Studies of the Transient Intermediates in the Photoreduction of Methylene Blue

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In the preceeding paper[1] some kinetic work has been reported on the photoreduction of thiazine dyes which proceeds in the absence of any reducing agent in the deaerated buffer solutions, especially in the phosphate buffer. In connection with these, the photoreduction of methylene blue by means of trimethylamine as a reducing agent has been re-examined.

The most characteristic feature of these reactions is that the essential process in the reaction is the interaction of the excited state dye with the ground state dye, perhaps producing semiquinone and half-oxidized dye; it seems that this is rather more common than it was at first supposed to be.

Further investigations now under way in our laboratory show that the photoreduction of methylene blue by allylthiourea (ATU) and other mild reducing agents often obeys a similar rate formula; the only clear-cut exception that has ever been found is the photoreduction by EDTA, in which the rate formula is satisfactorily of the first order of dye.

In order to get more direct insight into the mechanism of such reactions, studies of the transient intermediates by the flash-technique have been undertaken. The aim of the present paper is to report on the general features and behavior of the transient intermediates observed in an aqueous solution of methylene blue. The transient spectra at various pH values, especially in borate and phosphate buffer and also in the solutions containing various reducing agents, such as trimethyl-amine (TMA), allylthiourea (ATU), EDTA and $K_4 \cdot [Fe(CN)_6]$, have been compared. The decays of the chief transient spectra has also been investigated.

Experimental

Materials. — *Methylene Blue.* — Merck's special grade sample was recrystallized four times from an

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[1] M. Koizumi, H Obata and S Hayashi, This Bulletin, 36, 108 (1964).

aqueous solution and dried in vacuo. The $\varepsilon_{\max}$ value in the aqueous solution was 72000. All the inorganic reagents were submitted to recrystallization or repeated distillation.

ATU.—Three recrystallizations from water.

EDTA.—A G. R. reagent was used without further purification.

The Preparation of the Sample Solution.—After it had been evacuated more than five times with freezing and thawing, the solution was poured into a reaction cell and was sealed. The experiments were performed at room temperature. The spectra before and after the flash experiment were taken by a Hitachi autorecording spectrophotometer or a Kotaki spectrophotometer. The concentration of dye was usually 0.5×10^{-5} M$\sim$$1.0\times10^{-5}$ M.

Apparatus.—Two quartz flash lamps filled with xenon were used for the photolysis. The electrode distance was 10 cm. The input energy was varied from 31.5 to 180J by changing the capacitance 10 μF$\sim$40μF and applied voltage, 2500$\sim$3000 V. Hoya O-54 filters were inserted between the light source and the reaction cell so as to cut off <540 mμ. A spectro-flash was worked at 10 μF and 3000 V. The time interval between the photolysis flash and the spectro-flash was controlled by a delay circuit in the range from 20 μsec. to 0.8 sec.

The half-width of the photolysis flash was about 40 μsec.

Transient spectra were taken on Fuji Neopan plates with a Shimadzu QF-60 quartz spectrograph.

In measuring the decays of the transient absorption spectra, the sample was irradiated by a xenon lamp (20 V., 7.2$\sim$8.0 amp.) instead of the spectro-flash used above, and the monitoring light beam at a fixed wavelength was received by a photomultiplier through a slit attached to the spectrograph. The signal was recorded with an Iwasaki synchroscope SS 5151 through a cathode follower.

The reaction cells were of Shibata SB glass tubes, 10 cm. long and 7 mm. in diameter, to both ends of which were cemented plane quartz plates. A shorter cell, 2.5 cm. long, was used in order to measure the transient decrease of the main absorption band of methylene blue.

Results

Transient and Permanent Bleaching by Flash Illumination.—In general, illumination by a flash causes transient bleaching to a large extent, the magnitude depending upon the intensity of the flash, but it produces only a rather small quantity of permanent bleaching unless no suitable reducing agent is present.

First the effect of the flash intensity upon the quantity of the transient bleaching in the plain aqueous solution and in the phosphate buffer solution will be described. The results were quite similar. At 125J the transient bleaching was almost complete, whereas at 31.5J a considerable quantity of the ground state dye remained. Figure 1 shows the results in the plain aqueous solution. In Fig. 1., $\Delta D'$ is the difference in plate blackness between the sample and pure water, a difference which is proportional to the absorbance of the sample.

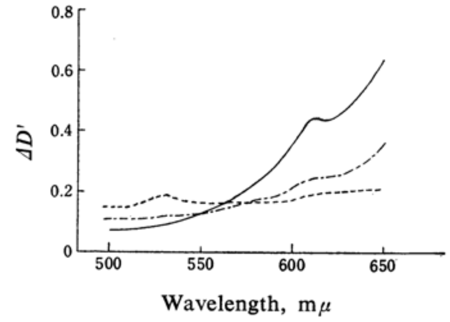

Fig. 1. The transient bleaching of the dye at different input energies.
——: Before flash, —·—·: 31J flash
----: 125J flash

The quantity of the transient intermediate corresponds quite well to the above results, as is shown in Fig. 2. In this figure, and also in Figs. 3, 4, 5, 6, 7 and 8, ΔD is the difference in the blackness of two plates, one of which was measured at a given time after the photolysis flash and the other, about five minutes later.

This value is proportional to the absorbance of the transient species. The amount of permanent bleaching by one flash of 125J in the phosphate buffer was about 4%, while in the plain aqueous solution it was about 3%, the difference between the two solutions being rather small in comparison with that for the steady illumination. There is no doubt that most of the transient species returns to the ground state dye. About 10% of the dye was permanently bleached by six flashes in the case of the phosphate buffer (pH $\sim$8.12), while the introduction of air restored the color of the dye almost completely. There was no shift of the main peak, in contradiction to the results for the steady illumination.

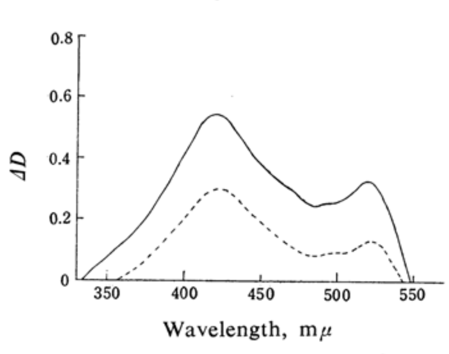

Fig. 2. The absorption spectra of transient species in a plain aqueous solution at different input energies.
——: 125J, ----: 31J

The addition of a 350-fold concentration of TMA (3.53×10^{-3} M) to the dye produced about a 40% permanent bleaching by one flash of 125J, but the quantity of bleaching per flash decreased for successive flashes. Along with the permanent bleaching, a new peak became apparent at 258～260 mμ. The introduction of air after almost complete bleaching led to a 100% recovery of the dye and the disappearance of the absorption in the ultraviolet region. There was no shift of the main peak. Comparing these results with the ones in the steady illumination, the general features are quite similar, though there are some differences in details.

When the degassed 0.01 N hydrochloric acid solution of methylene blue was mixed with the degassed solution of ATU, the bleaching occurred even in the dark, and only a very little recovery of color was observed upon the introduction of air. The addition of a few drops of a 3% hydrogen peroxide solution and subsequent shaking restored the initial color. The above-mentioned decolorization did not occur appreciably when the solution was not acidic. Further studies by means of the steady illumination seem to be desirable.

By the addition of a 100-fold quantity of EDTA (10^{-3} M) to that of methylene blue, flash illumination brought about remarkable permanent bleaching, as was expected on the basis of the steady illumination results reported by Oster.(2) The quantity of bleaching per flash was larger in the phosphate buffer solution with a high pH than in the plain aqueous solution, the degree of permanent bleaching in the former reaching about 80%. The product with the absorption peak at 258 mμ is considered, without doubt, to be leuco dye, just as in the case of TMA. The introduction of air caused a 100% recovery of the dye. No shift of the main peak was observed in the course of either bleaching or recovery

Although the photoreduction of methylene blue by EDTA, ATU, and other mild reducing agents has been studied extensively by many workers,(3) including Oster,(2) using a steady illumination, the present results by flash illumination seem to require a reinvestigation by the steady illumination; this is now being undertaken in our laboratory from a somewhat different point of view.

Transient Spectra in the Absence of Any Reducing Agent. — All the transient spectra described below are those at 20 μsec. after the main flash and are for the energy input of 125J unless otherwise described.

In an acidic solution of 0.01 N hydrochloric acid the transient absorption spectra spread over 330～540 mμ, peaking at 375 mμ, as is shown in Fig. 3. Its lifetime is so short that it seems to survive only while the photolysis flash is still on; i. e., τ is estimated to be less than 30 μsec. These results agree quite well with those of Parker,(4) and there is scarcely any doubt that this transient species is the triplet dye.

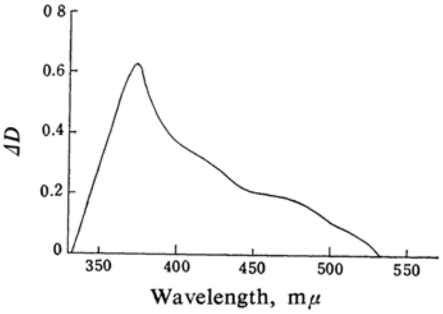

Fig. 3. The absorption spectrum of transient species in a 0.01 N HCl solution.

In the plain aqueous solution, the main absoption spectra spread from 340 to 550 mμ, with rather sharp peaks at 415 and 520 mμ, as is shown by the full line in Fig. 4. Besides, there is a weak transient absorption in ultraviolet, at 260～280 mμ and in the infrared region near 800 mμ. At 100 μsec., however, the shape of the spectra changes to some extent, as is shown by the broken line in Fig. 4. This implies that there are at least two transient species in the region near 415 mμ,

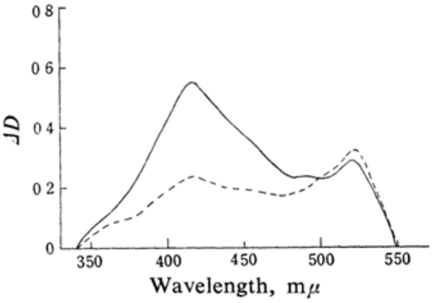

Fig. 4. The absorption spectra of transient species in the plain aqueous solution.
——: 20 μsec. after flashing
----: 100 μsec. after flashing

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

3) G. Holst, *Z. physik. Chem.*, **A179**, 172 (1937); **A182**, 231 (1938). M. Pestemer, *Z. Elektrochem.*, **58**, 121 (1954); G. Oster and N. Wotherspoon, *J. Chem. Phys.*, **22**, 157 (1954); R. Havemann and H. Pietsch, *Z. physik. Chem.*, **211**, 257, 267 (1959); S Matsumoto, This Bulletin, **35**, 1861, 1866 (1962).

4) C. A. Parker, *J. Phys. Chem.*, **63**, 26 (1959); C. G. Hatchard and C. A. Parker, *Trans. Faraday Soc.*, **57**, 1093 (1961).

each with a different lifetime. The shorter one will most certainly be a triplet, but it is possible that even at 20 μsec., the spectra are still a superposition of the two. The transient spectra in a borate and in a phosphate buffer solution (pH 8.2) are quite similar to those in the plain aqueous solution, with two peaks at 415 and 520 mμ.

The fact that in a strong acidic solution a peak exists at 375 mμ, while at a higher pH ($\geqq$7) a peak shifts to 415 mμ, irrespective of the presence of buffering substances, strongly suggests the existence of an acid-base change in the transient species, almost certainly a change in the triplet dye. Hence, the systematic study of the dependence of the transient spectra on pH was made from pH=2.2 to 10.2. For lower pH values, sodium acetate-hydrochloric acid or only hydrochloric acid was used, while for higher pH values, the sodium acetate-sodium hydroxide system was used. As had been expected, the variation in the spectra is very regular, with a clear isosbestic point at 400 mμ.

Figure 5 gives the relation between the differences in the plate blackness, differences which are certainly proportional to the absorbance, and the pH at three wavelengths, 415, 375 and 400 mμ. From these results one gets pK=6.7.

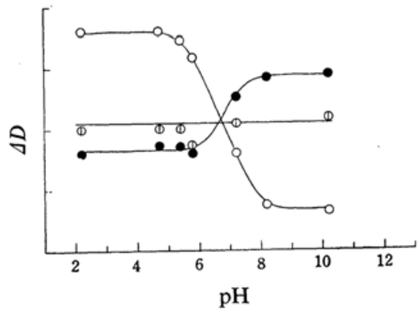

Fig. 5. The acid-base equilibrium of transient species. The change in the difference of plate blackness at various wavelengths, observed 20 μsec. after flashing, as a function of the pH value.
● 415 mμ ◑ 400 mμ ○ 375 mμ

The Transient Spectra in the Presence of Some Reducing Agents.—The transient spectra of the solution containing a 350-fold concentration of TMA compared to that of dye are shown in Fig. 6. Broadly speaking, they are similar to those for the plain aqueous solution or for the buffered solutions, with the absorption in the 250～280 mμ region, with a peak at 260～263 mμ, and in the 340～540 mμ region, with two peaks at 415～420 mμ and at 520 mμ. A slight difference is that the height at 520 mμ is less prominent in comparison with that of a solution containing no amine. The general features are also similar to those in a borate buffer solution (pH=8.2) to which TMA has been added, except that the 520 mμ peak is a little more prominent, all the spectra resembling those of the plain aqueous solution rather more.

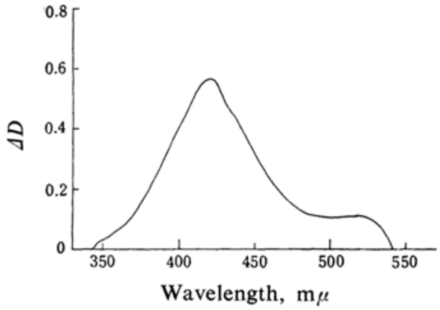

Fig. 6. The absorption spectrum of transient species in the plain aqueous solution containing 3.53×10^{-3} M TMA.

The transient spectra of the solution to which ATU has been added are also similar to those of the plain aqueous solution of methylene blue, with two peaks at 415 and 520 mμ.

The addition of a 100-fold quantity of EDTA (1.0×10^{-3} M) to that of methylene blue causes a notable change in the transient spectra as compared with those of the plain aqueous solution. Thus, the peak at 520 mμ becomes less prominent and a shoulder appears near 370～380 mμ, although the other peak still exists at 415～420 mμ (Fig. 7). With 3000 times more EDTA than dye (dye, 1.3×10^{-5} M, EDTA, 4.0×10^{-2} M), the peak near 520 mμ completely disappears and the transient spectra extend from 340 to 475 mμ. Moreover, the shape of the spectra changes appreciably in the course of time, as is shown in Fig. 8. The curve is rather flat in the neighborhood of the maximum at 20 μsec., but at 200 μsec., two maxima, at 375 and 413 mμ, become apparent.

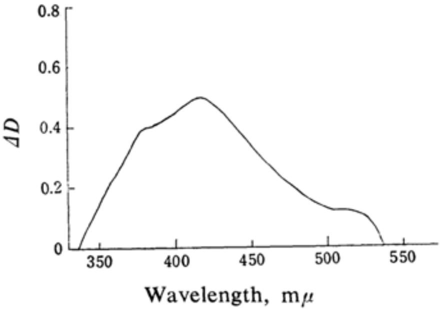

Fig. 7. The absorption spectrum of transient species in the plain aqueous solution containing 1.0×10^{-3} M EDTA.

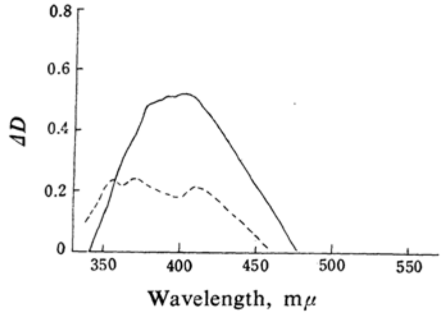

Fig. 8. The absorption spectra of transient species in the phosphate buffer solution (pH 8.1) containing 4.0×10^{-2} M EDTA.
——: 20 μsec. after flashing
----: 200 μsec. after flashing

When a concentration of $K_4[Fe(CN)_6]$ 50 times that of the dye is added to the plain aqueous solution of methylene blue, the transient spectra extend from 340 to 540 mμ, with a peak at 413 mμ. The peak at 520 mμ is completely lacking.

When leuco methylene blue (0.9×10^{-5} M) is added to the solution of methylene blue (1.5×10^{-5} M), the intensity at 415 mμ about doubles and the peak at 520 mμ is completely absent.

Lastly, when various amounts of oxygen are added to the solution, the transient absorption still appears in the 340～540 mμ region at 20 μsec.; on the other hand, at 100 μsec. the peak at 415 mμ declines remarkably, while quite a strong absorption is still existent in the ～520 mμ region.

Table I summarises the peaks of the transient spectra observed 20 μsec. after flashing in the visible region for various systems. s, m and w mean, respectively, that the intensity is strong, moderate and weak.

TABLE I

Solution	Peak
0.01 N HCl	375(s)
Plain aq. solution	415(s) 520(m)
Borate or phosphate	415(s) 520(m)
TMA	420(s)
TMA and phosphate	420(s) 520(w)
ATU	415(s) 520(m)
EDTA	415(s) 520(w or none)
$K_4[Fe(CN)_6]$	415(s)
Leuco methylene blue	415(v. s)
O_2	415(s) 520(m)

The Decay of the Transient Spectra.—In an 0.01 N hydrochloric acid acidic solution, the decay at 375 mμ obeyed the simple first order rate formula, giving a lifetime of about 30 μsec.

For the plain aqueous solution, the decays were examined at 415, 520 and 800 mμ. The initial part of the decay at 415 mμ obeyed the first order law approximately, giving a τ-value of 60～90 μsec. The same was true for the entire decay at 800 mμ, τ being 70～90 μsec. The approximate agreement of τ-values justifies the assignment of these two to the same transient species, most certainly to the triplet state. The latter part of the decay at 415 mμ showed a gradual deviation from the first order plot, but it seemed to fit another first order plot and a rough estimation gave a τ-value of about a few hundred μsec. The latter part of the decay at 520 mμ gave a τ-value of 200～260 μsec., roughly coinciding with the value for 415 mμ. Figure 9 shows a decay at 415 mμ.

It is very remarkable that the decay at 415 mμ for borate and phosphate buffer solution shows a clear hump at the earlier period as shown in Fig. 10. It is very difficult to express this decay curve analytically, but it is quite certain that the transient species survives longer than in the plain aqueous solution. It is to be noted that the above hump in the decay curve disappears when the energy input

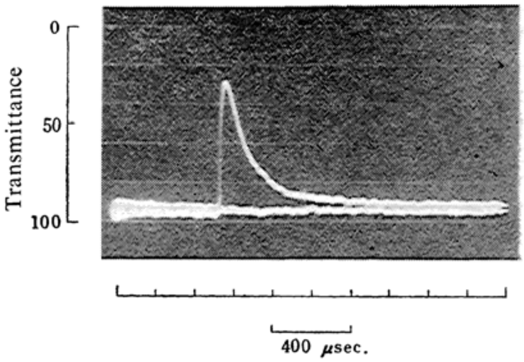

Fig. 9. The change in the transmittance at 415 mμ in the plain aqueous solution of 0.7×10^{-5} M methylene blue (input energy 125J).

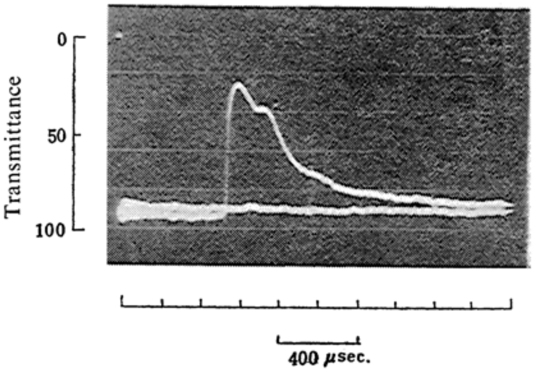

Fig. 10. The change in the transmittance at 415 mμ in the borate buffer solution (pH 8.2) of 0.7×10^{-5} M methylene blue (input energy 125J).

of the main flash is decreased to 31.5J. As has been mentioned before, almost all the dye turns into transient species at 125J, while at 31.5J more than half of the dye remains in the ground state. There is no doubt that the different results are due to these different situations (see Discussion).

The decay curve at 415 $m\mu$ when TMA or ATU was added displayed a small hump similar to that in the buffer solution.

Another notable finding is that the decay curve at 415 $m\mu$ when EDTA was added was simply monotonous. Although the entire decay curve did not obey the simple first order law, its earlier portion and the later one gave the lifetimes of the respective transient species, as respectively, about 170 μsec. and about 430 μsec.

Summarizing the results of the decay at 415 $m\mu$, one can say that the decay is anomolous, with a hump in the early stage when there are two peaks at 415 and 520 $m\mu$ in the transient spectra, the only exception being the plain aqueous solution. On the other hand, the decay in question is simply monotonous when the peak at 520 $m\mu$ is low or is not present in the transient spectra.

When various amounts of oxygen were added to the solution, the decay at 520 $m\mu$ became slower with the increase in the oxygen concentration.

Discussion

Broadly speaking, the general features of the flash photobleaching correlate well with those of the steady-light photobleaching. Thus, in the evacuated state the bleaching is reversible; i. e., the introduction of air restores the olor of the dye, indicating that the product is lecuo dye.

However, there are a few discrepancies which uggest that some different processes are ocurring in the flash experiment. For example, n the presence of oxygen the bleaching is rreversible in the steady illumination, while n the case of a flash there is not any slight leaching observed, even by ten successive lashes. Further, the addition of amine to the hosphate buffer solution of methylene blue ncreases the steady-light bleaching more than enfold, while in the flash experiment the uantity of bleaching only doubles that in the bsence of amine, even when a large amount f amine is added.

There is scarcely any doubt that all the ansient spectra observed in the present inestigation are due to some intermediates from ye in the photoreduction.

In the acidic solution there is only one intermediate, with a peak at 375 $m\mu$ with $\tau\approx$ 30 μsec. As has already been reported by Parker,[4] there is scarecely any doubt that this is due to the triplet state.

In the neutral solution quite different spectra than those of the acidic solution come into existence, and they show a rather complicated behavior. Thus in the plain aqueous solution, for example, the peaks at 415 and 520 $m\mu$ do not decline in a parallel way, the former declining a little faster at an early stage while at a later stage it seems that both decline at approximately the same rate. From the earlier part of the decay at 415 $m\mu$, τ is estimated to be several ten μsec., while from the later part it is estimated as a few hundred μsec. Further, the decay curve at 415 $m\mu$ displays a notable anomaly in the phosphate and borate buffer and τ becomes a little longer.

From these facts there is no doubt that there are at least two transient species existing in this wavelength region. The one with the shorter τ may be a basic species of the triplet state. The effect of pH value on the transient spectra shown in Fig. 5 strongly supports this view. It seems worth mentioning that the pK-value obtained, pK=6.7, coincides with the sharp change in the rate of photoreduction observed by Oster[2] in steady-light photoreduction by EDTA.

However, it is questionable what kind of molecular change corresponds to this spectra change, because there is no corresponding different species existent in the ground state from pH $\approx$0 to pH $\approx$11. Although the acid-base property in the singlet excited state often differs from that in the ground state so greatly that the pK of the former is several units apart from that of the latter, there is no such difference in the triplet, according to Jackson and Porter.[5] Their results, however, are concerned with rather a limited group of compounds; it is possible that different types of substances behave differently. At any rate, we must in the future decide whether the 375 $m\mu$ band belongs to the protonated triplet bivalent cation and the 415 $m\mu$ band to the univalent cation, or whether the 375 $m\mu$ band is the univalent cation and the 415 $m\mu$ band a neutral molecule with OH^- attached, or whether quite a different interpretation must be made. Here, we would like to attribute tentatively the 375 and 415 $m\mu$ bands (with shorter τ) to two different species of the triplet. The fact that the transient species in the infrared region has $\tau=70\sim90$ μsec., which is about equal to that of the earlier stage of

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[5] G. Jackson and G. Porter, *Proc. Roy. Soc.*, **260**, 13 (1961).

415 mμ absorption, also supports the triplet hypothesis.

As to the transient species which follow the triplet state, it is notable that there are two peaks, at 415 and 520 mμ, and that these transient spectra sometimes behave in a like manner, while in other cases they behave quite differently. Thus, when TMA or ATU is added, two peaks are observed, while when EDTA is added only the 415 mμ band appears; the 520 mμ band then becomes very weak or completely disappears. It is very natural, therfore, to consider that the two are produced by the same process in some cases but that only 415 mμ is produced in other cases and that the 415 mμ band is more intimately connected with the bleaching, because an effective reducing agent such as EDTA produces only this species.

In connection with this finding, it must be recalled that in the case of TMA and ATU, the rate formula for the steady illumination reaction closely approximates the second order law, while in case of EDTA the first order rate law holds.

From the above results it may be inferred that in the former case the excited dye (triplet, D^t), interacting with the ground state dye, D, produces semiquinone and a half-oxidized state, while in the latter case, the excited dye, by reacting with a reducing agent, produces only semiquinone. On this basis, the 415 and 520 mμ bands may be attributed respectively, to semiquinone and a half-oxidized state.

This hypothesis is further supported by the fact that the addition of leuco methylene blue greatly increases the peak at 415 mμ and completely eliminates the peak at 520 mμ. This implies that leuco dye, interacting with the triplet dye, produces semiquinone by the reaction $D^t + DH_2 \rightarrow DH + DH$ and that this occurs quite efficiently. It must be added here that the inhibiting action of leuco dye often referred to in the steady illumination experiment(1) is almost certainly due to such a reaction. The dehavior of the system containing oxygen also supports the above hypothesis. Thus, the finding that the absorbance by the existent oxygen at 415 mμ rapidly decreases while the decay at 520 mμ becomes slower is easy to understand if one considers that the former is a half-reduced form and the latter, a half-oxidized form.

It is well known that an analogous mechanism has been proposed by Lindqvist(6) for fluorescein on the basis of his extensive studies by the flash technique, but there have been no such examples reported since then.

As additional support for the above view, mention may be made of the features of the decay curves. The most conspicuous result is that in some cases a clear hump appears in the early stage of the decay. Such a phenomenon can be explained only if a certain transient species with a certain value of ε changes to another species with a negligible ε and is then transformed again into a third species with a rather large ε. The most plausible process satisfying the above conditions is considered to be the following one; the triplet dye with a certain value of ε goes back to the ground state, and the ground state dye thus produced, interacting with the remaining triplet dye, produces some new species with a larger ε. Thus D^t, at first, will perhaps go to the ground state by $D^t + D^t \rightarrow 2D$ or by a monomolecular process, but as the ground state dye accumulates, the $D^t + D \rightarrow 2D$ process will begin to make a greater contribution; accompanying this, the $D^t + D \rightarrow X(D^+ \cdots D^-)$ reaction will also occur. The later part of the above scheme is just the one proposed for the case when the second order rate formula is applied to the steady illumination experiment. Although exact analysis is very difficult, the conditions needed for the hump to appear may be something like the one described below.

Denoting a loosely-combined pair of semiquinone and a half-oxidized state as X and identifying the concentration of the triplet dye D^t, the ground state dye D, and X by, respectively, $[D^t]$, $[D]$ and $[X]$, the above scheme and the corresponding rate formulas may be written as follows, on the assumption that at $t=0$, $[D^t]_0 = a$, $[D]_0 = 0$, and $[X]_0 = 0$:

$D^t \rightarrow D$ $\quad k_1$

$D^t + D \rightarrow 2D$ (deactivation) $\quad k_2$

$D^t + D \rightarrow X$ (reaction) $\quad k_3$

$X \rightarrow$ leuco dye or ground state dye $\quad k_4$

$$-\frac{d[D^t]}{dt} = k_1[D^t] + k_2[D^t][D] + k_3[D^t][D]$$

$$\frac{d[D]}{dt} = k_1[D^t] + k_2[D^t][D] - k_3[D^t][D]$$

$$\frac{d[X]}{dt} = k_3[D^t][D] - k_4[X]$$

The absorbance of the system at 415 mμ [Ab] can then be given as follows if the molecular extinction coefficients of D^t, D, and X are, respectively, ε_1, $\varepsilon_2 = 0$ and ε_3:

$$[Ab] = l\{\varepsilon_1[D^t] + \varepsilon_3[X]\}$$

and

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

$$\begin{aligned}\frac{\mathrm{d}[\mathrm{Ab}]}{\mathrm{d}t} &= l\left\{\varepsilon_1\frac{\mathrm{d}[\mathrm{D^t}]}{\mathrm{d}t}+\varepsilon_3\frac{\mathrm{d}[\mathrm{X}]}{\mathrm{d}t}\right\}\\ &= l\{-\varepsilon_1 k_1[\mathrm{D^t}]-\varepsilon_1 k_2[\mathrm{D^t}][\mathrm{D}]\\ &\quad -\varepsilon_1 k_3[\mathrm{D^t}][\mathrm{D}]+\varepsilon_3 k_3[\mathrm{D^t}][\mathrm{D}]\\ &\quad -\varepsilon_3 k_4[\mathrm{X}]\}\end{aligned}$$

where l is the length of the reaction system.

At the earliest stage of the decay, $[\mathrm{D}]\approx 0$; hence, $\mathrm{d}[\mathrm{Ab}]/\mathrm{d}t<0$, as is naturally expected.

For the hump to appear, $\mathrm{d}[\mathrm{Ab}]/\mathrm{d}t>0$ must hold in some region of the decay curve.

This requires

$$(\varepsilon_3 k_3-\varepsilon_1 k_2-\varepsilon_1 k_3)[\mathrm{D^t}][\mathrm{D}] > \varepsilon_1 k_1[\mathrm{D^t}]+\varepsilon_3 k_4[\mathrm{X}]$$

When X disappears very fast, a steady state condition may hold with regard to X and it will be possible to put $k_3[\mathrm{D^t}][\mathrm{D}]$ as approximately equal to $k_4[\mathrm{X}]$. In this case the above condition never holds. However, if X is much more stable than $\mathrm{D^t}$, then, by putting approximately $k_4\approx 0$, the above conditions change as follows:

$$\varepsilon_3>\varepsilon_1\left(\frac{k_2}{k_3}+\frac{k_1}{k_3[\mathrm{D}]}+1\right) \qquad (1)$$

In fact, k_1 is $\sim 10^5\,\mathrm{sec}^{-1}$, while k_4 may be put approximately as $\sim 10^3\,\mathrm{sec}^{-1}$ from the later part of the decay curve. The situation is considered not to be very different from the above conditions. Provided that $k_1\approx 10^5$, $k_2\approx k_3\approx 10^{10}$, and $[\mathrm{D}]\approx 10^{-6}\,\mathrm{M}$, then (1) requires that $\varepsilon_3/\varepsilon_1>10$.

Thus it is expected in general that there are rather few chances for such a hump to appear, but it is not inconceivable. Borate and phosphate buffer solutions and solutions to which TMA or ATU has been added, are supposed to satisfy the above condition when the exciting light intensity is great enough. However, in the plain aqueous solution, in which X has a rather short lifetime, the condition would fail.

Further, when the intensity of the exciting light is small, the deactivation of X by the remaining ground state dye is large, as is expected from the steady illumination results, and so condition 1 can not hold again. In the case of EDTA, for which the rate formula is of the first order, $\mathrm{D^t}$ is directly reduced by EDTA, and so it is quite natural that the decay is simply monotonous.

To sum up, the results obtained in the flash experiments can be very satisfactorily correlated with the results obtained in the steady illumination experiments from the view point that the photoreduction of thiazine dyes in a neutral or an alkaline solution proceeds, in some cases, by a bimolecular process, $\mathrm{D^t}+\mathrm{D}\rightarrow\mathrm{D}^-+\mathrm{D}^+$ and, in other cases, by a direct electron transfer from the reducing agent to $\mathrm{D^t}$.

It is to be hoped that more quantitative studies on the rate will be made and that the rate constant for each elementary reaction will be evaluated.

Summary

The intermediate species which are produced in the course of the photoreduction of methylene blue have been investigated by flash photolysis in plain aqueous, borate and phosphate buffer solutions with and without reducing agents. In an acidic solution, an absorption band with the lifetime of 30μsec. appears at 375 mμ, while in neutral and basic solutions, two bands appear, at 415 and 520 mμ, with lifetimes of 70∼90 μsec. The bands at 375 and 415 mμ are considered to be due, respectively, to the acidic and basic forms of the triplet state, since the transient spectra change with pH, making an isosbestic point.

The band at 415 mμ is overlapped by another one with a lifetime longer by several hundred microseconds; this is presumed to be semiquinone. The intensity of the band at 520 mμ decreases upon the addition of reducing agents and increases upon the introduction of oxygen.

Hence, this band may be due to the half-oxidized form of the dye. These results support the reaction scheme that the excited state of the dye reacts with the ground state, which has been proposed in the investigation of steady light photobleaching.

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