Studies of the Photoreduction of Thiazine Dyes in Aqueous Solutions

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In 1961 Usui, Obata and Koizumi[1] found that methylene blue in suitable buffer solutions, without any reducing agents added, is photoreduced to leuco dye by the visible light. According to them, the quantum yield of photoreduction reaches as much as 10^{-3} in the phosphate buffer, but it is to be noted that the formation of leuco dye is accompanied by some degree of demethylation and a slight decomposition of the dye. Following this paper, Usui and Koizumi[2] attempted a kinetic treatment and found that in the borate buffer solution the reaction proceed approximately as of the first order with respect to the dye, while in the phosphate buffer solution, the reaction is a simple second order one with respect to the dye. It seemed that there is some difference between the reaction mechanisms of these two cases, but this was not conclusive in view of the rather tentative nature of the treatment.

The present paper is concerned with one, examining whether other kinds of dye show a similar photoreduction or not, and two, studying further the kinetic nature of the reaction in question.

The result is that, so far as we have examined, there are no kinds of dye other than thiazine dyes that show such a reaction. It seems that the reaction is specific for thiazine dyes. As to the second question, another example was found which behaves in a manner similar to methylene blue in the phosphate buffer solution. From the present investigation it has become more certain that in suitable cases the photoreduction of thiazine dyes process in a simple second order with regard to the dye.

I. A Search for Other Dyes which Display a Similar Photoreduction.—The following dyes were examined, each in plain aqueous, borate and phosphate buffer solutions.

a) Indophenol dye
  1) 2, 6-Dichlorophenol indophenol (Merck or Wako-junyaku)
b) Thiazine dyes
  2) New methylene blue (Tokyo-kasei)
  3) Methylene green (Tokyo-kasei)
  4) Toluidine blue (Tokyo-kasei)
  5) Thionine
c) Oxazine dyes
  6) Nile blue (Tokyo-kasei)
  7) Capri blue (Tokyo-kasei)
d) Phenazine dye
  8) Neutral red (Merck)

Experimental

Purification.—All the dyes were recrystallized from an ethanol solution several times. Sodium borate, sodium monohydrogen phosphate, and potassium dihydrogen phosphate (all G. R.) were recrystallized 3∼4 times from an aqueous solution.

Apparatus and Procedures.—The sample solution in a spherical ampule (2 cm. radius) was evacuated 5∼7 times by freezing and thawing and then poured into a reaction cell, which consisted of a spectrophotometer cell (1×1×4 cm.) and the upper part of which was connected to the above ampule by a glass tube. The experiment was performed in a thermostat at 30°C. The light source was a 500W/100V. projection lamp which usually operated at 75V. A suitable combination of filters were used for each system. At various time intervals, the sample cell was taken out and the absorption spectra were measured with a Hitachi spectrophotometer. The pH of the buffer solution was measured with a Hitachi pH-meter.

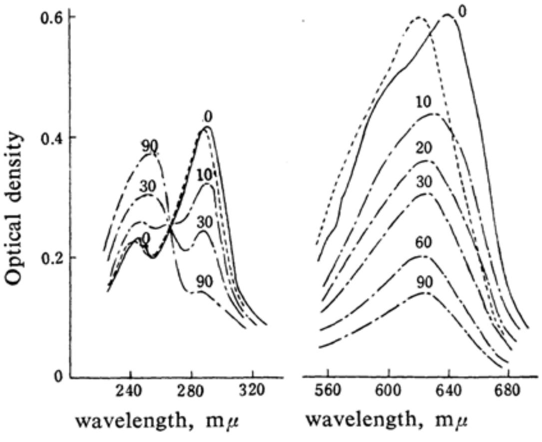

Fig. 1. Spectral change of toluidine blue in the photobleaching; dye conc. 2×10^{-5} M, phosphate buffer 1.0×10^{-2} M, pH 7.7. Numbers denote the time of illumination (min.); dotted line, after the introduction of air into the bleached solution.

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

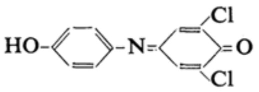
[2] Y. Usui and M. Koizumi, ibid., 34, 1651 (1961).

The following two tests were employed to decide whether or not the bleaching is due to photoreduction; 1) the measurement of the absorption spectra of the illuminated sample in the visible and ultraviolet regions, and 2) the recovery of color by the introduction of air. For example, when a phosphate buffer solution of toluidine blue is illuminated, the spectra decline in the visible region and a new absorption band appears at 255 mμ with an isosbestic point at $266\sim 7$ mμ, this band being just the same as that of the chemically-prepared leuco dye. This is shown in Fig. 1.

Furthermore, when air is introduced after the reaction, the absorption spectra in the visible region increase again, as is shown by a dotted curve. From these facts one can conclude that the photoreduction occurs in the case of toluidine blue. The phenomena described above are not observed in the case of irreversible decomposition.

Results

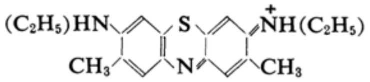
i) 2,6-Dichlorophenol Indophenol (2,6-DPIP)



This dye was studied because it has an oxidation-reduction potential as high as 0.217 V. (at $pH=7$). Its absorption spectra in the aqueous solution have three peaks, at 270, 310 and 600 mμ, while the reduced dye (prepared chemically) has one peak, at ~ 265 mμ.

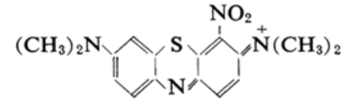
The stock solution must be kept in a refrigerator.[3] The dye was scarcely bleached at all by the visible light in the deaerated condition. Thus, the plain aqueous solution and the borate buffer solution ($pH=9.2$, 8.0), as well as the phosphate buffer solution ($pH=8.9$, 5.7), showed no change of absorption spectra even when illuminated as long as 430 min.

ii) New Methylene Blue (NMB)



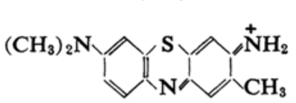
The absorption spectra have two peaks, at 285 and 630 mμ. The dimer peak exists at 585 mμ. The bleaching occurred, but very slowly, without any accompanying shift. The influence of the buffer solution on the rate was rather small. When the air was introduced after the reaction, $40\sim 50\%$ of the dye was restored.

iii) Methylene Green (MG)



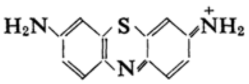
The λ_{max} lies at 275 and 650 mμ. The bleaching was moderately fast, with a shift of the maximum from 650 to $610\sim 620$ mμ. A new band appeared at 260 mμ. The rate increased in the order: plain aqueous solution < borate < phosphate. The introduction of air after the reaction restored $50\sim 60\%$ of the dye. The quantum yield of bleaching was ca. 3×10^{-4}.

iv) Toluidine Blue (TB)



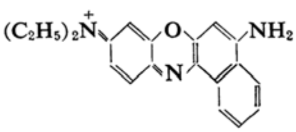
The photobleaching was rather rapid, with a shift of λ_{max} from 640 to 620 mμ. The order of the rate was: water < borate < phosphate. The introduction of air after the reaction recovered about 85% of the dye in case of the borate buffer solution, while in the phosphate buffer practically a 100% recovery was realized.

v) Thionine (Th)



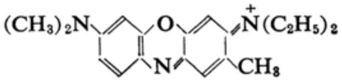
A gradual photoreduction occurred. The details will be described elsewhere.

vi) Nile Blue



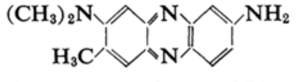
The rate of bleaching was very slow, and there was scarcely any recovery of dye by the introduction of air. It is to be noted that the borate buffer solution, when kept in the dark, showed a remarkable change in the absorption spectra and orange needle-like crystals precipitated.

vii) Capri Blue



The absorption spectra have two peaks, at 260 and 655mμ. The bleaching was very small, even with a 600 min. illumination. There was no effect of buffering on the rate, and no shift of the peak. The air-introduction recovered $20\sim 30\%$ in all cases examined.

viii) Neutral Red



This dye has two peaks, at 275 and 530 mμ. The plain aqueous solution and the phosphate buffer solution, when illuminated, showed a very small change in the absorption spectra. In the case of the borate buffer, the absorp-

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[3] I. Tachi and T. Tukamoto, *J. Agr. Chem. Soc. Japan*, 25, 340, 386 (1951).

TABLE I

Thiazine dyes				
Methylene blue	(MB)	(0.01)	◎	notable shift of λ_{max}
Toluidine blue	(TB)	(0.03)	◎	
Methylene green	(MG)	(—)	◎	
Thionine	(Th)	(0.06)	○	no shift of λ_{max}
New methylene blue	(NMB)	(−0.02)	○	
Oxazine dyes				
Capri blue		(ca. −0.07)	×	
Nile blue		(−0.12)	×	
Miscellaneous				
2, 6-Dichlorophenol indophenol		(0.22)	×	
Neutral red		(−0.33)	×	

tion spectra changed remarkably immediately after the buffer solution had been added to the dye solution (the resultant pH was 8∼9), and in addition, some precipitate was produced. It is to be added that, in the plain aqueous solution (pH≈6), mere evacuation caused a shift of λ_{max} from 530 to 465 mμ, and the introduction of air restored the original absorption spectra. The above absorption spectra in the evacuated solution resembled those of the phosphate buffer solution (pH≈7). These phenomena seem to deserve further study.

ix) Résumé

Table I summarizes the results so far obtained. In this table, the numbers in parentheses denote the oxidation-reduction potential and the signs ◎, ○ and × represent, respectively, that the reaction is prominent, moderate and practically non-existent.

From the above table one can say that the reaction in question is rather specific for thiazine dyes; it is notable that the reaction is especially prominent when there is an accompanying demethylation. The effect of the buffer solution is quite similar to that observed in methylene blue, the rate increasing in the order of plain aqueous solution < borate buffer < phosphate buffer. The magnitude of the reaction cannot be correlated with the oxidation-reduction potential. Generally, the photoreduction is more or less accompanied with the irreversible decomposition of the dye, which prevents the complete recovery of the oxidized form. Toluidine blue, however, is very fortunately an exception, the recovery of this dye in the phosphate buffer solution being practically 100%. This dye is therefore expected to be particularly suitable for quantitative studies, including kinetic studies. These will be described in II. As to toluidine blue in the phosphate and borate buffer solutions, the detection of hydrogen peroxide was undertaken by the method of Meloan, Manck and Huffman,[4] but the sensitivity was not good enough for our purposes.

II. Kinetic Studies, Chiefly of Toluidine Blue

The most reliable data obtained from the experiment concern the change of the absorbance at λ_{max}. However, a slight shift of λ_{max}, accompanied by a fall in absorbance, makes it necessary to examine the interrelation between these two quantities.

From the analogy of methylene blue, there is scarcely any doubt that the shift in the present case is due also to demethylation from the side dimethylamino groups. Therefore, the absorption spectra at various times of illumination were analysed by the minimum ratio method of Blaisdell. This gives the absorption spectra of the demethylated dye which is shown in Fig. 2. It is clear that the demethylated product consists only of one species, with a maximum at 620 mμ. In view of the

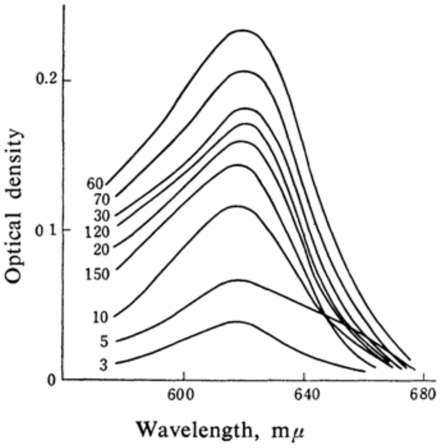

Fig. 2. Absorption spectra of the demethylated product (oxidized form) obtained by the Blaisdell method.

Toluidine blue	2.7×10^{-5} mol./l.
Phosphate buffer	1.7×10^{-2} mol./l.
pH	7.71

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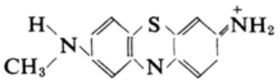
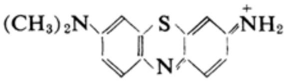
[4] C. E. Meloan, M. Manck and C. Huffman, *Anal. Chem.*, 33, 104 (1961); 32, 791 (1960).

fact that the difference of λ_{max} between azur A and azur C is ca. 20 mμ, it is very reasonable to consider that only one methyl group is detached in the case of toluidine blue. Table II gives the absorbance of the remaining toluidine blue at various times of illumination, D_0-D_t, and the absorbance of the demethylated product, D'_t.


Azur C


Azur A

From the above table one can see that, at an earlier stage of reaction, the ratio of the absorbance of the demethylated product to the total decrease in the absorbance of the original dye is near 0.5 in the case of phosphate, while in the case of borate it is less than 0.3. If one takes into account the fact that the restoration of dye by the introduction of air is almost complete in phosphate, it appears that, in this case, demethylation participates in the photoreduction, probably in the ratio of one to one.

Table II

a) Dye 2.7×10^{-5} M, phosphate buffer 1.0×10^{-2} M, pH=7.71

Time of illumination min.	D_t	D_0-D_t	D_t'
0	0.720	0	0
3	0.638	0.08	0.037
5	0.571	0.15	0.064
10	0.495	0.22	0.12
20	0.366	0.35	0.16
30	0.300	0.42	0.18
60	0.169	0.55	0.24
70	0.121	0.60	0.21
120	0.087	0.63	0.17
150	0.072	0.65	0.14

b) Dye 1.7×10^{-5} M, borate buffer 1.0×10^{-2} M, pH=9.4

Time of illumination min.	D_t	D_0-D_t	D_t'
0	0.480	0	0
3	0.413	0.07	0.016
5	0.397	0.08	0.025
11	0.375	0.10	—
20	0.330	0.15	0.045
30	0.309	0.17	—
60	0.255	0.22	0.05
90	0.198	0.28	0.083
120	0.154	0.33	0.10
210	0.144	0.34	0.094

In Figs. 3 a, b and c the values of D/D_0, where D and D_0 are, respectively, the absorbance at the maximum wavelength at time t and at time $t=0$, are plotted against λ_{max} at various times. The figure concerns the phosphate buffer solution. From the results one may say that, in the case of the phosphate buffer, the shift accompanies the decline in almost the same ratio always, irrespective of the concentration of dye, the temperature, or the intensity of the exciting light.

Such a situation does not hold in the case of the borate buffer, where the relation between the shift and the bleaching is different from case to case. The increase in dye concentration and pH seems to favor the shift much more than the bleaching. Some data are shown in Fig. 4. These results, quite similar to those obtained for methylene blue, suggest some differences, between the processes occurring in the phosphate and borate buffer solutions.

On the basis of the above results it seems quite rational to undertake the kinetic treatment for the phosphate buffer based on the

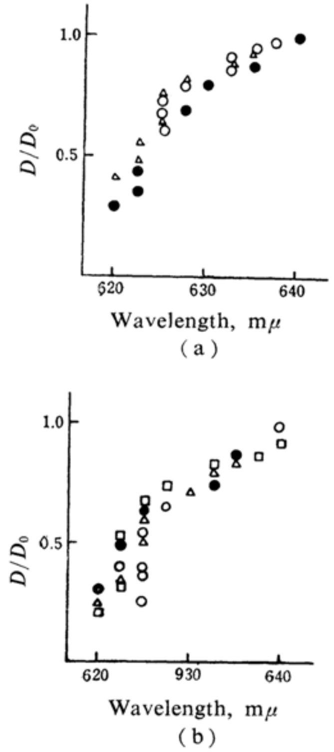


Fig. 3. Relation between D_0/D and a shift of λ_{max} in phosphate buffer.
a) Effect of light intensity
● I_0 △ $0.49\,I_0$ ○ $0.13\,I_0$
b) Effect of dye concentration
○ 0.7×10^{-5} mol./l. △ 2.1×10^{-5} mol./l.
● 1.4×10^{-5} mol./l. □ 3.6×10^{-5} mol./l.

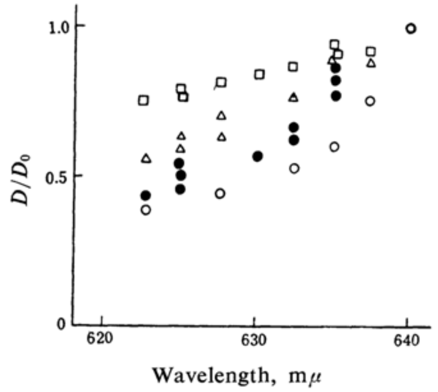


Fig. 4. Relation between D/D_0 and wavelength shift in borate buffer (constant pH).
Dye concn.
○ 0.5×10^{-5} mol./l. △ 1.7×10^{-5} mol./l.
● 1.0×10^{-5} mol./l. □ 2.6×10^{-5} mol./l.

decline of the absorption spectra of the remaining dye. This treatment, of course, concerns both photoreduction and demethylation, but these two processes, as has been mentioned above, go parallel in the case of toluidine blue in the phosphate buffer, irrespective of the experimental conditions. If demethylation plays an essential role in the photoreduction, as will be suggested later, the present treatment is most satisfactory. It must be added that the inner filter effect of the demethylated product will not cause much error because in a later stage, where the effect is expected to become notable, the concentration of the total dye (oxidized form) becomes much smaller.*

It must be added that the composition of the product consisting of demethylated dye and leuco dye obeys very rapidly the oxidation-reduction equilibrium and that a part of demethylated oxidized dye, after being produced by the reaction, will turn into the leuco form, while the corresponding quantity of leuco toluidine blue goes back to the oxidized form. These phenomena have already been treated to some extent in a previous paper;[1] the present finding that the recovered dye after the introduction of air has exactly the same $\lambda_{\max}$ as that of the remaining dye prior to aeration clearly demonstrates the existence of such an equilibrium as that discussed in the previous paper. In the following treatment, however, the effect of this phenomenon on the reaction rate will be disregarded in favor of a first approximation (see Appendix).

i) The Rate Formula.—A simple second-order rate formula was found to hold for the concentration of the remaining toluidine blue. However, as is shown in Fig. 5, the rate constant decreases as the concentration of dye increases. Some examples of the second order rate constant at various dye concentrations are given in Table III.

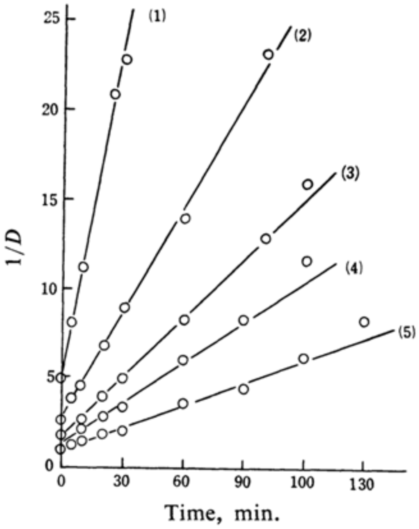


Fig. 5. Application of the second order rate formula. Effect of concentration of dye in phosphate buffer (constant pH).
Dye concn.
(1) 0.7×10^{-5} mol./l. (4) 2.7×10^{-5} mol./l.
(2) 1.4×10^{-5} mol./l. (5) 3.5×10^{-5} mol./l.
(3) 2.1×10^{-5} mol./l.

TABLE III. KINETIC DATA AND THE CONDITION OF THE SOLUTION

Phosphate buffer, pH=7.71
Na_2HPO_4 9.0×10^{-3} M
KH_2PO_4 1.0×10^{-3} M

Dye concn. $\times10^{-5}$ M	k $\times10^{-2}$ mol^{-1} l. sec^{-1}
0.7	3.07
0.9	2.03
1.4	0.935
1.8	0.735
2.1	0.534
2.7	0.375
3.6	0.238

A plot of $1/k$ against C_0 and a plot of $1/kC_0$ against C_0 are given in Figs. 6 a and b. The latter gives a good linear plot. Therefore, one can write the following equation as an experimental rate formula:

$$-\frac{\mathrm{d}c}{\mathrm{d}t}=kC^2=\frac{\alpha C^2}{\beta C_0(\gamma C_0+\delta)} \tag{1}$$

or

$$\frac{1}{kC_0}=\frac{\beta\gamma}{\alpha}\,C_0+\frac{\beta\delta}{\alpha} \tag{2}$$

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* If the concentration of the two components, C_1 and C_2, are very small, the quantity of light absorption by one component is $\varepsilon_1\times C_1\times I_0$, irrespective of the other components.

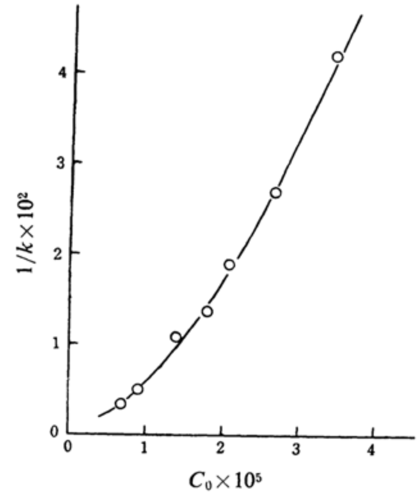


Fig. 6a. Relation of velocity constant to the initial dye concentration in phosphate buffer.

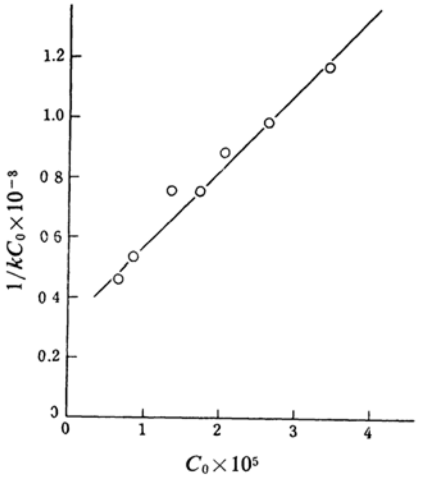


Fig. 6b. Relation of the velocity constant to the initial dye concentration in phosphate buffer.

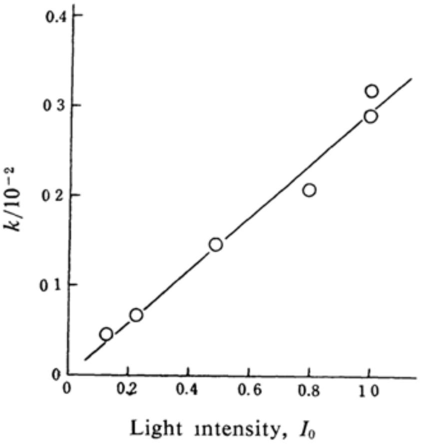


Fig. 7. Effect of incident light intensity on the rate constant in phosphate buffer.

From the above experimental data, one obtains;

$$\beta\gamma/\alpha = 2.4\times10^{7}\ \text{mol. l. sec}^{-1}$$

$$\beta\delta/\alpha = 0.3\times10^{3}\ \text{sec}^{-1}$$

ii) The Effect of Intensity.—The intensity of the illuminating light was varied by means of neutral filters, the transmission of which is 80, 49, 23 and 13%. The results are shown in Table IV and Fig. 7.

TABLE IV

Dye, ca. 2.5×10^{-5} M; Phosphate, 1.0×10^{-2} M

I_0	k $\times10^2$ mol^{-1} l. sec^{-1}
1.0	0.285
1.0	0.320
0.8	0.205
0.8	0.195
0.49	0.142
0.23	0.065
0.13	0.045

iii) The Effect of pH.—This is rather small, as Table V shows:

TABLE V

Dye, 1.8×10^{-5} M

pH	$k\times10^2$ mol^{-1} l. sec^{-1}	log k
9.10	1.18	2.07
7.71	0.735	1.87
7.00	0.595	1.78
5.93	0.239	1.38

iv) Temperature Effect.—Table VI.

TABLE VI

Dye, 2.1×10^{-5} M; pH=7.74

Temp., °C	$k\times10^2$ mol^{-1} l. sec^{-1}
20	0.421
30	0.534
40	0.627

From the above data, the apparent activation energy was evaluated as 4.2 kcal.

v) Quantum Yield (γ).—The following values were obtained. In the phosphate buffer (pH=7.74) and at 1.0×10^{-5} M of dye, $\gamma=6\times10^{-4}$. In the borate buffer (pH=9.40) and at 1.7×10^{-5} M of dye, $\gamma=4.4\times10^{-4}$. (The light quanta absorbed is 4.0×10^{14} sec^{-1} cc^{-1})

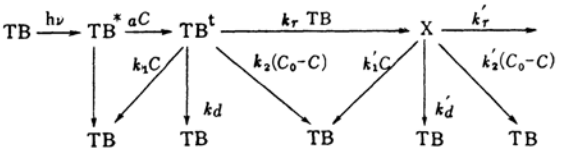
vi) Kinetic Results for Other Systems.—In the case of toluidine blue in a borate buffer, a simple second-order rate formula could not reproduce the experimental results. This is natural, since in this case there is an accompanying irreversible reaction and since, moreover, the degree of demethylation depends on the experimental conditions. Most of the other cases could not be treated by a simple second-order rate formula either. An example for which a simple second-order rate formula holds approximately, however, was methylene green in a phosphate buffer solution.

Discussion

Usui and Koizumi tentatively applied, and with some success, the second-order rate formula to the photoreduction of methylene blue in a phosphate buffer. At that time they supposed that the success was rather fortuitous because the overall reaction, consisting of reversible and irreversible bleaching and, furthermore, of demethylation, was so complicated that the treatment could not be expected to be more than approximate. Now in the case of toluidine blue in a phosphate buffer, for which the second-order rate formula proved to hold satisfactorily again, the situations, though still complicated, are considered to be much better than in the case of methylene blue. Thus, first, the recovery of dye by the introduction of air being almost complete, there is no irreversible decomposition of dye occurring in this case. Second, the shift of $\lambda_{\max}$ is much less than that of methylene blue and corresponds to the elimination of one methyl group. Lastly, the fact that the quantity of demethylation is about half of the total decrease in the original dye, irrespective of the experimental conditions, suggests that demethylation is coupled in some way with the photoreduction. In view of these results, there is scarcely any doubt that a bimolecular process with regard to the dye is taking place in this case. Furthermore, the fact that the rate is proportional to the light intensity indicates that the photoreduction in this case occurs between some excited species and the ground state dye.

It must be added here that, for the simple second-order rate formula to hold, the absorption of light must be proportional to the concentration of dye, and so the concentration of dye must be low enough. Some of the experimental results for cases in which the concentration is rather large do not fulfil the above condition in an early stage of the run, and it seems rather strange that the same rate formula still appears to hold in this region.

Disregarding this point for the moment, however, the scheme will be discussed below on the basis of the experimental rate formula. The following scheme, which is somewhat different from that proposed before,[1] can interpret the present experimental rate formula. Although of course, the scheme is not conclusive, yet the authors believe that, in view of various facts, some of which will be published in a following paper,[5] the scheme is established almost definitely, at least in its essential point, i. e., in the point that it involves a bimolecular process with regard to dye. The success of the following analysis also favors the scheme.



In this scheme, C_0 and C are the concentration of TB at time $t=0$ and t, and aC is the rate of the production of the triplet dye, the absorption of light being assumed to be proportional to the concentration of dye. a can be written alternatively as $I_0 \times \varepsilon \psi_{\mathrm{S-T}}$, and where I_0 is the incident light intensity, ε the molar absorbance, and $\psi_{\mathrm{S-T}}$, a probability of intersystem crossing to the triplet state. Further, in the above scheme, the triplet dye is assumed to disappear via the following four processes; spontaneous deactivation k_d, reaction and deactivation with the ground state dye (the rate constant of the former being written k_r and the total sum of the reaction and deactivation, k_1), and, lastly, the deactivation by some reaction product, perhaps leuco dye. The effect of leuco dye was experimentally confirmed as in the case of methylene blue. The reaction between the triplet dye and the ground state dye will produce another transient species, X, which perhaps consists of half-hydrogenated dye and half-oxidized dye. If these two components can separate away or if the half-oxidized state is removed by a suitable (reducing) agent, then a net photoreaction will result. The rate constant connected with this net reaction will be written as k'_r. Most of X in the absence of any reducing agent will, however, return to two ground state dye molecules spontaneously (k'_d) or by interacting with the ground state dye, $k'C$, or with the leuco dye, $k'_2(C_0-C)$.

From the above scheme one can write:

$$-\frac{\mathrm{d}c}{\mathrm{d}t}=\frac{ak_r k_r' C^2}{\{k_d+k_1C+k_2(C_0-C)\}\times \{k_d'+k_1'C+k_2'(C_0-C)\}} \qquad (1)$$

If one assumes here that the deactivation of the triplet dye and X is encounter-rate-determining, one may approximately put:

$$k_1 \approx k_2 \qquad k_1' \approx k_2' \qquad (2)$$

If one further assumes that $k_d k_d' \approx 0$, then;

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[5] S. Kato, M. Morita and M. Koizumi, This Bulletin, 37, 117 (1964).

$$-\frac{\mathrm{d}c}{\mathrm{d}t}=\frac{ak_rk_r'C^2}{k_2k_2'C_0{}^2+(k_2k_d'+k_2'k_d)C_0}=kC^2 \quad (3)$$

$$k=\frac{ak_rk_r'}{k_2k_2'C_0{}^2+(k_2k_d'+k_2'k_d)C_0} \quad (4)$$

These are just the same form as the experimental rate formula.

From 4,

$$\frac{1}{kC_0}=\frac{k_2k_2'}{ak_rk_r'}C_0+\frac{k_2k_d'+k_2'k_d}{ak_rk_r'}$$

Hence, the experimental parameters $\beta\gamma/\alpha$ and $\beta\delta/\alpha$ can be expressed as follows:

$$\frac{\beta\gamma}{\alpha}=\frac{k_2k_2'}{ak_rk_r'} \qquad \frac{\beta\delta}{\alpha}=\frac{k_2k_d'+k_2'k_d}{ak_rk_r'}$$

From the experimental values of $\beta\gamma/\alpha$ and $\beta\delta/\alpha$, one can estimate the rate constants for each elementary reaction as follows. Thus, if one tentatively assumes that $k_2 \approx k_2' \approx 10^{10}$ $\mathrm{mol^{-1}\,l.\,sec^{-1}}$, then:

$$10^{20}=2.4\times10^7\,ak_rk_r'$$

$$10^{10}(k_d+k_d')=3.2\times10^2ak_rk_r'$$

Hence,

$$k_d+k_d' \approx 1.3\times10^5\,\mathrm{sec^{-1}}$$

$$ak_rk_r' \approx 4\times10^{12}$$

The values of k_d+k_d' are consistent with the results of the flash experiment, which gives, for methylene blue in the buffer solution, $k_d \approx 10^5\,\mathrm{sec^{-1}}$ and about a one order smaller value of k_d'. From the quantity of light absorption, one puts

$$aC=a\times10^{-5}=\frac{4\times10^{17}}{6\times10^{23}}\phi_{\mathrm{S-T}}$$

and so a may be approximately $7\times10^{-2}\phi_{\mathrm{S-T}}$ $\mathrm{sec^{-1}}$. Therefore,

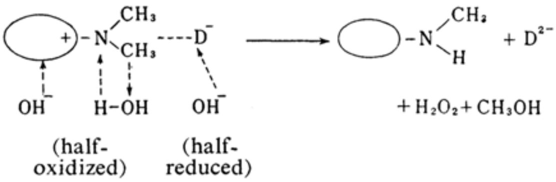
$$\phi_{\mathrm{S-T}}k_rk_r' \approx 6\times10^{13}$$

According to Oster, $\phi_{\mathrm{S-T}}$ is ca. 0.21 for methylene blue; if one uses the same value, k_r and k_r' may be, respectively, of the order $10^{10}\,\mathrm{mol^{-1}\,l.}$ and $10^4\,\mathrm{sec^{-1}}$, as is to be expected. Thus, the above scheme, although it involves some processes which must be verified, can lead to the experimental rate formula. The most dubious point to be ascertained is the deactivation of both D^t and X by the ground state dye and by leuco dye, which takes place as an encounter-rate-determining process.

Putting together the above kinetic analysis and the fact that the quantities of demethylation and the photoreduction are about equal, irrespective of the experimental conditions, it seems natural to consider that, in the case of toluidine blue in a phosphate buffer, the triplet dye interacting with the ground state dye produces a transient species which consists perhaps of the half-oxidized and half-reduced form, that a part of the former is demethylated, and that a corresponding quantity of the latter goes to leuco dye. Thus, it may be said that the reduction is promoted by the coupling with demethylation. The scheme will hold more or less for the photoreduction of thiazine dyes in general. The transient electron transfer between the triplet dye and the ground state dye is somewhat similar to that which Lindqvist[6] proposed for the photoreduction of xanthene dyes on the basis of the flash experiments.

It is quite unknown how the formation of leuco dye and demethylation occur. The following is only to show one conceivable scheme.

Writing X as $D^+\cdots C^-$ (D^+ and D^- are, respectively, the half-oxidized and the half-reduced state):



Such a scheme seems to be reasonable since demethylation will afford some quantity of free energy, which will in turn make the decomposition of water energetically feasible; further, this is consistent with the well-known fact that demethylation easily occurs in the oxidative condition. If the transient electron transfer between the triplet dye and the ground state dye occurs generally in thiazine dyes, then one may suppose that some reducing agents, especially weak ones, will selectively attack the half-oxidized form. Therefore, the overall rate formula is expected to be approximately of the second order with regard to dye. From such a point of view, we have reexamined the data of the photoreduction of methylene blue by means of trimethylamine.[7]

It was found that the application of the simple second-order rate formula $-\mathrm{d}C/\mathrm{d}t=kC^2$ can satisfactorily reproduce most of the experimental results, at least in the initial stage, until 60% or more photoreduction proceeds. The later part deviates in most cases in the concave direction upward; this suggests the participation of the first-order reaction. Some examples are shown in Table VII.

As has been shown above, the second-order

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[6] L. Lindqvist, *Arkiv. för Kemi*, **16**, 79 (1960).

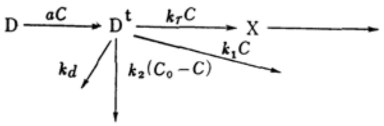
[7] H. Obata and M. Koizumi, This Bulletin, **30**, 136, 142 (1957). Details will be reported in the near future.

TABLE VII

Borate buffer pH=9.2
Trimethylamine 5×10^{-4} M

Exciting light	Concn. of MB($\times10^{-5}$)	$k\times10^{-4}$ l. mol^{-1} sec^{-1}	$kC_0\times10^2$ min^{-1}
λ=662 mμ	1.57	0.21	3.3
	1.20	0.38	4.55
	0.57	0.83	4.73
	0.28	1.58	4.43
λ=685 mμ	1.50	0.48	7.2
	1.17	0.63	7.25
	0.55	1.36	7.47
	0.30	2.40	7.2

rate constant in this case is inversely proportional to the initial concentration of dye. If one assumes that trimethylamine attacks the half oxidized component of X very effectively, then the mechanism can be written as follows:



Hence,

$$-\frac{dc}{dt}=\frac{ak_rC^2}{k_1C+k_d+k_2(C_0-C)}$$

If one puts $k_1\approx k_2$ and $k_d\approx0$, then:

$$-\frac{dc}{dt}=\frac{ak_rC_2}{k_2C_0}$$

Thus, the above experimental results have been interpreted. It is to be added that no shift and, hence, no demethylation occurs in this case, and so there is less ambiguity in the kinetic treatment than in the case of toluidine blue in the phosphate buffer. One reason why Obata and Koizumi misinterpreted the results was that the second-order rate formula was very difficult to understand at that time. Of course, some reducing agents will attack D^t directly, and in such cases an overall rate formula will be simply proportional to the absorption of light. Further, it is expected that in some cases the two mechanisms will work simultaneously.

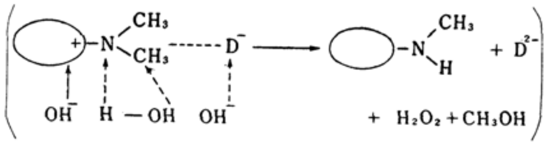
We are now reinvestigating various so-called reducing agents from this point of view. According to our preliminary experiments, EDTA is an example of a reducing agent which directly attacks the triplet dye, while allyl thiourea, ethanol, and trimethylamine belong to the other class.

Summary

Judging from the experiments performed in our laboratory, the photoreduction of dye without the addition of any reducing agent takes place only in thiazine dyes, and the reaction is particularly prominent when demethylation occurs simultaneously.

The reaction proceeds in the following way:

$$D + h\nu \rightarrow D^*$$
$$D^* \rightarrow D^t$$
$$D^t + D \rightarrow X \quad (X=D^-\cdots\cdots D^+)$$



The part in parentheses is still doubtful. The above conclusion is consistent with the results of the flash experiment which will be described in a following paper. It has been proposed that some reducing agents attack D^t directly, while others attack the half-oxidized state.

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Appendix

When the photoreduction occurs in such a way that a part of half-oxidized state is demethylated and an equal quantity of the half-reduced form is fixed as a leuco dye, then some of the former will turn into leuco dye, while the same quantity of the latter returns to the oxidized dye. Such an interchange will decrease the rate to some extent. If one takes a special case where 1) demethylated dye is not photoreduced at all, and 2) the ratio of the oxidized form to the reduced form is exactly the same for the original dye and the demethylated dye, the rate formula will have to be modified to some extent.

Putting the concentration of the original dye, demethylated dye, as x_1, x_2, and that of each reduced form as y_1, y_2, all at time t, one can write:

$$x_1+x_2+y_1+y_2=C_0 \tag{1}$$

$$x_2=y_1 \tag{2}$$

and

$$\frac{y_1}{x_1}=\frac{y_2}{x_2}=\frac{y_2}{y_1}=K \tag{3}$$

Hence,

$$y_1=Kx_1,\ y_2=Ky_1=K^2x_1,\ x_2=Kx_1 \tag{4}$$

Suppose that the coupled reaction of leuco dye formation and demethylation (process i) obeys the following rate formula:

$$-\frac{dx_1}{dt}=f(x_1) \tag{5}$$

then, due to the oxidation-reduction equilibrium between the original and demethylated dye (process ii), the rate formula observed experimentally will be altered as follows: From 5, x_1 decreases during

Δt by $\Delta x_1 = f(x_1)\Delta t$, while x_2 and y_1 increase by $f(x_1)\Delta t/2$ during the same period. However, by process ii a certain portion of the increase of x_2 (oxidized demethylated) and y_1 (reduced methylated) turns into a reduced demethylated form (y_2) and into an oxidized methylated form (x_1). This quantity, $\Delta' x_1 = |-\Delta' x_2| = |-\Delta' y_1| = \Delta y_2$, can be calculated using relation 3 as follows, because Eq. 3 is considered to hold instantaneously:

$$\frac{x_2 + \dfrac{f(x_1)}{2}\Delta t - \Delta' x_1}{y_2 + \Delta' x_1} = \frac{x_1 - f(x_1)\Delta t + \Delta' x_1}{y_1 + \dfrac{f(x_1)}{2}\Delta t - \Delta' x_1}$$

From this, using relation 4,

$$f(x_1)\Delta t\left\{\frac{x_2 + y_1}{2} + y_2\right\} = \Delta' x_1\{x_1 + y_1 + x_2 + y_2\}$$

Thus,

$$\Delta' x_1 = \frac{2y_2 + x_2 + y_1}{2C_0} f(x_1)\Delta t$$

This is the amount which returns to the original dye during Δt due to the equilibration of the oxidized and reduced forms. Hence, the net rate of reaction is written as follows:

$$-\frac{\mathrm{d}x_1}{\mathrm{d}t} = \left\{1 - \frac{2y_2 + x_2 + y_1}{2C_0}\right\} f(x_1)$$

This can be rewritten as follows by means of relation 4:

$$-\frac{\mathrm{d}x_1}{\mathrm{d}t} = \left\{1 - \frac{K(K+1)x_1}{C_0}\right\} f(x_1) \qquad (6)$$

From Eqs. 1 and 4,

$$K = \sqrt{\frac{C_0}{x_1}} - 1$$

Therefore, Eqs. 6 becomes:

$$-\frac{\mathrm{d}x_1}{\mathrm{d}t} = \sqrt{\frac{x_1}{C_0}} f(x_1) \qquad (7)$$

Thus, in the above ideal case the resultant rate formula has the factor $\sqrt{x_1/C_0}$.

However, the above treatment concerns an ideal case under very specified conditions; in most cases it is believed that demethylated dye will also be photoreduced. It is believed that the correction term involved in Eq. 6 may be neglected and that the original rate formula will still hold approximately.