Flash Photolysis of Methylene Blue. I. Reversible Photolysis in the Absence of a Reducing Agent and in the Presence of Certain Kinds of Reducing Agents

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It was shown in a previous paper[1] on the photoreduction of methylene blue by *N*-phenylglycines that kinetic data and some other properties of the system indicate a mechanism via a long-lived excited state (presumably the lowest triplet state) of the dye. As it was desired to detect such an intermediate in a more direct way and, simultaneously, to get more information on the mechanism of the photoreduction, a flash photolytic investigation was undertaken. Parker[2] has applied this method to methylene blue in an acid medium

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[1] S. Matsumoto, This Bulletin, 35, 1860, 1866 (1962).

[2] C. A. Parker, *J. Phys. Chem.*, 63, 26 (1959).

with ferrous ions as reductants. He observed two transients of different lifetimes and assigned them to the triplet state and to the semiquinone of the dye. He investigated the reaction, however, only in a 0.1 N sulfuric acid solution. Details of the absorption curves were not given.

For the present work the flash photolysis of methylene blue was carried out in solvents of various pH values, both in the absence and in the presence of a reductant. In Part I, only those cases are treated in which the solution recovers its original absorption and little or no permanent spectral change is observed after a flash illumination. In this case, ferrous sulfate, *p*-phenylenediamine or benzohydroquinone is used as the reductant. When *N*-phenylglycines were used as reductants, or when organic solvents were used, flash photolysis resulted in a more or less apparent permanent spectral change of the solution. Such cases will be treated in Part II of this paper.

Experimental

The exciting flash lamp was made of a quartz tube (1 cm. in external diameter) wound in a helix (3.4 cm. in inner diameter and 6 cm. long). It was filled with xenon to 150 mmHg. To recored the transient absorption spectra, an argon-filled (200 mmHg) linear flash lamp was used. The condensers for these lamps were 20 and 10 μF respectively, and they were charged up to 4000 V. in most cases. The reaction cell was also made of quartz (2.2 cm. in external diameter and 7 cm. long). It was covered with an ordinary-glass tube (wall thickness, 1 mm.), which served as a filter for the short-wavelength part of the exciting flash. The interval between the firings of the two flashes could be varied, by means of an electronic delay circuit, from 3 to 5000 μsec. The spectra were photographed with a Shimadzu Quartz spectrograph QF-60. In ordinary cases, a "Fuji Processpan Plate for Spectrograph" was used, but in some cases a "Fuji Neopan Plate" or a "Fuji Neopan Sheet Film SSS" was preferable. Near-infrared spectra were recorded on a "Fuji Infrared Plate 820" or on "Kodak Infrared Sheet Film." The densities on the plates were traced with a Nalumi recording microphotometer NLM-VII. For a preliminary study of tracing the change in absorption at certain wavelengths, the spectro-flash lamp was replaced by a 40 W./12 V. incandiscent lamp operated by a floating-charged battery. The light was made parallel by a lens, went through the reaction cell and an interference filter, and fell on the photo-cathode of a photomultiplier. Ordinary precautions to minimize the effect of scattered light, namely the use of baffles[3] and the use of a compensating circuit,[4] were adopted, but the relatively long duration ot the exciting flash interfered with a detailed kinetic study of the early stage of the photoreaction. Such kinetic study requires a much improved exciting lamp.[3]

Oxygen was removed from solutions by flushing them with pure nitrogen as in previous studies.[1] Although this method of deaeration is imperfect for the complete removal of oxygen, the results were reasonably reproducible and may be compared with the results of the previous experiments[1] performed under the same conditions.

The methylene blue was a G. R. reagent from the Koso Chem. Co.; $\varepsilon_{max}=8.15\times10^4$. The other reagents were also of G. R. grade from the same company and were used without further purification.

Results

Flash Photolysis in the Absence of a Reductant.—*A Transient in an Acid Medium.*—The transient spectrum which appears in a 0.1 N sulfuric acid solution is shown in Fig. 1. It may be seen that in this case the reaction is simple and that only one transient is formed. It has an absorption peak near 375 mμ and decays, in agreement with Parker's statement, almost in parallel with the exciting flash. This must be the triplet state of the dye. A weak second peak is present near 280 mμ. The spectrum is the same in 1 N sulfuric acid. In a more concentrated acid, the region of the absorption seems to remain the same, but its shape is obscured by an increase in the absorption of the ground state of the dye in this region.

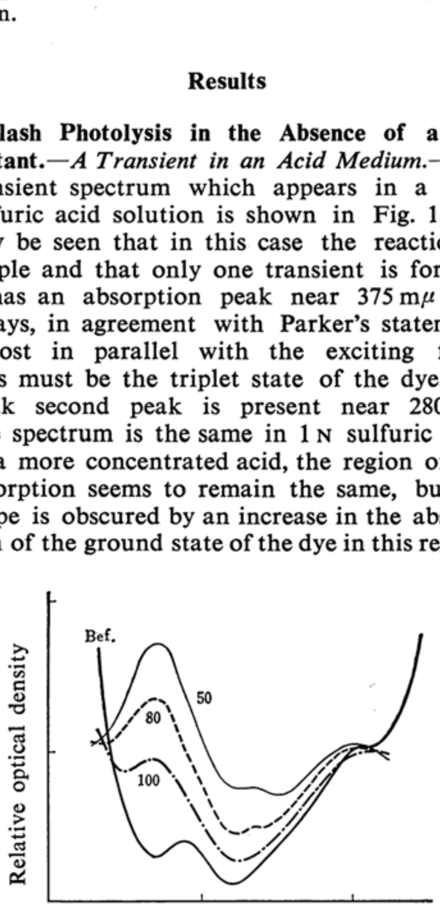

Fig. 1. Transient spectra in 0.1 N H_2SO_4 in the absence of reductant. M. B. 10^{-5} M. Figures beside curves indicate delay time Δt in μsec.

A Transient in Plain Water.—In this case, the transient spectrum is quite distinct from that in an acid medium (Figs. 2, 3 Plate 3). The absorption persists longer and shows peaks near 282 mμ, 420 mμ, and 520 mμ. It also extends to near-infrared region, where no absorption is observed in an acid medium.

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3) H. Linschitz and K. Sarkanen, *J. Am. Chem. Soc.*, **80**, 4826 (1958).

4) S. Claesson and L. Lindqvist, *Arkiv Kemi.*, **11**, 535 (1957).

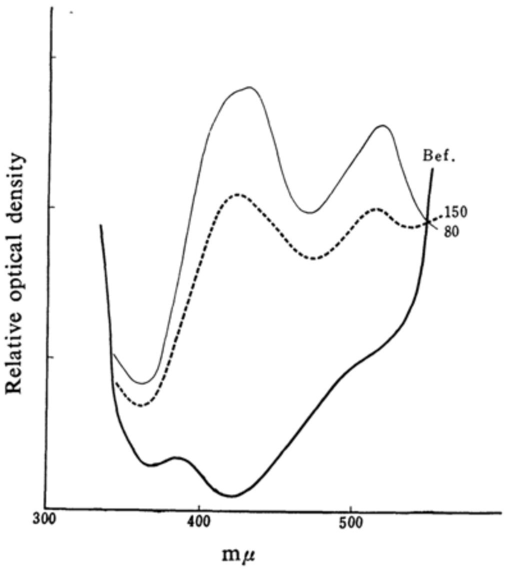

Fig. 2. Transient spectra in water in the absence of reductant. M. B. 10^{-5} M

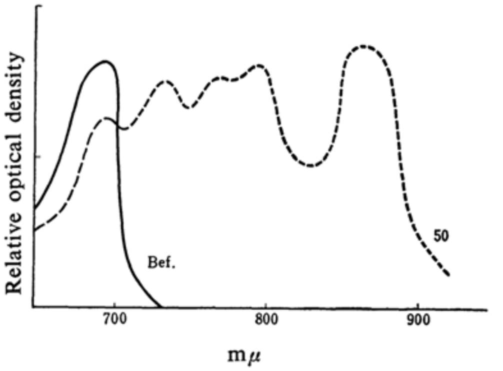

Fig. 3. The same as in Fig. 2. Near-infrared region.

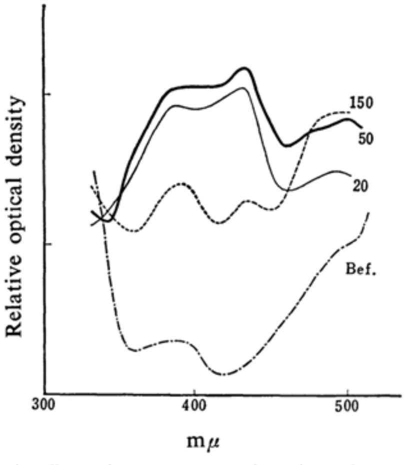

Fig. 4. Transient spectra in the absence of reductant. Solv.: buff. pH 6.8; M. B. 10^{-5} M

Another difference and complication is that the absorption around 520 mμ grows a little more slowly than that in the other region of the spectrum (Plate 1). This shows the superposition of at least two transient spectra, but their separation is difficult at present because the duration of the flash is not sufficiently short compared with the transient lifetimes. Preliminary experimentation shows the decay at 414 mμ to be unimolecular with a half-life of about 130 μsec.

A Transient in Buffer Solutions of Different pH Values.—The spectra at earlier stages of photolysis (e. g. when $\Delta t=20$ μ sec., Δt is the time interval between the firings of the exciting flash and the spectro-flash) vary regularly according to the pH value of the solvent. Below a pH value of 6.0, only the one found in 0.1 N sulfuric acid (acid form) appears, while above 7.4 only the one found in water (neutral form) appears. At intermediate pH values the two forms appear superposed, and at a pH value near 6.8 both forms seem to appear in equal amounts (Fig. 4). It seems, therefore, that the spectrum which appears initially in the photolysis of methylene blue in water[5] corresponds to the same species as that appearing in an acid medium, only with different protonation. Its $\mathrm{p}K_A$ value is approximately 6.8. No other protonation type of the species is found in the pH range from 1 to 12. It may also be seen from Fig. 4 that the increase in the absorption around 500 mμ at the later stage of the flash photolysis is much more pronounced in buffer solutions than in plain water.

A Transient in Dilute Acid Solutions.—When the photolysis was carried out in dilute sulfuric acid solutions, a queer result was obtained. The spectrum in 5×10^{-5} N sulfuric acid resembles that in a buffer solution of pH 6.8 rather than that in pH 4.3. The use of hydrochloric acid in place of sulfuric acid gives the same result. In accord with these results, near-infrared absorption appears in the photolysis in sulfuric or hydrochloric acid when the acid is more dilute than about 10^{-4} N, whereas it appears in buffer solutions only when the pH value is higher than about 6.2. This descrepancy may be explained most simply by the assumption that the neutral form of the triplet is first formed in the course of the photolysis and that in a very dilute acid solution the speed of the protonation producing the acid form of the triplet is so slow that the equilibrium between the two forms is not attained during the short lifetimes of the

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[5] The transient spectrum in a buffer solution of pH 7.0 contains some acid form. This discrepancy may be related to the facts described in the next sub-section.

transients. In buffer solutions, rather concentrated buffer agents may catalyze the acid-base equilibrium of the triplet. It was found that, in a dilute acid solution, the ratio of the absorption at 370 mμ to that at 420 mμ somewhat increases when $\Delta t=20\sim80\ \mu$ sec.

Flash Photolysis in the Presence of Reductants.—*A Transient in an Acid Medium in the Presene of Ferrous Ions.* — The photolysis in 0.1 N sulfuric acid has been investigated by Parker²⁾; in agreement with his result, the transient spectrum in the presence of this reductant is very similar to the triplet spectrum in this medium, only with a much longer lifetime. The broad band has a maximum around 375 mμ (Fig. 5). An experiment at this wavelength shows the disappearance to be roughly bimolecular (Fig. 6). This transient must be the semiquinone of methylene blue, as stated by Parker. The semiquinone also shows absorption from ca. 450 mμ down to the near-infrared region, just as with the transient observed in water in the absence of a reductant, whereas in an acid medium no absorption is observed in the absence of a reductant. Plate 2 shows that at a certain period of the photolysis the absorption in the visible region is weak, although in time the absorption of the original dye is restored. This suggests that some part of the methylene blue is trapped in a rather stable form, with no absorption in the visible region. This may be the leuco form of the dye. This supposition is supported by the appearance of an absorption around 255 mμ⁶⁾ (Plate 4). Figure 5 shows that the peak of the semiquinone absorption is displaced towards 390 mμ in media of pH> 3. Here, a less protonated form of the semiquinone must be present. No further change is observed in buffer solutions with pH values lower than 5.3 where the ferrous salt could be used without the trouble of hydrolysis. In 10 N or more concentrated sulfuric acid, a second change in the transient spectrum is observed. The peak of the absorption seems to be moved a little to a longer wavelength (ca. 390 mμ), and the absorption at wavelengths longer than 500 mμ observed in 0.1 N sulfuric acid is lacking. Another feature is that the the absorption appears delayed after the flash and persists much longer than in a 0.1 N acid solution. In an intermediate acid solution, e. g., in 3 N sulfuric acid, an intermediary situation is observed.

Transients in the Presence of p-Phenylenediamine or Benzohydroquinone. — The photolysis in the presence of *p*-phenylenediamine is simple. The spectrum and its change with time in a buffer solution of pH 7.0 is shown in Plate 7. This general apperance does not change in the pH range from 5 to 8. It shows peaks at 390 mμ and 460 mμ and one below 330 mμ. The first coincides with that of the semiquinone of methylene blue, while the latter two probably belong to the semiquinone of the diamine.⁷⁾ Absorption also appears in the near-infrared region. As the pH of the solvent is lowered below 5, the transient becomes progressively faint and appears somewhat delayed after the appearance of an absorption due to the triplet. This result is in harmony with the facts that the diamine shows acid-base equilibria⁸⁾ of pK_A 6.0 and 2.5 and that only the neutral diamine molecule seems to have a strong deactivating effect on the dye triplet.⁹⁾ The absorption near 280 mμ given in Plate 5 shows a close resemblance to that of the triplet, but

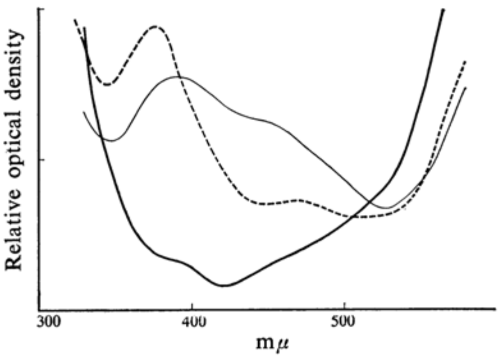

Fig. 5. Transient spectra in the presence of Fe^{2+} in acid medium. M. B. 10^{-5} M; $FeSO_4$ 0.01 M; $\Delta t=50\ \mu$sec.
— Before flash
- - - In 0.1 N H_2SO_4
— In a buff. pH 5.3

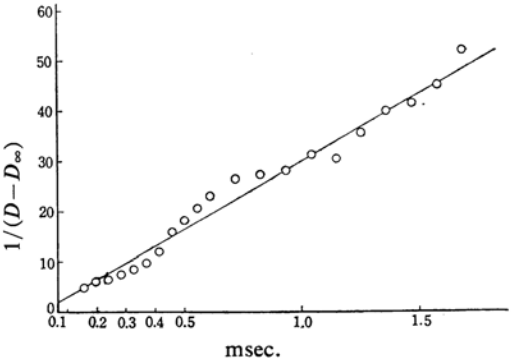

Fig. 6. Bimolecular decay of the transient absorption at 380 mμ.

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

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

8) N. A. Lange, "Handbook of Chemistry," 8th Ed., Handbook Publishers, Inc., Sandusky, Ohio (1952), p. 1235.

9) S. Matsumoto, unpublished.

no definite distinction is possible. In Plate 5 it may also be seen that, in this case, the recovery of the ground state of the dye occurs directly from the semiquinone and that little leuco dye is formed, in contrast to the case with ferrous ions and the case with hydroquinones described below. In media with pH values higher than 8.8, the transient spectrum is different (Fig. 7). The absorption maximum at 390 mμ is displaced to a wavelength near 430 mμ, and the absorption at wavelengths longer than 500 mμ is stronger than in solvents with lower pH values.

The result is more complex when hydroquinone is used as the reductant. Figure 8 shows a transient increase in absorption around 490 mμ and at wavelengths longer than 530 mμ preceding the growth of a strong and persistent absorption around 430 mμ in a buffer solution of pH 7.4. The latter may be mainly due to

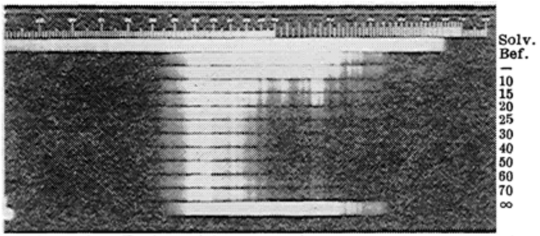


Plate 1. Delayed increase in absorption around 520 mμ in water in the absence of reductant.
M. B. 3×10^{-5} M

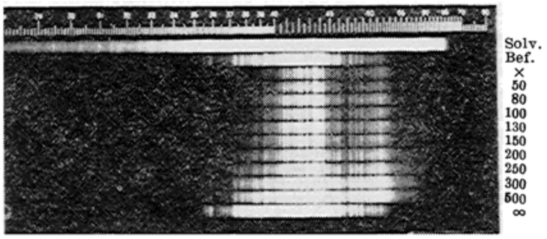


Plate 2. Transient spectra in the presence of $FeSO_4$ (0.1 M) in 0.1 N H_2SO_4.
M. B. 2.5×10^{-5} M

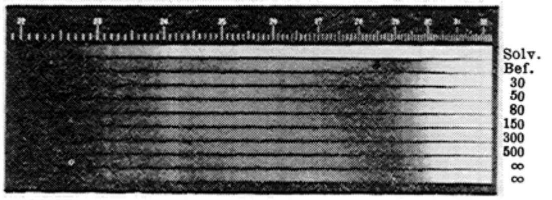


Plate 3. Transient absorption in the range 220 ~320 mμ in the absence of reductant.
Solv. H_2O ; M. B. 5×10^{-6} M

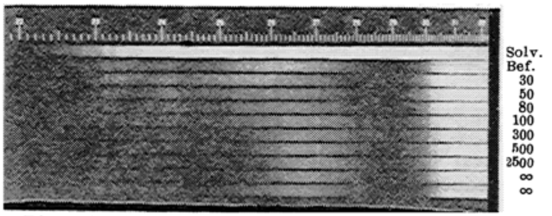


Plate 4. The same as in Plate 3 in the presence of $FeSO_4$, 0.002 M.
Solv. 0.1 N H_2SO_4 ; M. B. 10^{-5} M

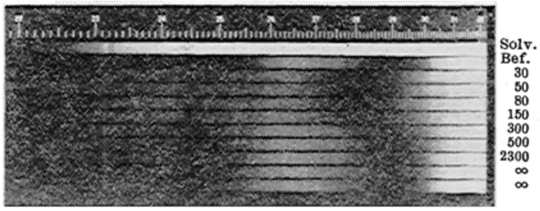
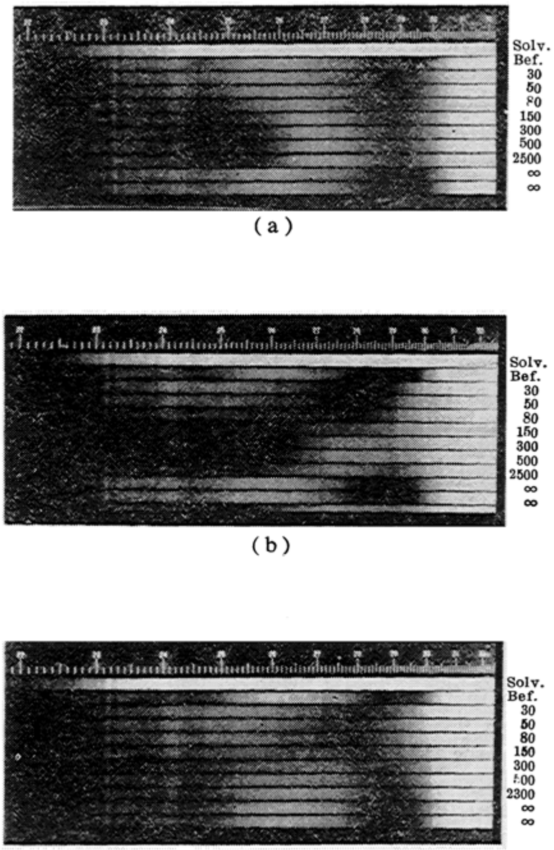


Plate 5. The same as in Plate 3 in the presence of PPD, 10^{-5} M.
Solv. buff. pH 7.4 ; M. B. 10^{-5} M



(c)

Plate 6. The same as in Plate 3 in the presence of HQ, 10^{-5} M.
M. B. 10^{-5} M ; Solv.: (a) 0.1 N H_2SO_4 (b) Buff. pH 7.4 (c) Buff. pH 9.2

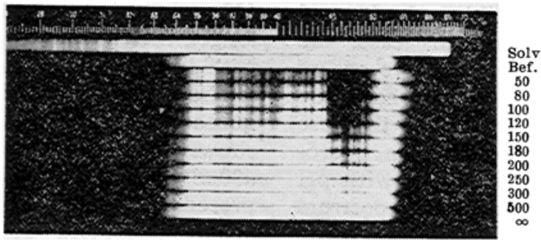
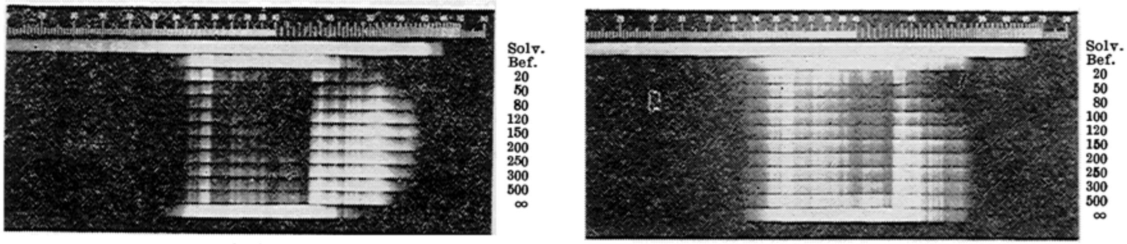


Plate 7. Transient spectra in the presence of PPD (5×10^{-5} M). Solv. buff. pH 7.0; M. B. 2.5×10^{-5} M



(a) (b)

Plate 8. Transient spectra in the presence of HQ (10^{-4} M). M. B. 3×10^{-5} M; Solv.: (a) Buff. pH 7.4, (b) Buff. pH 9.2

the semiquinone produced from hydroquinone,[10] probably superposed by the absorption of methylene blue semiquinone. In the earliest stage, the peak at 400 $m\mu$ is more marked than that at 430 $m\mu$. The order is upset at later stages. This may indicate the more rapid deformation of the dye semiquinone than that of the benzosemiquinone. The reaction may be a dismutation of the semiquinone or its reduction by other molecules of hydroquinone. Probably the latter predominates, as will be seen below (Plate 6b). At some stage the absorption in the region of methylene blue absorption reaches its minimum and then increases. This stage is analogous to a later stage of the photolysis with ferrous ions, when semiquinone has been converted into leuco methylene blue (cf. Plates 2 and 8a). The general pattern is the same in the pH range 5∼8. Figure 9 shows transient spectra at various pH values. The difference between the spectra at pH 6.5 and 9.2 is remarkable (cf. also Plates 8a and 8b). The spectral shift is similar to the case with *p*-phenylenediamine, but the increase in absorption at wavelengths longer than 500 $m\mu$ is more pronounced at an early period than in the latter case. It is also seen that, at pH 9.2, there is no period when methylene blue and its semiquinone disappear simultaneously. At pH values higher than 9.2, the general features of the spectrum and its change with time remain the same, but in an extremely alkaline solution, e. g., a 0.01 N sodium hydroxide solution, an irreversible bleaching of the dye becomes noticeable. In acid media (pH< 5), transient spectra and their change with time

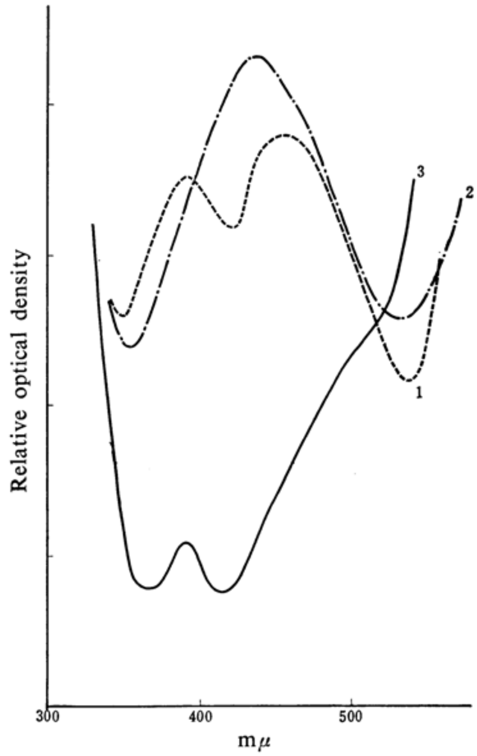


Fig. 7. Shift in transient absorption at a pH near 8, *p*-phenylenediamine being added as reductant, 10^{-4} M. M. B. 3×10^{-5} M; $\Delta t=50$ μsec. Curve 1: pH 7.0. Curve 2: pH 9.2. Curve 3 shows spectrum before flash.

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[10] H. Linschitz, J. Rennert and T. M. Korn, *J. Am. Chem. Soc.*, **76**, 5839 (1954).

are quite similar to those observed when ferrous ions were used, except for an absorption around 400 mμ due to benzosemiquinone. The spectrum at pH 3.2 is red-shifted compared with that at pH 1, parallel with the results obtained in the case of ferrous ions. In view of the results obtained in the cases of ferrous ions and *p*-phenylenediamine, the changes observed at other pH values must be due to the characteristics of the hydroquinone component. In a buffer solution of pH 3.8, the semiquinone of benzoquinone seems to have an absorption around 400 mμ, one which is shifted to 430 mμ in a buffer solution of pH 5.3. Plates 6a—6c show the transient spectra in the wavelength region from 220 mμ to 320 mμ at various pH values. It may be seen that, below a pH value of 8.4, leuco methylene blue is formed in a process of reversible photoreaction. At a pH value of 9.2, however, the recovery of the original dye occurs without the formation of leuco methylene blue. At a pH value of 8.4, an intermediary situation is observed. This pH dependence parallels that of the spectrum of methylene blue semiquinone at longer wavelengths. It may also be seen that, although at pH values higher than 9 little formation of leuco methylene blue is observed, the formation of an intermediate (presumably the semiquinone of the dye) is a clear-cut process and the yield of the intermediate is good. On the contrary, the conversion in an acid medium seems to be less perfect.

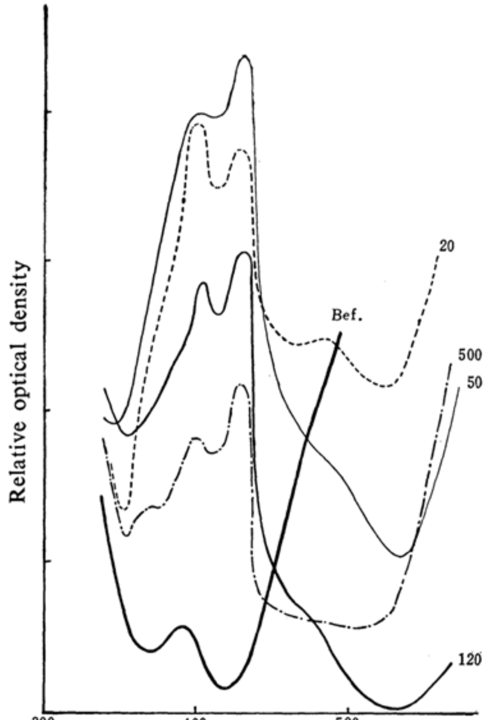

Fig. 8 Transient spectra in the presence of hydroquinone in a buffer of pH 6.5.
M. B. 3×10^{-5} M; HQ 10^{-4} M

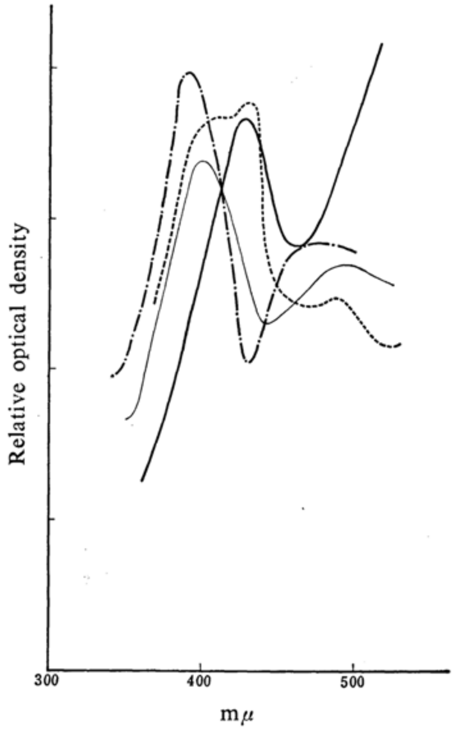

Fig. 9. Transient absorption in the presence of hydroquinone at various pH values.
M. B. 3×10^{-5} M; HQ 10^{-4} M; $\Delta t=50$ μsec.

━ pH 9.2 --- pH 6.5
— pH 3.8 -·- In 0.1 N H_2SO_4

Discussion

The results just described seem to be related to the fact that the quantum yield of the photoreduction of methylene blue by *N*-phenylglycines reaches a maximum at a pH near 7.8. As was seen above, the transient spectrum that appears after a flash illumination of methylene blue in a neutral or alkaline medium persists longer than the spectrum in an acid medium. Whether this means a longer lifetime of the neutral form triplet itself compared with that of the acid form, or whether it can be accounted for solely by a superposition of another intermediate with a long life, it can not be decided at present. Evidently the delayed increase in the absorption around 530 mμ indicates such a second transient, but the clear-cut separation of the spectra is difficult because of the long duration of the exciting flash. This requires a detailed kinetic study with an improved flash apparatus. At any rate, the neutral-form triplet must have a different (probably an increased) reactivity or reaction probability towards reductants compared with that of the acid form. For this reason, the velocity of photoreduction increases with the pH value in the range below about 7.8. At still higher pH values, the semiquinone of methylene blue loses a proton to change into its conjugate base. The latter has a very slight tendency to be reduced further and returns smoothly to the original dye cation (Plate 6c). This would explain the sharp decrease in the rate of photoreduction at the higher side of the pH region. If the methylene blue cation is denoted by D^{+}, the neutral and acid forms of the triplet may be denoted by D^{+t} and DH^{++t} respectively. In the same way, various forms of semiquinone, which appears in solvents of different acidities, may be designated D, DH^{+}, DH_2^{2+}, and DH_3^{3+}. Only DH^{+} and DH_2^{2+} were seen to be reduced or to dismutate rapidly to form the leuco dye, and D would have to be protonated before it is reduced further if it were the first reduction product.

The assignment of the absorption at 520 mμ (in plain water), of those at 490 mμ and at longer wavelengths than 530 mμ (Fig. 8), and of that one at longer wavelengths than 500 mμ (Plate 8b) can be only speculative at present. The first may be assigned to a labile complex between a pair of half-oxidized and half-reduced methylene blue ions produced by a redox-reaction between an excited ion and a normal one stabilized by some negative ions,[11] or to a sort of charge-transfer complex between an excited dye ion and a normal one. The second and the third absorption may also be assigned to the same species or to a similar charge-transfer type of complexes between excited dye ions and reductant molecules.

Summary

Transient spectra which appear in the flash photolysis of methylene blue in aqueous solvents of various pH values have been surveyed both in the absence and in the presence of a reductant. In the absence of a reductant, the triplet state of the dye has been observed to appear in two forms, with different degrees of protonation. The form found in neutral water seems to be formed directly from the excited singlet state of the dye. The pK_A value of the acid-base equilibrium has been found to be approximately 6.8. A second transient has been found to be produced in water and in buffer solutions of a moderate pH value at a later stage of the photolysis. Its formation is more marked in buffer solutions, and it may be supposed to be a sort of charge-transfer complex between an excited dye ion and another dye component. The photolysis in the presence of ferrous ions, *p*-phenylenediamine or benzohydroquinone is nearly reversible, and the absorption of the original dye is restored after the illumination except in an extremely alkaline solution. The transitory formation of leuco methylene blue has been observed when ferrous sulfate or hydroquinone was used as the reductant below a pH value of 8.4, while that was not the case with *p*-phenylenediamine. The formation of leuco methylene blue from semiquinone has been found to be a bimolecular dismutation process in an acid medium when ferrous ions are the reductant, but when hydroquinone is used as the reductant at moderate pH values, it also reduces the methylene blue semiquinone. When hydroquinone is used as the reductant in a neutral or alkaline solvent, an increase in absorption is often observed at some longer wavelengths; this has tentatively been attributed to a complex between excited dye ions and reductant molecules. A comparison of the transient spectra obtained in various conditions has shown that the semiquinone of methylene blue exists in variously protonated forms in the following ranges: $pH>9$, $pH=3\sim8$, in $0.1\sim1$ N sulfuric acid, and in very concentrated sulfuric acid. The pH dependence of the quantum yield of the photoreduction obtained earlier has been tentatively related to the change in the degree of protonation of the triplet state and the semiquinone.

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[11] Suggested by Professor Masao Koizumi.