air, the irradiation causes a gradual decrease of the absorption spectra of methylene blue without any noticeable change of the maximum position.

When the concentration of oxygen is decreased down to 1.41×10^{-5} M, the bleaching caused by irradiation is still irreversible, the introduction of air not causing any recovery of dye. It was found that the absorption maximum shifts towards violet more and more, when the oxygen concentration is decreased.

When the solution is completely degassed, the bleaching still occurs and with a much greater rate than that of the oxygen-containing solution. However, the shift of the absorption maximum is somewhat less than in the case in which the oxygen concentration is small. The notable fact in this case is that the color appreciably recovers when the air is introduced after the irradiation. The position of the absorption maximum of the aerated solution exactly coincides with that of the partially bleached solution before aeration.

The rate of bleaching is proportional to the quantity of light absorption, that is

$$k't=-\ln(e^{acd}-1)+\text{const.} \qquad (1)$$

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[1] H. Obata, K. Kogasaka and M. Koizumi, This Bulletin, 32, 125 (1959).

except the two cases, i. e., the completely evacuated solution and the one dissolving the least amount of oxygen.

In evaluating the value of k' from Eq. 1, α-value was conventionally chosen as ε-value in order to compare the results with those of the pure aqueous solution[1], and the remaining quantity of methylene blue was obtained by use of Blaisdell's[2] method. The above equation does not hold for the two exceptional cases mentioned above, the rate in these cases decreasing more rapidly than calculated by Eq. 1. In Table I are given the values of k' and the shift of maximum absorption ($\Delta\lambda$) after 50 min. k' for the two exceptional cases were tentative as they were calculated from the initial slope of the curve drawn according to Eq. 1.

The concentration of oxygen was analyzed by a specially designed apparatus.

TABLE I

Concn. of O_2, M	k', min^{-1}	$\Delta\lambda$, mμ
0	(2.0×10^{-1})	5
$3.2_0 \times 10^{-5}$	(1.4×10^{-2})	9
6.76×10^{-5}	1.14×10^{-2}	8
8.58×10^{-5}	1.08×10^{-2}	5
1.13×10^{-4}	1.14×10^{-2}	3
1.53×10^{-4}	1.32×10^{-2}	3
3.40×10^{-4}	1.66×10^{-2}	0

As seen from Table I the value of k' is not affected so much by the concentration of oxygen. The results conform well with those of the pure aqueous solution, where it was found that the rate is almost constant down to about 4×10^{-5} M of oxygen whence the rate begins to decrease. The order of the k' value is also the same as that of the pure aqueous solution, in view of the difference of the light intensity[1].

2. Bleached Product of Methylene Blue in the Deaerated Solution.—In order to make clear the cause for photobleaching in the evacuated solution and the recovery of color by aeration, the absorption spectra of the solution irradiated for various times were examined from 200 to 700 mμ. Figure 1 shows the results obtained when the phosphate buffer solution of methylene blue was irradiated.

It is evident that accompanied with a decline of the main absorption of methylene blue, a new absorption appears in the ultraviolet region with the maximum at 255 mμ. Further it is observed that the change in ultraviolet region takes place with an isosbestic point at about 273 mμ. The absorption at 255 mμ coincides essentially with that of the leuco methylene blue chemically prepared, and it was almost definitely established by Obata[3] that the bleached product is nothing but the leuco form of dye which is but partially demethylated. It is to be added here that the dotted line in Fig. 1 which represents the absorption spectra of the recovered dye after aeration, has exactly the same maximum position as that of the partially bleached solution (see Sec. 6).

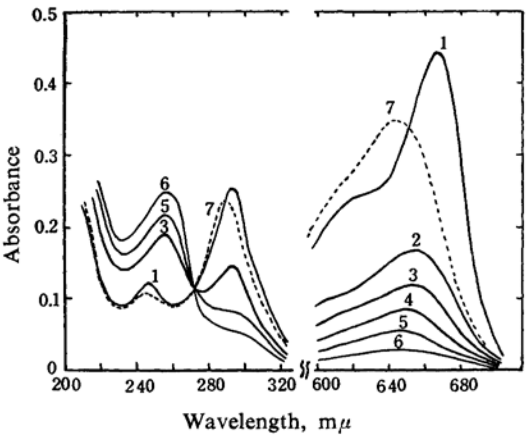

Fig. 1. The absorption spectra of the irradiated solution of methylene blue in deaerated phosphate buffer. $[Na_2HPO_4]=1.2\times10^{-2}$ M, $[KH_2PO_4]=2.8\times10^{-3}$ M, pH=7.57.

1. Initial
2. 5 min.-irradiation
3. 10 min.-irradiation
4. 20 min.-irradiation
5. 50 min.-irradiation
6. 80 min.-irradiation
7. Aeration

In connection with the formation of leuco dye, it seems to be worthy of reconfirming and studying further what Lewis[4] et al. formerly observed. Thus they perceived a slight fall of the absorption at 660 mμ with the rise of temperature ($\Delta D/\Delta T$=0.03/40°C) in the aqueous solution of methylene blue at three different concentrations, 9.0×10^{-6}, 1.9×10^{-6}, 6.3×10^{-7} M, and they attributed this change to the formation of a colorless form according to the equilibrium; $D^+ + H_2O \rightleftarrows (DOH)H^+$. They, however, did not study about the change in the ultraviolet region. Hence we carefully examined the absorption spectra of the aqueous dye solution 6.3×10^{-7} M, at various temperatures from 20 to 75°C in the region 200 ~700 mμ, using a long path cell (10 cm.).

At first the aerated solution was examined at several temperatures, varying the temperature from low to high and then in the inverse direction. Three such independent experiments were done but no change beyond experimental

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

[3] Part II of this series.

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

error was observed. In some cases rather opposite changes were observed; thus ε_{max} increased with the rise of temperature and decreased to its original value with the fall of the temperature. This would perhaps be due to the adsorption and desorption of dye on the cell wall. Thus the aerated solution did not show any sign of the phenomenon that Lewis et al. had claimed to perceive.

Next, the three analogous experiments were performed using the evacuated solution, and this time, a small depression beyond the experimental error was observed in the visible region with the rise of temperature. In addition to this change a clear reversible change was observed in the neighborhood of 250 mμ. A typical result is shown in Fig. 2. It is seen that a new peak appears when the temperature is raised. Thus the formation of the colorless form that Lewis had postulated seems to be substantiated in the case of the degassed solution.

This colorless form might somehow be related to the formation of leuco dye, but there is no doubt that the change of spectra in the irradiated solution is not due to what Lewis et al. supposed to be, as was confirmed by Obata³⁾.

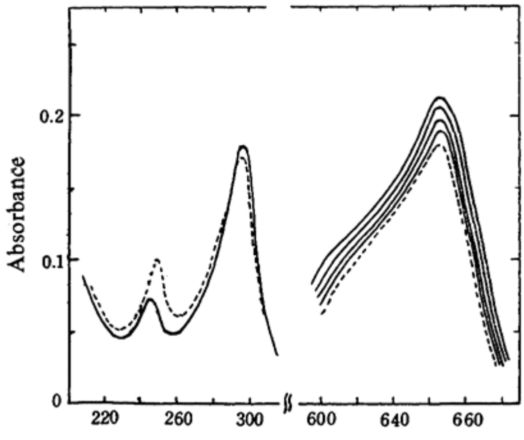

Fig. 2. Reversible temperature dependence of the absorption spectrum of methylene blue in aqueous solution degassed. (6.3×10^{-7} M) Visible region; from upper to lower 20, 30, 40, 50 and 60°C (dotted line) Ultraviolet region; 20, 60°C (dotted line)

3. Studies about the Solution Containing Various Kinds of Salts.—Since it has become established in Sec. 2 that methylene blue is photoreduced in the presence of certain salts, the next problem is to examine whether the similar phenomena be observed for other salts and how the rate will depend upon the type of salts, including the plain aqueous solution. The salts examined were sodium borate, sodium hydrogen phosphate, potassium dihydrogen phosphate, sodium sulfate, sodium chloride, sodium iodide, hydrochloric acid and sodium hydroxide.

The results obtained were quite similar at least qualitatively in all respects, thus in regards to the spectral change, recovery of color by aeration and the maintainance of the maximum position when recovered. But the rate depends very much upon the types of salts and a quite large difference is also observed in the magnitude of the spectral shift.

In Fig. 3 some typical cases are shown.

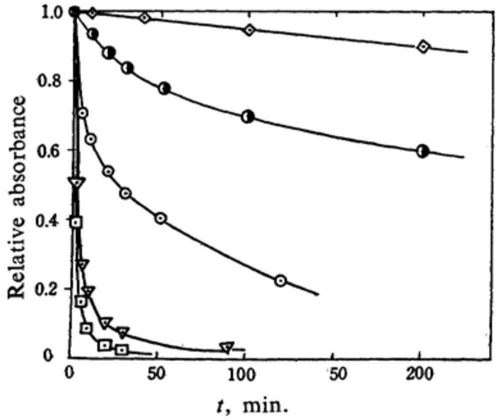

Fig. 3. Photobleaching of methylene blue in deaerated aqueous solutions containing various inorganic compounds at 30°C.

◇ HCl 1.0×10^{-3} M (pH=3.0)
◑ Na_2SO_4 1.0×10^{-2} M (pH=6.1) (NaI, NaCl and the plain aqueous solution are similar to Na_2SO_4)
⊙ Borate buffer (pH=7.55)
▽ NaOH 1.0×10^{-3} M (pH=10.9)
▣ Phosphate buffer (pH=7.57)

In the case of phosphate buffer and sodium hydroxide the rate is far greater in the order of magnitude, next comes the borate buffer and in the case of other salts and plain aqueous solution, the rate is less than one hundredth of the first class. The rate is still slower in the case of hydrochloric acid.

Experimental results are given in Table II in which ΔD and $\Delta\lambda_{max}$ are respectively the depression and the shift of the absorption maximum per twenty minutes. As for the shift, details will be described further in Sec. 6. All these solutions are of course stable in the dark.

It is well-known that methylene blue is very weak against alkali, and for caution's sake, the spectral change of methylene blue in

TABLE II

Condition	D_0	ΔD	$\Delta\lambda_{\max}$ mμ
Phosphate buffer soln.	0.550	0.515	17
Borate buffer soln.	0.621	0.304	2
NaOH (1×10^{-3} M) aq. soln.	0.619	0.543	8
NaI (1×10^{-2} M) aq. soln.	0.910	0.062	0
NaCl (1×10^{-2} M) aq. soln.	0.830	0.075	0
Na_2SO_4 (1×10^{-2} M) aq. soln.	0.580	0.070	0
Plain aq. soln.	0.986	0.088	1
HCl (1×10^{-3} M) aq. soln.	0.774	0.010	0

sodium hydroxide alkaline solution in the dark was examined.

In the case of 1 N solution, the absorption spectra in the visible region rapidly change into broader ones with a maximum near 590 mμ, whether the solution was degassed or not. When not deareated, this band remains constant after 41 hr. standing in the dark. But under the degassed condition the band gradually declines accompanied with a growth of a new peak at 250 mμ, and upon introduction of air it almost recovers its original height. When the concentration of sodium hydroxide is 10^{-3} N, there is no marked change observed in either aerated or deaerated solutions at room temperature. But when the temperature of the deaerated solution is raised to 60°C, a similar spectral change as in the case of 1 N sodium hydroxide (deaerated) takes place.

From the above observations it looks as if the spectral changes in the alkaline solutions are somewhat related to those in the neutral solutions caused by the temperature change. They may deserve further study but here it is enough to say that at 10^{-3} N sodium hydroxide scarcely any spectral changes occur in the dark at room temperature.

4. Scrutiny about any Possible Effect of Impurity.—In view of the rather unusual character of the present reductive reaction which proceeds without any reducing agent in the ordinary sense, it seems essential to scrutinize cautiously about the possible effect of any contaminating impurity which might act as a reducing agent, especially in cases of phosphate and borate where the rate is appreciably great. For this purpose the following experiment was performed.

Air was introduced to the nearly completely photobleached solution and the recovered dye was eliminated from the solution by adsorbing it on charcoal and filtering the solution through glass wool. Into the buffer solution thus pretreated, methylene blue was newly dissolved and the reaction was once more repeated. The rate proved to be almost the same as that of the preceeding experiment. From the above experiment any possible effect of impurity which might be contained in the buffer solution can be neglected.

Since it is very difficult to prepare a very pure sample of dye, the following special method was adopted in order to scrutinize the possible effect of any impurity contained in the dye.

According to Oster⁵⁾ the irradiation of the solution containing methylene blue, EDTA, *o*-phenanthroline and ferric salt, produces the complex between Fe^{2+} and *o*-phenanthroline and the reaction persists as long as ferric salt exists in the solution. This is because the leuco form of methylene blue produced by the photochemical reaction between EDTA and dye, reduces the ferric salt, the dye being recovered by this reaction. The similar reaction should necessarily be expected in our case since the leuco dye is produced by the irradiation. Accordingly the solution containing methylene blue, borate buffer, Fe^{3+} (6.0×10^{-4} M) and *o*-phenanthroline (2.3×10^{-3} M) was degassed and then irradiated. The result was that an absorption spectrum of Fe^{2+}-phenanthroline complex ($\lambda_{\max}=510$ mμ) did appear and that the concentration of the complex produced, attained more than ten times than that of the dye. Hence it can safely be concluded that most part of the reaction is not due to any impurity contained in the dye sample. The details of this experiment will be described in Part III.

5. Search for other Reaction Products.—Since the dye is reduced to the leuco compound, it is naturally expected that some oxidizing substances be produced. Hence it was undertaken to detect hydrogen peroxide and oxygen.

After the reaction was performed using a cell with a breakable joint, the solvent was distilled in vacuum through the joint upon the mixed powder of ferric salt (12 mg.) and potassium ferricyanide (16 mg.). In case of borate buffer, the existence of hydrogen peroxide was in fact ascertained by a green tint, while in case of phosphate buffer no change was observed.

In case of phosphate buffer, a very small amount of gas collected (about 30% of the employed dye) after the irradiation was examined by a mass-spectrometer*. Oxygen was detected but it was interpreted to be due to the air since nitrogen and argon were also detected and the ratio of the three patterns practically coincided with that of the air. The

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5) G. K. Oster and G. Oster, ibid., **81**, 5543 (1959).

* We are indebted to Professor T. Titani of Tokyo Metropolitan University for this analysis.

gas in question might have been due to the leak in the gas collection apparatus.

Thus according to this analysis no oxygen was detected. But this negative result does not necessarily deny the formation of oxygen. Because if oxygen be produced, there is no doubt that part of it must be consumed in the irreversible photo-decomposition of dye mentioned in Sec. 1. Moreover, when the phosphate buffer solution of dye partially bleached was kept in the dark for a long time, it recovered its color very gradually and this perhaps may be attributed to the existence of a very small quantity of oxygen produced during the irradiation.

6. Relation between the Quantity of Demethylation and That of the Produced Leuco Dye.— The shift of absorption spectra accompanied with the photobleaching can be attributed most certainly to the dimethyl amino groups[3]. Now to know the relation between these two quantities, the ratio D/D_0 (D and D_0 are respectively, the optical density of maximum absorption at time t and at $t=0$) is conventionally plotted against the position of maximum absorbance for all the results so far obtained. Figure 4 shows the plots for the phosphate buffer. In this case shift and bleaching apparently proceed in a parallel way and the position of absorption maximum in the final stage seems to tend toward the neighborhood of 640 mμ. When the absorption spectra of a partially bleached solution is divided by Blaisdell's method into two parts, i.e. the part due to the remaining methylene blue and that of other compounds, then the absorption of the latter always has its maximum near 640～645 mμ as shown in Fig. 5.

This strongly suggests that throughout irradiation or in most periods of irradiation except

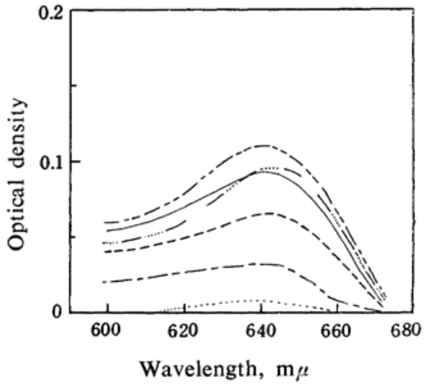

Fig. 5. The absorption spectra of the demethylated component separated by Blaisdell's treatment (in phosphate buffer pH=7.58).

		Irradiation time, min.	Opt. density remaining methylene blue at 665 mμ
a)		0	0.550
b)	···—···	1	0.278
c)	—— — ——	2	0.173
d)	———	4	0.090
e)	------	6	0.065
f)	—-—-—	11	0.035
g)	········	20	(0.028)

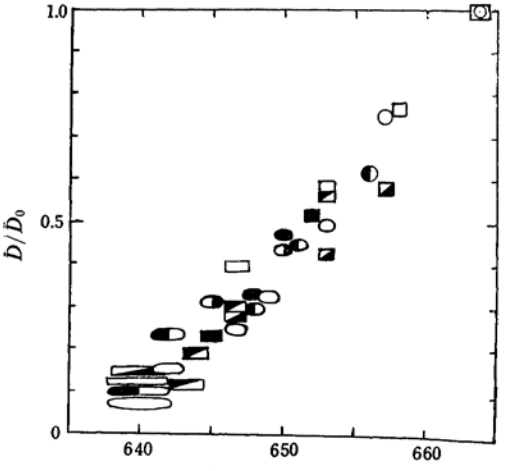

Fig. 4. Plot of D/D_0 against $\lambda_{\max}$ for the various conditions in phosphate buffer.

●	[Dye] 4 $\times 10^{-6}$ M	■	pH=8.81
◑	8 $\times 10^{-6}$	◩	8.07
◐	1.2$\times 10^{-5}$	◪	7.71
○	1.6$\times 10^{-5}$	□	6.94
	all pH=constant		all [Dye]=constant

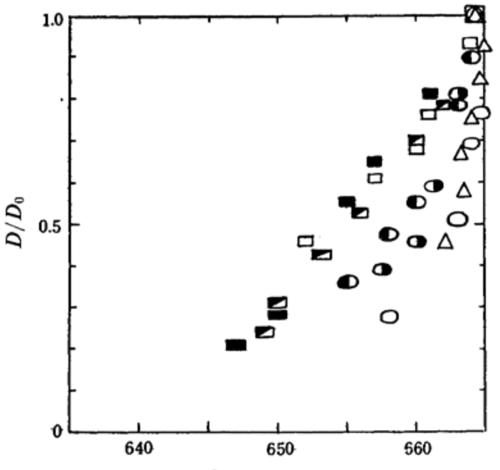

Fig. 6. Plot of D/D_0 against $\lambda_{\max}$ for the various conditions in borate buffer.

●	[Dye] 4 $\times 10^{-6}$ M	■	pH=9.14
◑	8 $\times 10^{-6}$	◩	8.30
◐	1.2$\times 10^{-5}$	□	7.85
○	1.6$\times 10^{-5}$		
	all pH=constant		all [Dye]=constant

the last stage, a single molecular species is predominantly formed as a result of demethylation. In case of other salts, the relation between bleaching and shift is somewhat different from that of the phosphate buffer, as shown in Fig. 6.

There are many cases in which demethylation is appreciably delayed than bleaching. In case of borate buffer, demethylation seems to be accelerated by the increase of pH. Summarizing all the results, demethylation apparently takes place with a rate about equal to bleaching at most, or with a much smaller rate. But in all cases the band maximum of demethylated compound lies near 640～650 mμ as in the case of phosphate buffer, suggesting again the formation of a single molecular species through demethylation. An example of borate buffer is shown in Fig. 7.

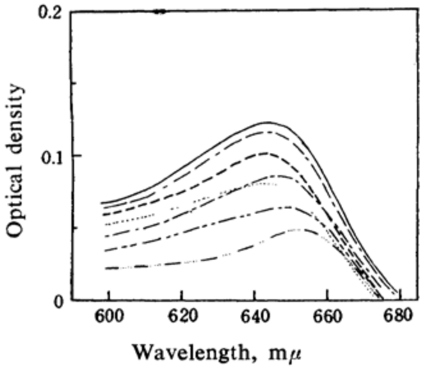

Fig. 7. The absorption spectra of the demetylated component separated by Blaisdell's treatment (in borate buffer pH=8.30).

		Irradiation time, min.	Opt. density remaining methylene blue at 665 mμ
a)		0	0.563
b)	·······–···	4	0.472
c)	-------	10	0.409
d)	——–—	20	0.340
e)	———--	50	0.209
f)	———	80	0.153
g)	------	152	0.093
h)	·········	212	0.078

Now correct data for the absorption maximum of mono-, di-*N*, *N'*-, di-*N*, *N*- and trimethylthionine can not be found in the literature, so that the number of demethylation can not be decided from Figs. 5 and 7, though the latter two seem rather plausible.

Suspecting the possibility that a small amount of hydrogen peroxide produced might cause demethylation to occur, the effect of the addition of hydrogen peroxide was examined. Such an investigation is also desirable from the standpoint of examining the effect of the reaction product upon the entire reaction.

The experiment was done using the aqueous solution of methylene blue and the intensity of the illuminating light was chosen so great that about 90% bleaching took place in about 10 hr. Plots of D/D_0 against $\lambda_{\max}$ are given in Fig. 8.

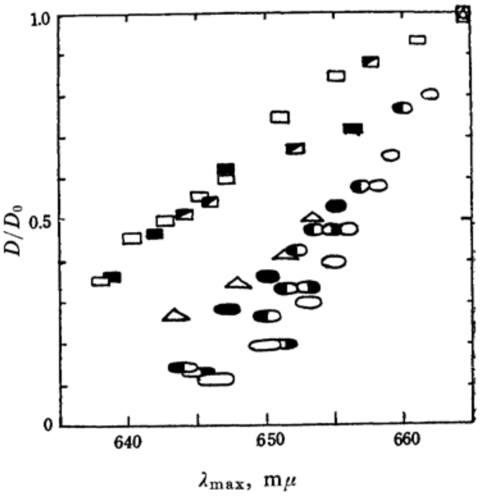

Fig. 8. Plot D/D_0 against $\lambda_{\max}$ for the various concentrations of H_2O_2 in plain aqueous solution.

◩	5.15×10^{-4} M	△	3.09×10^{-5} M
■	1.03×10^{-4} M	◐	2.58×10^{-5} M
□	1.03×10^{-4} M	◑	2.06×10^{-5} M
●	7.20×10^{-5} M	○	0

It is evident that demethylation is much accelerated when the added hydrogen peroxide exceeds 7.22×10^{-5} M. But when the concentration of hydrogen peroxide is 3.9×10^{-5} M or 2.5×10^{-5} M, there is no noticeable effect observed. Accordingly, it is clear that demethylation occurring without added hydrogen peroxide is not due to hydrogen peroxide produced during the reaction. In the presence of a large quantity of hydrogen peroxide irreversible photodecomposition becomes marked and at 5.15×10^{-4} M of the added hydrogen peroxide, the reaction consists only of the irreversible photodecomposition. But the contribution of such an irreversible decomposition is negligible when the concentration of hydrogen peroxide is $\sim10^{-5}$ M.

Of course, it was ascertained that the addition of hydrogen preoxide in the above order of magnitude does not cause any dark reaction. In connection with the above experiments, the effect of the addition of pyruvate was examined and it was found that the recovery of dye exceeds 85～90% of the original dye (measured by D/D_0) when it is added, as against 80% when it is not added.

Discussion

The present reaction as a whole is very complicated and as to the dye, there occur simultaneously three types of reaction; demethylation, formation of leuco compound and the irreversible decomposition. The magnitude of the dye decomposed irreversibly is estimated at most to be about 20～30% of the entire dye disappearing (irreversible decomposition plus leuco formation), but the correct value can not be obtained since the extinction coefficient of the demethylated compound is unknown.

The fact that the magnitude of recovered dye when pyruvate is added to the plain aqueous solution (in this case no shift occurs) reaches about 90%, strongly supports the view that the greater part of the irreversible decomposition is due to some oxidative substance produced during the photoreduction. At the present stage it is not certain whether and how much the irreversible decomposition is essentially connected with the formation of leuco compound but 10% or less would be plausible from the above data.

As for the relation between demethylation and leuco formation, the following conclusions can be drawn from the experimental results. Thus the two reactions are not directly interrelated with each other but rather take place seemingly at least, as two independent simultaneous reactions; because the addition of pyruvate can almost completely suppress demethylation without essentially affecting the rate of leuco formation.

From the absorption spectra of the oxidized form of demethylated dye, the number of demethylation is perhaps one or two. The spectrum as seen in Figs. 5 and 7, rises at first and reaches a maximum when the optical density of the remaining methylene blue falls near one third of the original value and then declines gradually. The absorption spectra of the solutions which have been partially photobleached and then recolored by the introduction of air, have almost the same maximum position as that of the solution before aeration. The latter fact indicates that the ratio between methylene blue and demethylated dye in the oxidized form is quite similar to that in the reduced form. This conforms well with the fact that oxidation potential of the demethylated compound is almost the same as that of methylene blue[6]. The variation of the concentration of the oxidized form of demethylated dye, as judged from its absorption spectra, can well be interpreted, if one assumes that an equilibrium is rapidly attained between the oxidized and reduced form of methylene blue and the corresponding forms of the demethylated dye. If one further assumes that demethylation and leuco-formation have about the same rate (this situation perhaps corresponds to the case of phosphate buffer approximately) then the concentration of the oxidized form of demethylated dye will simply be given as follows.

Putting the mole fractions of the oxidized and reduced form of methylene blue as y_1 and x_1 respectively and the corresponding quantity for the demethylated dye as y_2 and x_2, then the total mole fraction of reduced form is $x=x_1+x_2$, and the total mole fraction of demethylated dye is x_2+y_2. From the assumption, $x_2+y_2=x_1+x_2$ or $y_2=x_1$.

If one assumes, to simplify the matter, that there is no irreversible decomposition, then $x_1+x_2+y_1+y_2=1$.

Since the percentage of the demethylated compound is the same in oxidized and reduced form, the following formula can easily be obtained, utilizing the above relations.

$$x_1/y_1=y_2/y_1=x_2/y_2=(1-y_1-y_2)/(y_1+y_2) \quad (2)$$

Hence

$$y_2=-y_1+\sqrt{y_1}$$

From the following figures calculated by Eq. 2 one can see how the changes in y_1 and y_2 are related with each other.

y_1	0.81	0.49	0.25	0.16	0.09	0.04	0.01
y_2	0.09	0.21	0.25	0.24	0.21	0.16	0.09

For the sake of completeness, the numerical figures will be given below when the rate of demethylation is 0.7 and 0.5 times that of bleaching, i. e. $x_2+y_2=0.7(x_1+x_2)$ and $x_2+y_2=0.5(x_1+x_2)$.

$x_2+y_2=0.5(x_1+x_2)$

y_1	0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.04
y_2	0.06	0.104	0.118	0.125	0.122	0.106	0.070	0.034

$x_2+y_2=0.7(x_1+x_2)$

y_1	0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.04
y_2	0.076	0.135	0.157	0.172	0.174	0.162	0.12	0.10

The real situation is that the irreversible decomposition always takes place more or less, but it is evident from the above data that the changes of absorption spectra of methylene blue and the demethylated dye in most cases (Figs. 5 and 7) correspond to the case that demethylation has roughly the same rate as that of bleaching.

The result that a small quantity of dissolved oxygen increases the degree of demethylation,

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[6] L. Rapkin, A. P. Struky and R. Wurmser, *J. Chim. Phys.*, **76**, 340 (1926); See also Part II of this series.

can be accounted for as due to the repeated reoxidation of leuco dye, since this process retards the leuco-formation, increasing the relative magnitude of demethylation.

In some cases demethylation is found to proceed noticeably at a later stage and this strongly suggests, as already mentioned, that the reduced dye is more susceptible to demethylation. Thus it is plausible that demethylation and bleaching are in this sense related with each other although they are in fact independent simultaneous reactions.

A brief structural discussion will be added at the end.

Since demethylation is related with the amino group of a side chain while the formation of leuco dye is connected with the ring nitrogen, it is natural that the two reactions take place independently. In general demethylation is considered to take place oxidatively so that it is plausible in the present case that the (transient) removal of the lone pair electrons participates in the course of demethylation. This view seems to account for the difference of salts in suppressing demethylation. Thus according to the results so far obtained less demethylation seems to occur in the presence of SO_4^{2-}, Cl^-, I^-, OH^- when compared with borate and phosphate, and this might be due to the attachment of such simple anions to the amino group which hinders the removal of lone pair electrons. The anticipation that the leuco form is more susceptible to demethylation is compatible with the above view, since the leuco dye is electroneutral and can not combine with an anion. This might be the reason why demethylation becomes more pronounced at a later stage.

As for the formation of leuco dye, it is most plausible to consider that the primary process for the reaction consists in the acceptance of an electron by the excited dye, but it is not known at the present stage what kind of molecular species acts as an electron donor. It is conceivable that the amino group of the dye molecule plays this role, but there seems to be another possibility that hydroxide ion loosely attached to or existing in the neighborhood of ring nitrogen behaves as an electron donor. The latter possibility might be supported by the existence of a colorless form $(DOH)H^+$ which Lewis first postulated and which the present paper further supports.

At any rate there is scarcely any doubt that the ultimate fission of water by the visible light is taking place in this case. Such a possibility was formerly considered as a very unlikely event from the standpoint(7) of energy, but recently experimental evidence for such a process to occur, seems to be rather strengthened(8); the present result, we believe, demonstrates unequivocally that it can really occur though the mechanism is still entirely unknown and is to be solved in future.

Summary

It was discovered that the irradiation of the aqueous solution of methylene blue containing various kinds of salts, produces the leuco form of dye; accompanied with this reaction, irreversible photodecomposition of dye and demethylation occurs to some extent. Hydroxide, phosphate and borate were found to be most effective in promoting this photoreduction. Careful studies were performed to counteract the possible effect of impurity. Since the present reaction was considered most certainly to be due to the ultimate fission of water, the analysis for Oxygen and hydrogen peroxide was undertaken. The former was not detected by means of a mass-spectrograph in case of phosphate, but the latter was chemically detected in case of borate buffer. The connection between the quantity of demethylation and that of the formation of leuco dye was investigated and clarified to some extent.

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