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Measurements of Optical Absorption Spectra under ^{60}Co-γ Irradiation. Aqueous Methylene Blue Solutions Containing Oxygen

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Aerated aqueous solutions of methylene blue (MB^+; 3×10^{-7} M) were subjected to ^{60}Co-γ rays in the presence of added methanol, ethanol, or D-glucose at dose rates up to 4×10^{15} eV g^{-1}s^{-1}, and the change in the optical absorption spectrum in the 300—700 nm region was studied using a rapid-scan spectrophotometer (scanning speed: 30 ms/100 nm). In the presence of high concentrations ($>10^{-2}$ M) of organic substances, it was found that radiation caused a decrease in the absorption due to MB^+ at 650 nm (the magnitude of the decrease was proportional to the radiation intensity), and that, on the removal of the radiation source, the original absorption spectrum was restored. This was interpreted as meaning that the radiation-produced leuco-form of methylene blue (MBH) was rapidly oxidized by oxygen. The rate constant, $k(MBH+O_2)$, was estimated to be $(0.6\pm0.2)\times10^4$ M^{-1}s^{-1} at neutral pH. In the absence of added organic substances, the permanent decoloration of MB^+ took place; this was interpreted as being due to the reaction of MB^+ with OH-radicals. From the competition kinetic study using 10^{-4} M methanol, the rate constant, $k(OH+MB^+)$, was estimated to be 1.1×10^{10} M^{-1}s^{-1}.

Short-lived species in radiation-induced reactions have been studied by pulse-radiolysis techniques,[1] which, in most cases, employ an electron accelerator. On the other hand, much less effort has been made to study the short-lived species during continuous ^{60}Co-γ irradiations. Some reasons for this are: (1) The radiation from available Co-60 sources is, very often, not intense enough to produce measurable concentration of the species to be studied. (2) One must eliminate the radiation effects on the measuring systems, which is more difficult in γ-radiolysis than in electron-beam radiolysis.

We have now constructed an apparatus which can be adopted to a commercially-available spectrophotometer, *e.g.*, a Hitachi rapid-scanning spectrophotometer RSP-2, and which makes it possible to obtain an optical absorption spectrum in the 300-700 nm range of the liquid sample during irradiation up to 4×10^{15} eV g^{-1}s^{-1}. The details of the apparatus will be described elsewhere.[2] Here we wish to report the results of such measurements in the case of aerated aqueous solutions of methylene blue.

It is well known that methylene blue in air-free solutions containing various added organic substances loses its color upon irradiation, and that this decoloration can be reversed by the admission of air.[3,4] It is of interest, therefore, to measure the steady-state concentration of methylene blue in aerated solutions during the γ-radiolysis.

Experimental

The radiation source, 15 kCi ^{60}Co, was of a cylindrical type (40 cm in height, 13.4 cm in outer diameter). It was hung on a hoist which could be operated so as to move horizontally at a constant speed (3.3 cm s^{-1}).

The intensity of the radiation energy absorbed by a dilute aqueous solution at a horizontal distance of D cm from the center of the present source is expressed by Eq. (1), on the basis of the Fricke-dosimetry:[5]

$$I\ (\text{eV g}^{-1}\text{s}^{-1}) = 15.6\times10^{17}/D^2 \qquad (1)$$

$$\text{where}\quad D\geq 20\ \text{cm}$$

The reaction vessel was a 20-cm quartz tube 4.0 cm in internal diameter. The optically-flat windows were attached to the tube ends. The vessel was completely filled with the sample solution through the side arms attached to the vessel.

The reaction vessel was subjected to radiations at a fixed position (see Fig. 1) and at an ambient temperature *ca.* 25°C. The absorption spectra in the range from 330 to 700 nm were obtained using a Hitachi rapid-scanning spectrophotometer RSP-2 (scanning speed: 30 ms/100 nm) and were recorded on an oscilloscope (Hitachi V-018). Variations in the optical density at selected wavelengths could also be recorded as a function of the time.

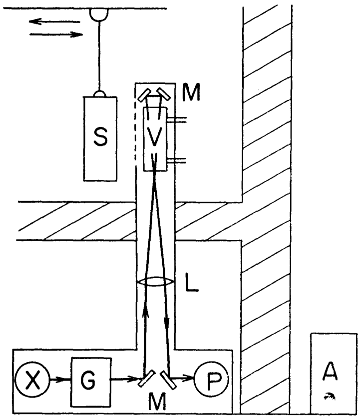

Fig. 1. Schematic drawing of optical arrangement:
X: a 150W Xe-lamp; G: monochrometer (a grating which can rotate for wave-length scanning); L: lens; V: reaction vessel; M: mirrors; P: photomultiplier; A: oscilloscope; S: radiation source. The unit, X, G, and P, makes a Hitachi rapid-scanning spectrophotometer RSP-2.

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[1] "Pulse radiolysis," Ed. by M. Ebert, Academic Press, London (1965).

[2] S. Ohno *et al.*, JAERI-Report, to be published.

[3] L. I. Grossweiner, *Radiation Res. Rev.*, **2**, 345(1970).

[4] A. J. Swallow, "Radiation Chemistry of Organic Compound," Pergamon Press, London (1960), p. 196."

[5] Measurements made by the ^{60}Co-source operating group of the Institute.

All the reagents were of an Analar grade and were used without further purification. The concentration of methylene blue were calculated using an extinction coefficient reported in the literature, *i.e.*, $0.77\times10^5\ \text{M}^{-1}\text{cm}^{-1}$ at 665 nm.[6] The optical path-length of the reaction vessel employed was 40 cm.

Results

Methylene Blue+Ethanol. Figure 2a shows the radiation effects on an optical absorption spectrum of an aerated solution of 3×10^{-7} M methylene blue in the presence of 0.1 M ethanol. Radiation (3.9×10^{15} eV g^{-1}s^{-1}) decreased the absorption at 650 nm by about 30%. However, this bleaching during the irradiation was found to be almost completely reversible, and, on the removal of the radiation source, the original absorption spectrum was restored.

Similar results were obtained when lower concentrations of ethanol (10^{-2} or 10^{-3} M) were used, though the recovery after the removal of the source was 90% when 10^{-3} M ethanol was employed.

Methylene Blue+D-glucose. Aerated aqueous solutions of 3×10^{-7} M methylene blue and 0.1 M D-glucose at various pH values (neutral, 0.1 N H_2SO_4, and 0.01 N NaOH) were examined. All these results were similar to those in the case of 0.1 M ethanol mentioned in the previous section. Both the decrease in absorption during irradiation and the subsequent recovery on removing the source could be repeated several times. The variation in the optical density at 650 nm with the time in the case of methylene blue in aerated 0.01 N NaOH containing 0.1 M D-glucose is shown in Fig. 2b. The radiation source was first brought near the sample (thus causing the decrease in absorption) and then removed from the sample at a constant speed of 3.3 cm s^{-1}.

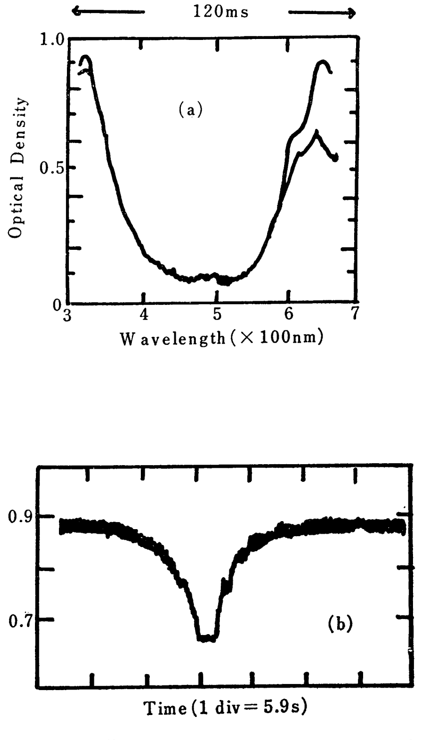

Fig. 2. Radiation effects on absorption spectra of aerated aqueous solutions of 3×10^{-7} M MB$^+$.
(a) 0.1 M ethanol present; Spectrum before irradiation (upper curve) and during the irradiation at dose rate of 3.9×10^{15} eV g^{-1} s^{-1} (lower curve); (b) Decrease in absorption and its recovery accompanying the transfer of the source (see text).

Irreversible Bleaching of Methylene Blue. A permanent decoloration of methylene blue occured when it was irradiated in an aerated, or in an argon-saturated, solution containing a low concentration (10^{-4} M) of methanol. The absorption spectra were recorded (*cf.* Fig. 3a) at various intervals after the source was placed at the fixed position (3.9×10^{15} eV g^{-1}s^{-1}). From the results shown in Fig. 3a, one may plot the decrease in the methylene blue concentration with the irradiation time, as in Fig. 3b. This bleaching was found to be irreversible. The initial yield for bleaching was 0.05 molecules per 100 eV of absorbed energy.

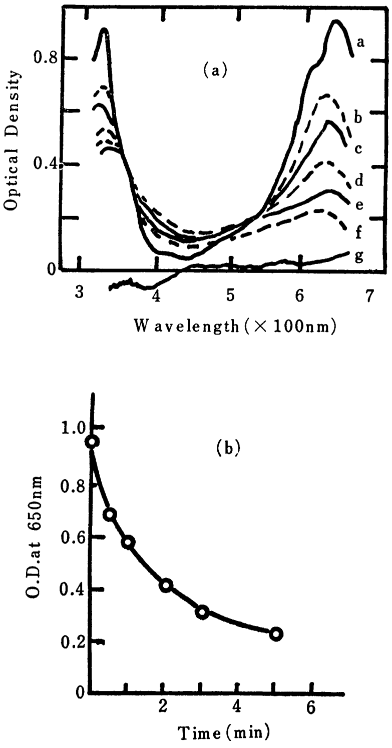

Fig. 3. Spectral change of an aerated solution of (3×10^{-7} M MB$^+$ + 10^{-4} M CH_3OH) with irradiation time at dose rate of 3.9×10^{15} eV g^{-1} s^{-1}.
(a) Spectrum before irradiation (a), and after 0.5(b), 1(c), 2(d), 3(e), and 5(f) min irradiation; Reference solution (g);
(b) Plot of optical density at 650 nm from the above results.

Discussion

When an aerated solution of methylene blue (MB$^+$; see Fig. 4) and high concentrations of organic substances are subjected to irradiation, the reactions which must be considered are very complex. Radiation decomposes water to give H, H_2, e_{aq}^-, H_3O^+,

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[6] J. P. Keene, E. J. Land, and A. J. Swallow, in Ref. 1, p. 227.

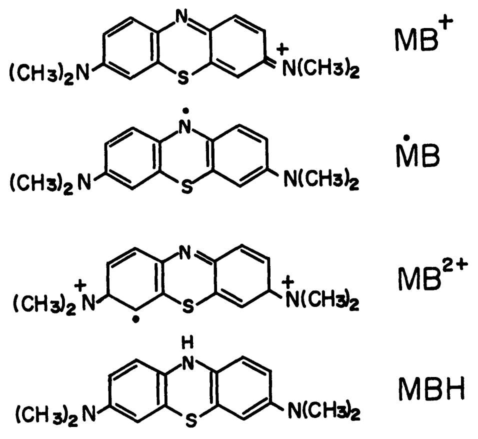

Fig. 4. MB^+ (methylene blue), $\dot{\mathrm{MB}}$(semiquinone-form), MB^{2+} (half-oxidized form), and MBH(leuco-form).

OH, and H_2O_2. In aerated solutions where the concentration of oxygen is $\sim 4\times10^{-4}$ M,[7] H and e_{aq}^- will predominantly react with O_2 to yield HO_2 or O_2^-. If the ratio of the concentration of the organic substance to that of MB^+ is high enough, all the OH-radicals react according to:

$$OH + RCH_2OH \rightarrow R\dot{C}HOH + H_2O \qquad (2)$$

where RCH_2OH represents ethanol, D-glucose, *etc.* The organic radicals are reducing agents:[3,4]

$$R\dot{C}HOH + MB^+ \rightarrow RCHO + \dot{M}B + H^+ \qquad (3)$$

where $\dot{\mathrm{MB}}$ represents the half-reduced semi-quinone form of the dye. Such species as $R\dot{C}HOH$ and $\dot{\mathrm{MB}}$ may react with themselves (*e.g.*, disproportionation) or with O_2, H_2O_2, O_2^-, or HO_2. These reactions have not yet been fully studied. However, one may assume that reactions involving these radicals are fast. Some relevant rate constants available from the literature are included in Table 1. The present study has failed to observe an absorption due to MB-semiquinone (λ_{max}: 420 nm, $\varepsilon=10{,}400\ \mathrm{M^{-1}cm^{-1}}$),[6] as may be seen from Fig. 2a. This may be because $\dot{\mathrm{MB}}$ is too short-lived to attain measurable steady-state concentrations under our experimental conditions. Therefore, it may be concluded that the reversible change in absorption at 650 nm during irradiation is related to the formation of the colorless leuco-base (MBH), which is the fully reduced form of the dye and which is well known to be rapidly oxidized by O_2 to MB^+.[3,4]

TABLE 1. SOME RELEVANT RATE CONSTANTS

Reaction	$k(\mathrm{M^{-1}\,s^{-1}})$	Ref.
$MB^+ + R\dot{C}HOH$ (D-glucose)	2×10^9	12
$MB^+ + e^-_{aq}$	2.5×10^{10}	6
$\dot{MB} + \dot{MB}$ (pH 7)	3.0×10^9	6
$MB^+ + OH$	1×10^{10}	11
$CH_3\dot{C}HOH + O_2$	4.6×10^9	13
$R\dot{C}HOH$ (D-glucose) $+ O_2$	1.6×10^9	13

Rate Constant for ($MBH+O_2$) Reaction. The radiation-induced chemical reactions which finally yield leuco-MB in the present system may be very complicated. However, they all occur in a very short time compared with the time required for the re-oxidation of MBH by O_2. Thus, the reaction sequence forming MBH may be simply expressed as:

$$MB^+ \xrightarrow{G_4 I} MBH \qquad (4)$$

where G_4 denotes the 100 eV-yield of MBH in the bulk of the solution, and I, the intensity of the radiation energy absorbed. The re-oxidation of MBH by O_2 may be:

$$MBH + O_2 \xrightarrow{k_{5a}} MB^+ + HO_2^- \qquad (5a)$$

Alternatively, it may be:

$$MBH + O_2 \xrightarrow{k_{5b}} \dot{MB} + HO_2 \qquad (5b)$$

followed by a further oxidation of $\dot{\mathrm{MB}}$, which can be assumed to be much faster than Reaction (5b). In either case, the rate of the disappearance of MBH may be expressed as $k_5[MBH][O_2]$. Thus, for the steady-state concentration of MBH (designated as $[MBH]_s$):

$$\begin{aligned} d[MBH]/dt &= 0 \\ &= G_4 I - k_5[MBH]_s[O_2] \qquad (6) \end{aligned}$$

We also obtain the relation:

$$[MB^+]_0 = [MB^+]_s + [MBH]_s \qquad (7)$$

where $[MB^+]_0$ is the initially-added MB^+ concentration, and $[MB^+]_s$, the steady-state MB^+ concentration observed during irradiation. Equation (6) will hold as long as a sufficient amount of the solutes (O_2, organic substance) remains in the solution under the irradiation. This condition will be fulfilled while the total absorbed dose does not exceed 10^{19} eV g^{-1} in the present case (4×10^{-4} M O_2 will be consumed at this dose if it disappears with $G=3$).

The G(MBH) values for deaerated solutions in the presence of sufficient amounts of organic substances have been reported to be 3.3,[8] 2.75,[9] and 2.9.[9] Thus, we may take a value of 3 for the value of G_4 in Eq. (6). The value of $[O_2]$ is 3.7×10^{-4} M for aerated aqueous solutions at 25°C.[7] Thus, one obtains:

$$[MBH]_s = \left(\frac{0.81\times10^4}{k_5}\right)\times I \qquad (8)$$

Figure 5 plots the $[MBH]_s$-values *vs.* I. These have been calculated using Eq. (7) and from the measurements of $[MB^+]_s$ at various I values (by varying the distance between the reaction cell and the radiation source) in the (3.7×10^{-7} M MB^++0.1 M ethanol) system. The reasonable fit of the data to a straight line demonstrates the validity of the above scheme. From the slope of the line, one obtains:

$$k_5 = (0.6\pm0.2)\times10^4\ \mathrm{M^{-1}s^{-1}} \quad \text{at neutal pH.}$$

Thus, the half-life of the leuco-form in the present

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[7] H. Hotta, A. Terakawa, and S. Ohno, This Bulletin, **33**, 442 (1960).

[8] E. Hayon, G. Scholes, and J. Weiss, *J. Chem. Soc.*, **1957**, 301.

[9] M. J. Day and G. Stein, *Radiation Res.*, **6**, 666(1957).

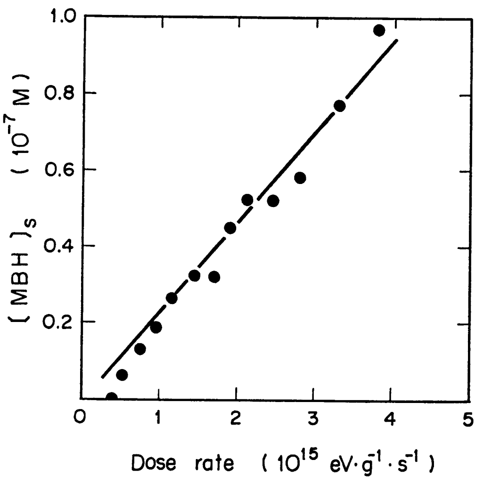

Fig. 5. Steady-state concentrations of leuco-form, $[MBH]_s$, as a function of dose rate, I.

system may be calculated to be $\ln 2/k_5[O_2]=0.3$ s.

Irreversible Decoloration of MB^+. It is generally accepted that the irreversible bleaching of MB^+ occurs by the attack of OH-radicals and that about three OH-radicals are required for one molecule of MB^+ bleached:[3)]

$$MB^+ + OH \rightarrow MB^{2+} + OH^- \qquad (9)$$

Thus, competition between Reactions (2) and (9) in the (3×10^{-7} M $MB^+ + 10^{-4}$ M CH_3OH) system would lead to the expression:

$$G(-MB^+)_{irrev.} = \frac{G_{OH}}{3}\left(\frac{k_9[MB^+]}{k_9[MB^+]+k_2[CH_3OH]}\right) \qquad (10)$$

One may use a value of 0.05 for $G(-MB^+)_{irrev}$ from Fig. 3b, a value of 2.5 for G_{OH},[1)] and a value of 5×10^8 $M^{-1}s^{-1}$ for k_2.[10)] The value of k_9 is then calculated to be 1.1×10^{10} $M^{-1}s^{-1}$, which is in fair agreement with the value obtained by Hutchinson.[11)]

Applicability of the Present Method. The apparatus used in this work may be used to observe the change due to irradiation in the optical absorption spectrum of any transparent material. An optical density change of 0.01 is detectable with the present apparatus. This means that the smallest concentration change which is detectable is approximately 10^{-8} M if a light absorbing species has an extinction coefficient of 10^4 $M^{-1}cm^{-1}$ (optical path-length of the cell: 40 cm). The presence of transient species during a continuous irradiation will be observed if the intensity of radiation is sufficient to produce a steady-state concentration higher than 10^{-8} M. The conditions required for this are determined by means of an equation similar to Eq. (6);

$$GI\times(\text{half-life}) \approx [\text{transient}] \qquad (11)$$

With numerical values appropriate to our present work ($G=3$, $I=3.9\times10^{15}$ $eVg^{-1}s^{-1}$, $[\text{transient}]=10^{-8}$ M), it may be concluded that optically-absorbing species with a half-life of 0.06 s or longer will be detected and their steady state concentration determined. Provided that the G-value, the rate of generation, is known from other methods (*e.g.*, chemical analyses), information on the rate constant for reactions of the transient and, thus, its lifetime in various media may be obtained.

This method does not utilize kinetic spectroscopy and can be readily used at laboratories with no electron accelerator. However, since the measurable species must have a lifetime of at least 0.06 s, the widespread application of the method cannot be expected at the present moment (*cf.*, the lifetime of the hydrated electron in water, ≈ 1 ms). Considerable refinement of this method would be possible by, for example, (1) improving the sensitivity of the detection system or (2) employing a larger radiation source. Of these two possibilities, the former one is of more practical interest. One may readily improve the sensitivity by employing a longer optical path-length of the cell. Alternatively, one may employ a highly sensitive detection technique, for example, a lock-in amplifier in conjunction with analyzing light choppers. Attempts to study shorter-lived species by the use of the present spectrophotometer method with some modifications are now in progress in our laboratory.

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10) M. Anbar and P. Neta, *Int. J. Appl. Rad. Isotopes*, **18**, 493 (1967).

11) F. Hutchinson, *Radiation Res.*, **9**, 13(1958).

12) E. A. Balazs, J. V. Davies, G. O. Phillps, and D. S. Scheufele, *J. Chem. Soc.*, **1968** C, 1424.

13) G. E. Adams and R. L. Willson, *Trans. Faraday Soc.*, **65**, 2981 (1969).