Flash Photolysis of Methylene Blue. II. Photolysis in an Aqueous Solvent in the Presence of N-Phenylglycines and Photolysis in Organic Solvents

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In Part I[1] of this paper the flash-photolytic reduction of methylene blue in aqueous solvents in the presence of several reducing agents was surveyed. Now it is desirable to investigate in this connection the reaction in the presence of *N*-phenylglycines, and to compare the results with those obtained by continuous illumination.[2] In this case an irreversible bleaching of the dye remains after flash illumination. This requires renewed preparation of a degassed solution after every flash to get a series of spectra corresponding to various delay times.

Photolysis in common organic solvents was also found to result in an irreversible bleaching. Preliminary results of photolysis in these solvents will be given also.

Experimental

The apparatus and procedure are the same as those described in Part I. The preparation of *N*-phenylglycines has been described in an earlier paper.[2] The organic solvents were G. R. reagents from the Koso Chem. Co., and were used without further purification.

Results and Discussion

Photolysis in Aqueous Solvents in the Presence of *N*-Phenylglycines.—*1) Resemblance to the Reversible Photolysis.*—Plates 1a and 1b give spectra in which the concentration of the reductant (*N*-*p*-chlorophenylglycine) is varied. It is seen that the spectrum changes from the type observed in pure water to the one observed when *p*-phenylenediamine[1] is used as the reductant. The absorption shows a peak around 410 mμ at the earliest stage of photolysis and somewhat blue-shifts later. This absorption may be mainly that of methylene blue semiquinone superposed by that of the triplet dye. This assumption is substantiated by the fact that the absorption red-shifts to 425 mμ at a pH value where the same change was observed with *p*-phenylenediamine[1] (Fig. 1).

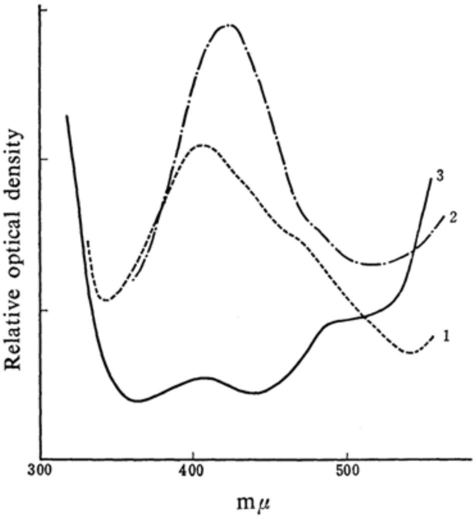

Fig. 1. Shift in transient absorption at a pH near 8, *p*-Cl-ϕ·G being added as reductant, 10^{-4} M. M. B. 10^{-5} M; delay time $\Delta t = 50$ μsec. Curve 1 : pH 7.4 ; Curve 2 : pH 9.2. Curve 3 shows spectrum before flash exctn.

In this case the absorption corresponding to the oxidation product of the reductant is uncertain. The spectrum in the 220∼320 mμ region resembles the spectra in the reversible reduction in Part I. The spectrum obtained with *N*-phenylglycine (Plate 2) has features intermediate between those with *p*-phenylenediamine and those with benzohydroquinone (cf. Plates 5 and 6b in Part I). Some part of the semiquinone is going to leuco methylene blue, while the other part is coming back to the original form of the dye. The spectrum with *N*-*p*-methoxyphenylglycine (Plate 3a) resembles that with *p*-phenylenediamine. In this case the photolysis is reversible, in accordance with the results described in a previous paper,[2] where the resemblance of the two reductants was stressed.

2) Relations between the Transient Spectra and the Structure of the Reductant. — Plates 3b—3g show the spectra obtained with typical derivatives of *N*-phenylglycine. The spectrum with *N*-phenylglycine is similar to that with

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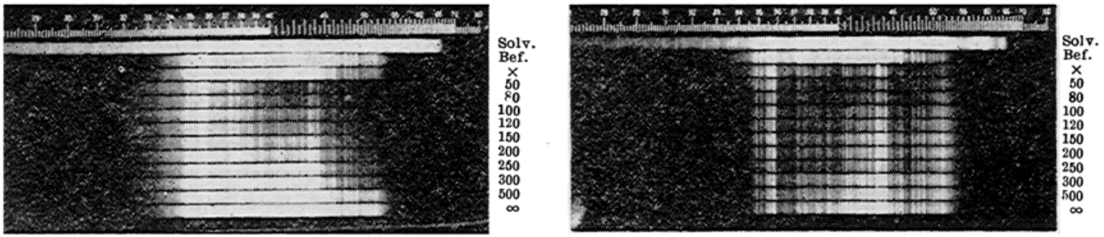
[1] S. Matsumoto, This Bulletin, 37, 491 (1964).

[2] S. Matsumoto, ibid., 35, 1860, 1866 (1962).



(a) (b)

Plate 1. Transient spectra in the presence of p-Cl·ϕ·G. Solv. buff. pH 7.0; M. B. 2.5×10^{-5} M; concn. of p-Cl·ϕ·G: (a) 10^{-6} M (b) 10^{-4} M.

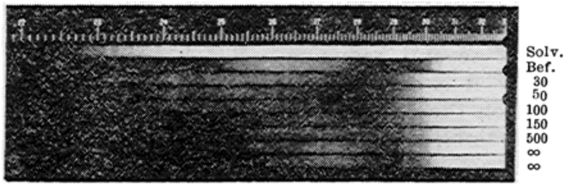


Plate 2. Transient absorption in the 220∼320 mμ in the presence of ϕ·G 10^{-5} M. Solv. buff. pH 7.4; M. B. 10^{-5} M.

N-p-chlorophenylglycine. In the case of *N-p*-bromophenylglycine, the transient absorption is faint. This must be due to the strong deactivating influence of the heavy atom-containing reductant on the triplet state of the dye. In the case *N-m*-bromophenylglycine, the formation of methylene blue semiquinone is more apparent, but the yield is still not as good as in the case of *N*-phenylglycine, to judge from the degree of the disappearance of methylene blue absorption. The situation is similar in the case of *N-p*-acetylphenylglycine.

The result with α-methyl-*N*-phenylalanine is characteristic. It is seen that the dye ion is converted into its colorless form almost invariably once it is excited by the flash. The relatively faint transient absorption in this case may indicate a low stationary concentration of the intermediate that is brought about by a strong reactivity of the reductant with the intermediate semiquinone. The absorption near 330 mμ may be due to the leuco methylene blue.[3] The spectrum in the 220∼320 mμ range (Plate 4) is in accord with these results. The above results, together with the results with *N-p*-methoxyphenylglycine described in section 1), support the general idea obtained earlier[2] about the reactivity of various derivatives of *N*-phenylglycine. One point to be modified concerns the mechanism of the deactivation of excited dye ions by some reductant. The excited state seems to be deactivated almost catalytically by *N*-phenylglycines containing heavy atoms at the ortho- or para-position of the benzene ring, while with *N*-phenylglycine having a methoxy substituent in either of these positions, a relatively long-lived intermediate (presumably the semiquinone of the dye) is formed which, in this case, is converted effectively into the ground state of the dye. The net result is an effective deactivation of the excited state. The mechanism of the strong inhibitory action of p-phenylenediamine also belongs to the latter category. This difference may, however, be superficial. Dye ions in their excited state may form a sort of charge-transfer complex, however short-lived, with reductant molecules before they are deactivated, as suggested by the effect of the substituent position.[2] In the case of *N*-phenyl-β-alanine, the disappearance of the methylene blue absorption and the increase in the semiquinone absorption is remarkable at first, but the recovery of the original absorption is rapid and complete compared with *N*-phenylglycine; the net quantum yield of the reduction of methylene blue is poor, in accordance with the previous results.[2] This observation stimulates a speculation as to the good quantum yield of photoreduction by *N*-phenylglycines. The half-oxidized form of *N*-phenylglycine may, for some reason, be liable to irreversible decomposition and may so become unable to reoxidize the semiquinone or the leuco base of dye, whereas the half-oxidized *N*-phenyl-β-alanine, with one more methylene unit between the amino- and the carboxyl-groups, may have a different stability and may remain efficient enough to reoxidize the semiquinone.

Photolysis in Organic Solvents.—The solvents used were methanol, ethanol, 1-propanol, 2-propanol, glycerol, acetone, pyridine and

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[3] A. T. Vartanyan, *Zhr. Fiz. Khimi.*, 29, 1304 (1955).

acetic acid. The solubility of the dye is slight in 1, 1-dimethylethanol and in less polar solvents. In these solvents flash illumination resulted in a more or less irreversible fading of the dye. The degree of fading observed visually after a same number of flashes was approximately in the order: 2-propanol < 1-propanol < acetic acid, glycerol < ethanol < methanol, pyridine <acetone. There seems to be no relation between the ease with which the solvent molecule is dehydrogenated by an ordinary oxidant and the above series. Probably the net quantum yield of photoreduction depends also on the succeeding reactions, by which the first oxidation product of the solvent is removed from the reacting system;

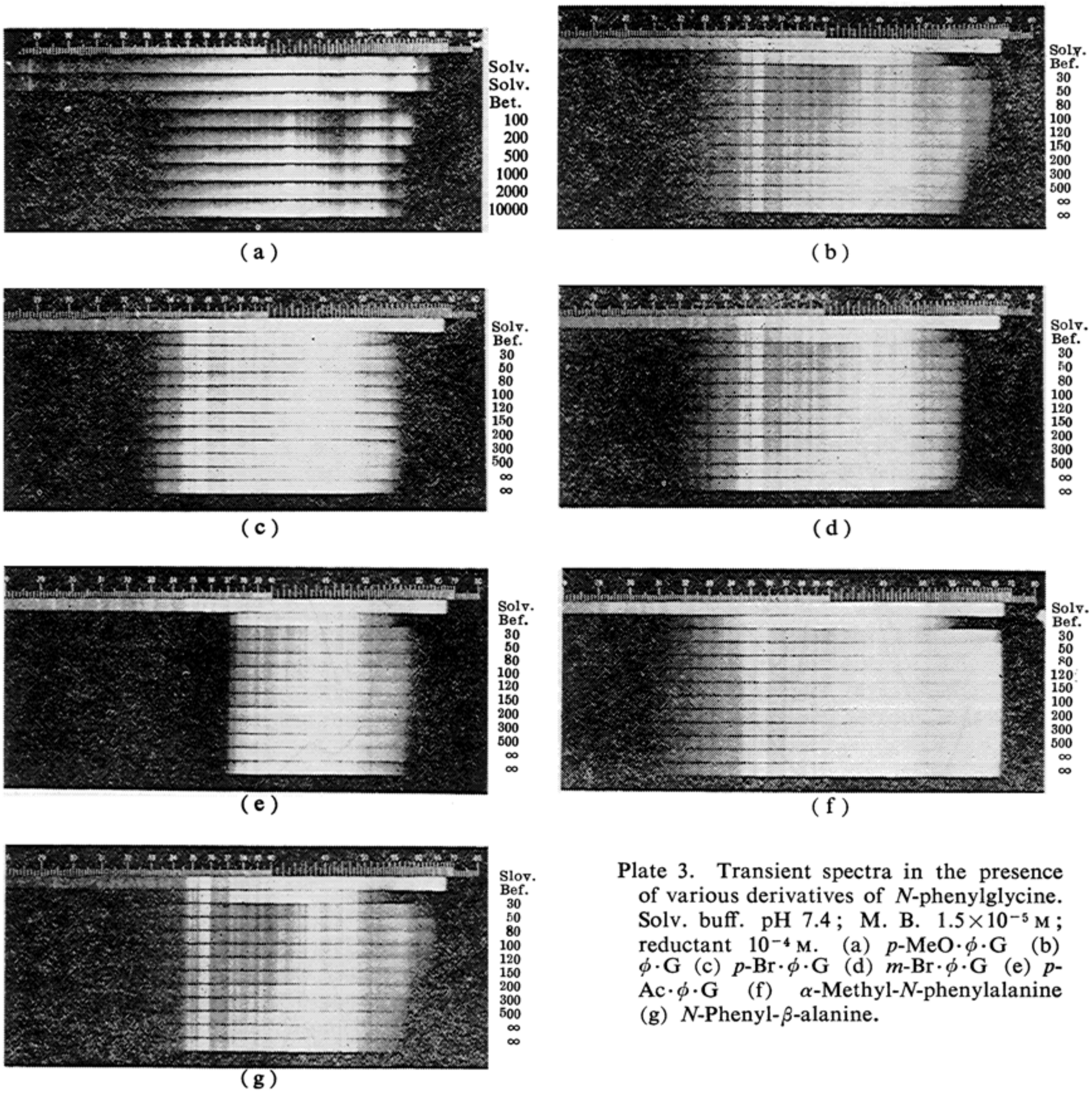


Plate 3. Transient spectra in the presence of various derivatives of *N*-phenylglycine. Solv. buff. pH 7.4; M. B. 1.5×10^{-5} M; reductant 10^{-4} M. (a) *p*-MeO·ϕ·G (b) ϕ·G (c) *p*-Br·ϕ·G (d) *m*-Br·ϕ·G (e) *p*-Ac·ϕ·G (f) α-Methyl-*N*-phenylalanine (g) *N*-Phenyl-β-alanine.

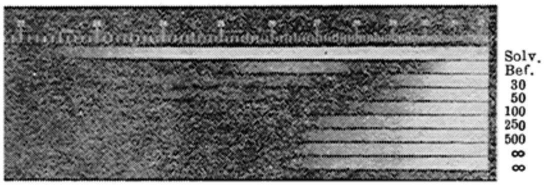
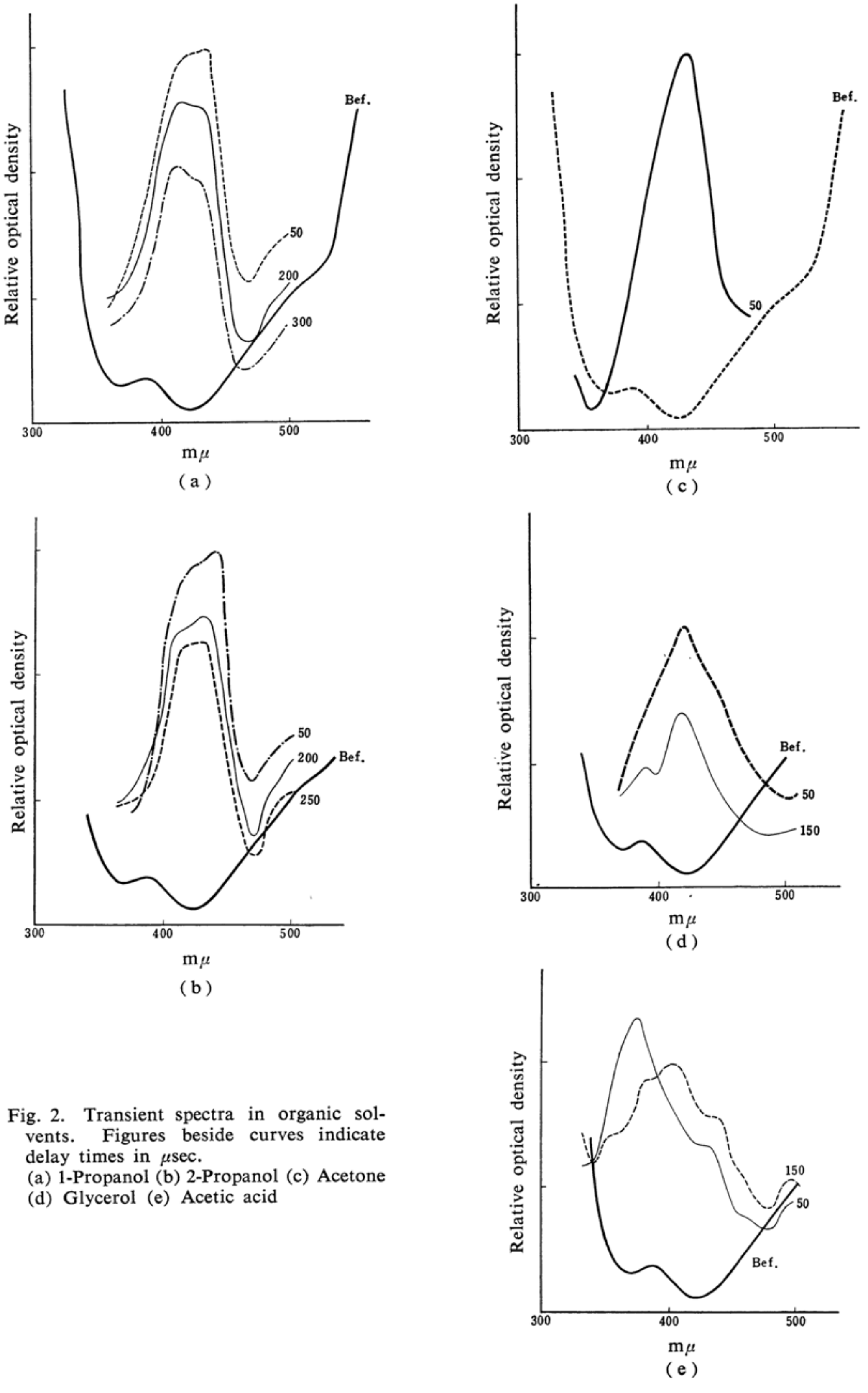


Plate 4. Transient absorption in the region $220\sim320\,\mathrm{m}\mu$ in the presence of α-methyl-*N*-phenylalanine 1.5×10^{-5} M.
Solv. buff. pH 7.4; M. B. 10^{-5} M



(a)

(c)

(b)

(d)

(e)

Fig. 2. Transient spectra in organic solvents. Figures beside curves indicate delay times in μsec.
(a) 1-Propanol (b) 2-Propanol (c) Acetone
(d) Glycerol (e) Acetic acid

the intermediary oxidation product will reoxidize the reduced dye back to the original form. The transient spectra appearing in these solvents are shown in Figs. 2a—2e. Except in acetic acid, the region of strong absorption is in the 400 mμ~430 mμ range. There is also a second peak around 515 mμ; a near-infrared absorption is also observed. As may be seen in the above figures, the absorption persists longer than that observed in plain water.

In the cases of 1- and 2-propanol, two absorptions seem to be superposed. The one which has a peak at a shorter wavelength appears later and persists longer. In these solvents and in glycerol there is a period during the reaction when the absorption in the visible range of the spectrum almost disappears. In time the methylene blue absorption is restored. This phenomenon is quite analogous to that observed in an aqueous medium when ferrous sulfate or hydroquinone is used as the reductant, and it must be attributed to the transitory formation of leuco methylene blue. All these observations suggest that the absorption around 400 mμ at a later stage of the reaction is mainly due to the semiquinone of the dye, although its peak is red-shifted compared with that in an aqueous medium. The spectrum in 1- or 2-propanol at early stages of photolysis may be nearly that of the triplet state. The spectrum observed in acetic acid shows reasonable shifts due to the acidity of the solvent (cf. Part I). Another difference between the transient absorption in an organic solvent and in an aqueous solvent is that, in the latter, a peculiar increase around 520 mμ at a later stage of photolysis is observed, while it is lacking in the former.

Summary

The flash photolysis of methylene blue in an aqueous medium with added *N*-phenylglycines or photolysis in organic solvents results in a more or less apparent bleaching of the dye. In the former case, the general features of the transient spectra are similar to those in the reversible photolysis treated in Part I. An absorption peak at 400 mμ in a solvent with a pH of 8.0 shifts to 430 mμ in a solvent with a pH of 8.8; it is to be attributed to the semiquinone of methylene blue. The absorption around 255 mμ increases at a later stage of the reaction, indicating the formation of leuco methylene blue. The photolysis seems to proceed in a way similar to that of the reversible cases. The results of flash photolysis with several derivatives of *N*-phenylglycine support the general idea of their reactivity obtained in a study with continuous illumination. There is a difference between the ways in which *N*-*p*-bromophenylglycine and *N*-*p*-methoxyphenylglycine retard the photoreduction, although this may be superficial. In the former case, some early intermediate (probably the triplet state of the dye) is deactivated almost catalyticaly, while in the latter case the deactivation takes place at the stage of a long-lived semiquinone-like intermediate. Peculiar features of photolysis with α-methyl-*N*-phenylalanine and *N*-phenyl-β-alanine have also been noted.

The degree of irreversible bleaching in an organic solvent has no regular relation to the ease with which the solvent molecule is dehydrogenated by an ordinary oxidant. The main part of the transient absorption in organic solvents is attributed to the semiquinone of the dye. The peak has somewhat red-shifted compared with that in water. Triplet absorption seems also to appear at an early stage of photolysis. The photolysis in 1- or 2-propanol reveals the reversible feature observed when ferrous ions were used as reductants.

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