The Formation of Stable Semiquinone of Methylene Blue by the Irradiation of Visible Light and γ-Rays.

By Masao KOIZUMI and Hiroshi OBATA

(Received May 15, 1958)

According to a recent paper of A. J. Swallow⁽1⁾, stable semiquinone radical is formed when a deaerated solution of methylene blue in 22.9 N sulfuric acid containing a small quantity of ethanol is irradiated by γ-rays. He considered that methylene blue, in this case, is reduced while ethanol is oxidized.

In connection with Swallow's results, we have taken up the following three questions.

1) Is it not possible to form semiquinone by the irradiation of Swallow's system with the visible light?

According to Swallow's view, ethanol is to be oxidized into aldehyde by OH radical produced from the radiolysis of water, hence semiquinone should remain unaffected by the existing aldehyde. Now it is well known that when alcoholic solution of methylene blue is irradiated by the visible light in vacuo, the dehydrogenation of alcohol by dye-ion takes place, the dye being transformed into the leuco compound. Therefore it is very natural to expect that the visible-light irradiation of Swallow's system will produce a stable semiquinone.

2) Is benzoic acid which is dissolved in concentrated sulfuric acid as a protonated form, more effective than alcohol for preparing semiquinone radical?

---

1) A. J. Swallow, *J. Chem. Soc.*, **1957**, 1553.

If ethanol acts as OH scavenger as Swallow seemingly supposed, benzoic acid, which is a strong OH scavenger in neutral or alkaline solution, is expected to be so in the case of strong acidic solution. In addition it would be interesting to examine the concentration effect of ethanol from the above viewpoint.

3) Which reaction will predominate when the solution of methylene blue in 22.9 N sulfuric acid but containing no ethanol is irradiated by γ-rays, the formation of semiquinone or the decomposition of methylene blue by OH radical? Or alternatively, does the reaction proceed in quite a different manner?

In case of a neutral solution of methylene blue, decomposition of dye and the formation of leuco compound take place simultaneously, the former being suppressed by the addition of OH scavenger. It therefore seems worth while to examine the case of strong acidic solution.

In order to examine these problems the following experiments were undertaken using a similar system to that of Swallow.

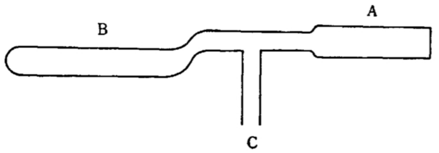

Fig. 1. Reaction cell.
Part A. Thickness 10 mm. plane parallel.
Part B. Diameter ca. 12 mm. (painted with black enamel).

Experimental

The reaction cells used are shown in Fig. 1. The solution is irradiated in Part B, the outside wall of which is painted with black enamel to interrupt the visible light. The absorption spectra of the solution is observed in Part A with the use of Hitachi Spectrophotometer. The solution, when necessary, is evacuated, transferred into B through C, and then the cell is fused off at C.

The irradiation of γ-rays was performed at the Department of Radiology in Tohoku University. Twenty Curie ^{60}Co injecting source was placed 25 cm. away from the samples and all the samples were irradiated for about 15 hours. The dose of γ-rays entering the solution during this period is estimated to be about 21000 Rentgen.

The irradiation of visible light was done by a projection lamp (75 V, 500 W) which was used at 55 V, and placed 4.5 cm. away from the cell.

The procedure for sampling was as follows. The solution of methylene blue 1×10^{-4}M in sulfuric acid (22.9 N) containing 0.001~0.6 M ethanol, for example, was prepared by mixing 0.25 cc. of 2×10^{-3}M aqueous methylene blue solution, 3.18 cc. of pure sulfuric acid and 1.57cc. of an aqueous alcoholic solution of appropriate concentration. The volume contraction during the mixing was disregarded. No special attention was paid to the formation of ester[2].

Below will be given the experimental results and discussions about the afore-mentioned three questions.

Results and Discussion

1) Photochemical Formation of Semiquinone.

— The results obtained for the

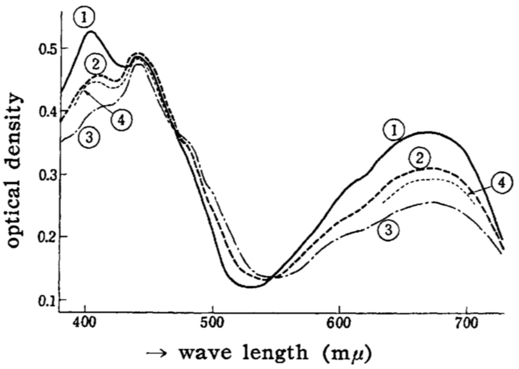

Fig. 2. Change of absorption spectra (visible light irradiation).
Methylene blue ca. 4×10^{-5}M
H_2SO_4 22.9 N
Alcohol 3.38 M
①—— initial
②- - - after 3.5 hr.' irradiation
③—·— after 8 hr.' irradiation
④······ exposed to oxygen after irradiation

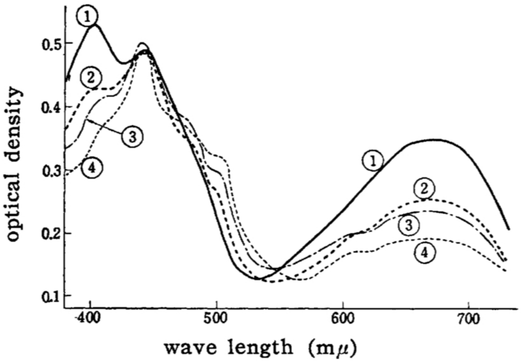

Fig. 3. Change of absorption spectra (visible light irradiation).
Methylene blue ca. 4×10^{-5}M
H_2SO_4 22.9 N
Alcohol 4.96 M
①—— initial
②- - - after 1 hr. irradiation
③—·— after 2.5 hr.' irradiation
④······ after 5.5 hr.' irradiation

---

[2] D. J. Clark and G. Williams, ibid., **1957**, 4218.

following two deaerated solutions are shown in Fig. 2 and Fig. 3.

a) Methylene blue ca. 4×10^{-5}M; sulfuric acid, 22.9 N; ethanol 4.96 M.

b) Methylene blue ca. 4×10^{-5}M; sulfuric acid, 22.9 N; ethanol 3.38 M.

The absorption curves of the illuminated solutions are quite similar to those reported by Swallow and in view of the investigation of Michaelis et al.[3], it can safely be concluded that the stable radical is very easily produced by the photochemical reaction. Perhaps the photochemical method would be a better one for preparing a stable free radical than that employing high energy radiation, since the reaction in the former case would be far more simple than in the latter.

2) Formation of Semiquinone by γ-ray Irradiation.—In Fig. 4 to Fig. 6 are shown the typical results obtained when the deaerated solutions of methylene blue in 22.9 N sulfuric acid containing ethanol of various concentrations or benzoic acid instead of ethanol are irradiated by γ-rays.

As to the effect of the concentration of ethanol, there exists scarcely any difference from 0.1 M up to 0.5 M. Below 0.01 M, the quantity of semiquinone formation seems somewhat smaller, but the reproducibility being not so good, an exact comparison is difficult. Roughly speaking, the effect of concentration is rather small from 0.001 to 0.5 M. The use of benzoic acid in place of ethanol is very effective for producing semiquinone and 0.005 M of the acid gives quite a similar result as that of 0.15 M or more of ethanol. Thus it is certain that benzoic acid which in this case must be dissolved as $C_6H_5COOH_2^+$ acts as a good OH-scavenger.

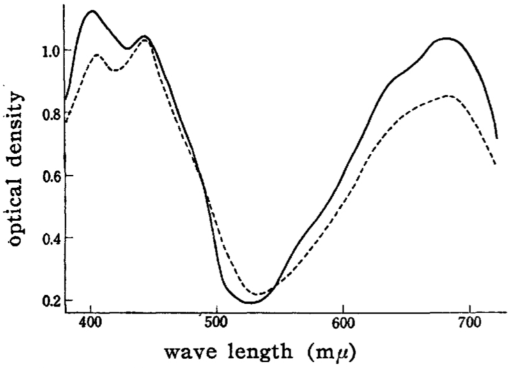

Fig. 4. Change of absorption spectra (γ-rays irradiation, 15 hr.)
Methylene blue 1×10^{-4}M
H_2SO_4 22.9 N
Alcohol 0.01 M
—— initial
······ after irradiation

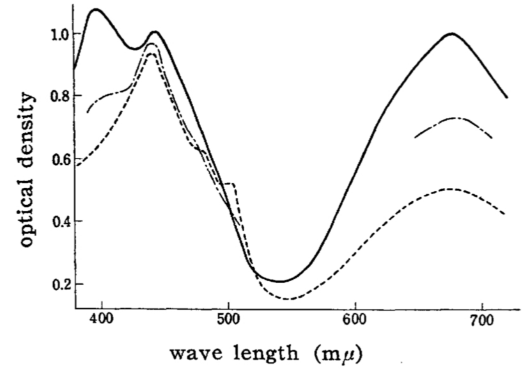

Fig. 5. Change of absorption spectra (γ-rays irradiation, 15 hr.)
Methylene blue 1×10^{-4}M
H_2SO_4 22.9 N
Alcohol 0.5 M
—— initial
······ after irradiation
–·– exposed to oxygen after irradiation

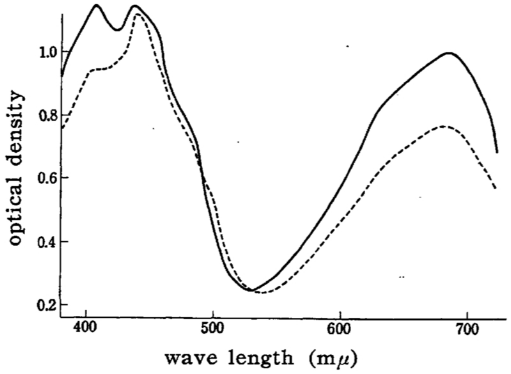

Fig. 6. Change of absorption spectra (γ-rays irradiation 15 hr.)
Methylene blue 1×10^{-4}M
Benzoic Acid 0.005 M
H_2SO_4 22.9 N
—— initial
······ after irradiation

The above results support the view that ethanol acts as a pure OH-scavenger and the necessary condition for the formation of semiquinone is perhaps the existence of OH-scavenger in a certain amount.

3) The Results when no OH-scavenger is added.—When the deaerated solution of methylene blue in 22.9 N sulfuric acid is irradiated by γ-rays, practically no

---

[3] L. Michaelis, M. P. Schubert and S. Granick, *J. Am. Chem. Soc.*, **62**, 204 (1948).

change is perceived in the original absorption curve. But the solutions of methylene blue in 22.9 N sulfuric acid not deaerated, when irradiated by γ-rays, show a small but definite decrease of absorption throughout the whole visible region as shown in Fig. 7 and Fig. 8, whether it contains 0.1 M ethanol or not. This decrease must undoubtedly be attributed to the destruction of some dye molecules.

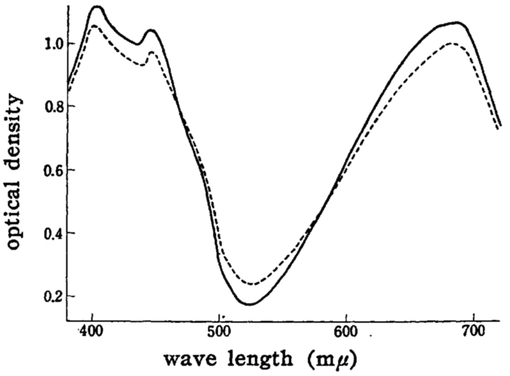

Fig. 7. Change of absorption spectra (γ-rays irradiation, 15 hr., in the presence of oxygen).

Methylene blue	1×10^{-4}M
H_2SO_4	22.9 N
Alcohol	0.1 M

—— initial
······ after irradiation

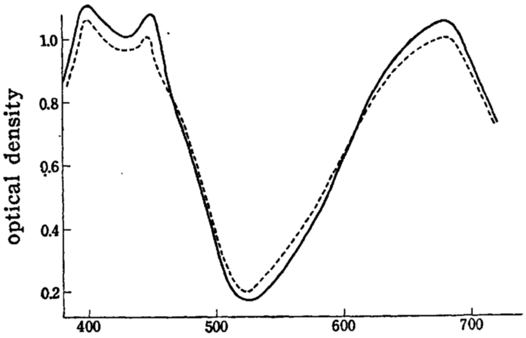

Fig. 8. Change of absorption spectra (γ-rays irradiation, 15 hr., in the presence of oxygen).

Methylene blue	1×10^{-5}M
H_2SO_4	22.9 N
Alcohol	not added

—— initial
······ after irradiation

These results are in sharp contrast with those for neutral solutions of methylene blue where the decomposition of dye and the formation of leuco-compound go hand in hand in vacuo. These noteworthy results would be interpreted as follows.

Let us take up the case of the deaerated system at first. If the attack of hydrogen atom and that of OH radical upon dye molecules take place simultaneously, both the formation of semiquinone and the irreversible destruction of dye molecules should occur as is the case in neutral solution. This contradicts the experimental results and the interpretation must be as follows. Hydrogen atom produced by the radiolysis of water first attacks the methylene blue molecule, converting it into semiquinone radical, and following this step, the attack of OH on the latter takes place recovering the original dye molecule. It seems worth while to note that OH attacks the semiquinone radical preferentially and in such a way as to reproduce the original dye, since this behavior is quite different from that of the neutral solution where dye molecules are believed to be attacked at various points by OH radical. In the presence of oxygen, the semiquinone radical intermediately produced would be reoxidized by OH radical (in case of no ethanol) or O_2 molecule (when ethanol exists), and the irreversible destruction would perhaps occur in a common way in the existent oxygen.

The above interpretation that hydrogen first attacks the dye molecule and then the produced semiquinone reacts with OH, seems at first rather curious. But the following consideration will make it very plausible.

The production of H and OH from water is generally believed to occur by the following processes.

$$H_2O \rightsquigarrow H_2O^+ + e \qquad (1)$$

$$\left.\begin{array}{ll} e + H_2O \rightarrow H + OH^- & (a) \\ e + H^+aq. \rightarrow H + aq. & (b) \end{array}\right\} \quad (2)$$

$$H_2O^+ + aq. \rightarrow OH + H^+aq. \qquad (3)$$

In the case of strong acidic solution, the hydrogen atom should be easily produced via 1 and 2b, but the existence of H^+aq. in large quantity would inhibit the reaction 3. Or it may be said that when the reaction 3 takes place, the reverse reaction would at once recover H_2O^+ again. Thus the oxidative radical that exists is not OH but H_2O^+ and this is expected to be removed by ethanol, but the dye ion which exists as a polyvalent cation would not be attacked by H_2O^+ owing to a strong electrostatic repulsion. This causes the reduction by hydrogen atom to proceed and H_2O^+ only attacks the semiquinone radical and by depriving its labile hydrogen atom stabilizes as H_3O^+. Such would perhaps be a correct interpretation for the fact that the dye is scarcely affected at all when OH scavenger does not exist.

Thus our results can be comprehended very well along Stein's general view[4] for the radiolysis of the aqueous solution.

Summray

In connection with Swallow's observation that methylene blue in 22.9 N sulfuric acid aqueous solution containing a small amount of alcohol is reduced to a stable semiquinone when irradiated with γ-rays in vacuo, some experiments were done to investigate the role of alcohol.

It was elucidated that alcohol acts most certainly as an OH scavenger. Benzoic acid was found to be an effective OH scavenger in this case just as in usual solutions.

In the absence of alcohol either the formation of semiquinone or the irreversible decomposition of dye scarcely ever takes place in vacuo while in the aerated solution, only the latter reaction slowly proceeds. A plausible interpretation of these results was given.

It was further found that the formation of stable semiquinone can easily occur on irradiation of Swallow's system with the visible light.

The authors express their thanks to Dr. Y. Koga, Professor of the Department of Radiology, Tohoku University, for permitting them to use the ^{60}Co injection source.

The cost of the present research has been partly defrayed by the Scientific Grant from the Ministry of Education.

Department of Chemistry, Faculty of
Science, Tohoku University
Katahira-cho, Sendai

---

[4] G. Stein, *Discussions Faraday Soc.*, **12**, 227 (1952).