BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 1329—1336 (1970)

# The Radiolysis and Photolysis of Methanolic Solutions of Eosin. I. The $\gamma$-Radiolysis of Neutral and Alkaline Solutions

Kazuie KIMURA, Takuji MIWA*1 and Masashi IMAMURA

*The Institute of Physical and Chemical Research, Yamato-machi, Saitama*

(Received December 1, 1969)

The $\gamma$-radiolysis of eosin has been studied in deaerated neutral and alkaline methanolic solutions. Eosin is reduced principally with $CH_2OH$ radicals in dilute solutions. In neutral solutions, $G(-\text{eosin})$ is about 2 at the eosin concentration of $10^{-5}$M and increases as the concentration of eosin is increased. The final products are leuco-eosin and an unidentified substance which has an absorption peak at 455 nm. In an alkaline solution ($10^{-5}$M), $G(-\text{eosin})$ is about 4 and the product is semireduced eosin, which is stable in the dark and which undergoes photo-debromination. The ESR spectrum of semireduced eosin has also been recorded. Comparative studies have also been made on uranin solutions.

The photochemistry of several xanthene dyes, eosin, erythrosin, and uranin in aqueous and alcoholic solutions was investigated by Imamura and Koizumi during the 1955—1958 period.[1-6] Thereafter, Koizumi and his co-workers have worked on the flash photolysis of these systems and have elucidated the primary processes in detail; the outlines have recently been reviewed by Koizumi.[7] The flash-photolysis technique has also been applied by Lindqvist[8,9] and by Grossweiner and Zwicker[10,11]

*1 Present address: Institute of Natural Color Technology, Chiba University, Yayoi-cho, Chiba.

1) M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955).

2) M. Imamura and M. Koizumi, *ibid.*, **29**, 899 (1956).

3) M. Imamura and M. Koizumi, *ibid.*, **29**, 913 (1956).

4) M. Imamura, *ibid.*, **30**, 249 (1957).

5) M. Imamura, *ibid.*, **31**, 62 (1958).

6) M. Imamura, *ibid.*, **31**, 962 (1958).

7) M. Koizumi, *Nippon Kagaku Zasshi*, **90**, 117 (1969).

8) L. Lindqvist, *Ark. Kemi*, **16**, 79 (1960).

9) V. Kasche and L. Lindqvist, *Photochem. Photobiol.*, **4**, 923 (1965).

10) L. I. Grossweiner and E. F. Zwicker, *J. Chem. Phys.*, **31**, 1141 (1959); **34**, 1411 (1961); **39**, 2774 (1963).

11) E. F. Zwicker and L. I. Grossweiner, *J. Phys. Chem.*, **67**, 549 (1963).

to aqueous solutions of these dyes. The photochemical oxidation or reduction of these dyes is initiated by the reaction between the dye in the triplet state and the oxidizing or reducing substance or the dye in the ground state, depending upon the concentration of dye.

In the radiolysis of dilute solutions, however, dyes in the ground state are attacked by intermediates produced from the radiolyzed solvent. For aqueous solutions, the attacking species are principally hydrated electrons and hydroxyl radicals, and the products are semireduced and semioxidized dyes respectively.[12-14] Subsequent reactions seem similar to those in the photochemical systems. These intermediate products for uranin and eosin in aqueous solutions have been investigated by the pulse-radiolysis technique,[12,13] and the overall yields for the bleaching of eosin by $\gamma$-rays have been reported.[14] No corresponding studies have yet been reported on alcoholic solutions, however. As the radiolysis of alcohols has been investigated to a considerable extent,[15] it seemed that it would be interesting and worthwhile to compare the results for aqueous and alcoholic solutions. It seemed that it would be interesting also to observe if the dehalogenation of halogenated dyes occurred in the radiolysis; it has been observed but not fully explained in the photolysis of aqueous alcoholic solutions containing alkali.[3] The photochemical dehalogenation reaction will be described at length in the succeeding paper. In this paper the results obtained for the $^{60}$Co-$\gamma$ radiolysis of neutral and alkaline methanolic solutions of eosin, along with the ESR spectra of semireduced eosin and uranin produced in alkaline solutions, will be presented.

## Experimental

Eosin (disodium salt of 2′,4′,5′,7′-tetrabromofluorescein) of Merck was recrystallized four times from a slightly alkaline methanol solution. Uranin (disodium salt of fluorescein) of Merck was also purified similarly. The molar extinction coefficient of the purified eosin was $1.17\times10^{5}$M$^{-1}$cm$^{-1}$ in methanol at 521 nm, while that of uranin was $8.7\times10^{4}$M$^{-1}$cm$^{-1}$ at 495 nm.

Methanol of a spectral quality (Dotite Spectrosol) was refluxed with sodium borohydride (1 g/400 m*l*) before distillation on a 40-theoretical-plate column. A middle fraction of the distillate was taken and was dried by refluxing it on magnesium methoxide prepared by dissolving 2—3 g of magnesium turnings in 150 m*l*. Purified methanol was kept *in vacuo*.

Nitrous oxide (standard gas of Takachiho Chem. Ind.; purity stated >99.9%) was further purified by distillation *in vacuo*. Extra-pure sodium and potassium hydroxides were used without further purification.

A concentrated eosin solution in methanol (stored in the dark) was diluted with purified methanol by distillation *in vacuo* and then degassed thoroughly by the microstill reflux method.[16] Nitrous oxide was added to the degassed solution manometrically; the concentration was calculated from the solubility data.[17]

Solutions were irradiated in the dark with 1.2 kCi $^{60}$Co-$\gamma$ rays at a dose rate in the vicinity of $1\times10^{16}$ eV m$l^{-1}$min$^{-1}$. An irradiation ampoule made of Pyrex glass was provided with a quartz optical cell (10 or 1 mm thick). The optical cell was shielded from $\gamma$-rays with lead blocks during $\gamma$-irradiation. Dye solutions were kept in the dark throughout the experiment because some irradiation products were found to be particularly sensitive to light. The dose rate was determined by Fricke dosimetry on the basis of $G(Fe^{3+})=15.5$, with a correction for the electron density of the solvent used. The absorption spectra were recorded on a Shimadzu SV-50A spectrophotometer at room temperature.

The ESR spectra were recorded at room temperature with a JEOL 3B-X spectrometer with a 100 kHz-field modulation. A capillary cell (1 mm *i.d.*) attached to the irradiation ampoule was heated before use to remove any paramagnetism induced by $\gamma$-irradiation.

## Results

**Neutral Solutions.** Upon the $\gamma$-irradiation of neutral eosin solutions in methanol in the concentration range of $9.38\times10^{-6}$—$1.64\times10^{-4}$M, the absorption peak of eosin at the longest wavelength (521 nm) decreases with a slight shift (2—3 nm) to the shorter wavelength. Isosbestic points are observed in the vicinities of 242, 355, and 460 nm. A typical spectral change induced by $\gamma$-irradiation for the $7.64\times10^{-5}$M solution is shown in Fig. 1.

Close examination revealed that a small absorption band which was very sensitive to light appears between 360 and 450 nm. This absorption spectrum is shown more clearly in Fig. 2, which was obtained with the eosin solution ($9.29\times10^{-5}$M) subjected to prolonged irradiation. Other product absorptions are observed at 289 and <260 nm.

The absorption spectra of irradiated eosin solutions did not change for at least one day so long as the solutions were kept in the dark. However, upon the absorption of light the 455 nm absorption band decreased, finally disappearing as the 521 nm band increased. The saturation by air or bubbling oxygen gas into the irradiated eosin solution caused a slight depression of both 455 and 521 nm absorption bands, but they were restored by bubbling nitrogen gas.

12) J. Chrysochoos, J. Ovadia and L. I. Grossweiner, *ibid.*, **71**, 1629 (1967).
13) P. Cordier and L. I. Grossweiner, *ibid.*, **72**, 2018 (1968).
14) A. F. Rodde, Jr., and L. I. Grossweiner, *ibid.*, **72**, 3337 (1968).
15) J. Teply, *Radiat. Res. Rev.*, **1**, 361 (1969).
16) L. M. Theard and M. Burton, *J. Phys. Chem.*, **67**, 59 (1963).
17) A. Seidell, "Solubilities of Inorganic and Metal Organic Compounds," 2, 4th Ed., American Chemical Society, Washington, D. C. (1965), p. 802.

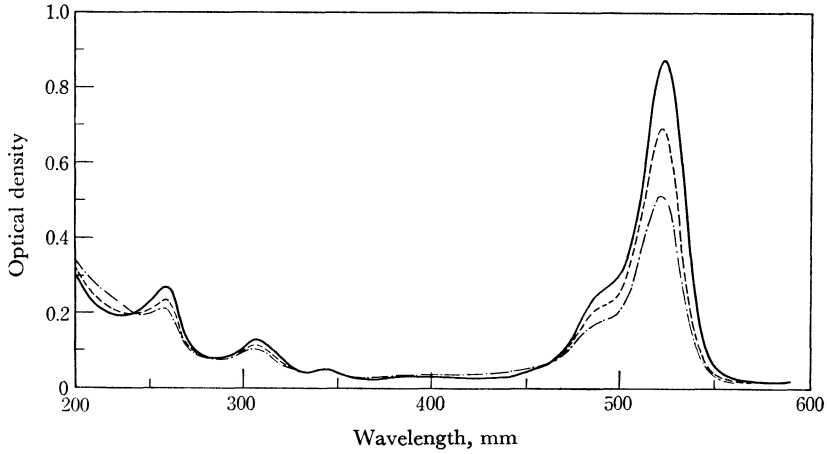



Fig. 1. Absorption spectra of $7.64\times10^{-5}$M eosin solution in MeOH before and after $\gamma$-irradiation ($l$=1 mm).
—— before irradiation, ------ after irradiation $6\times10^{17}$ eV/m$l$, -·-·- $1.2\times10^{18}$ eV/m$l$.

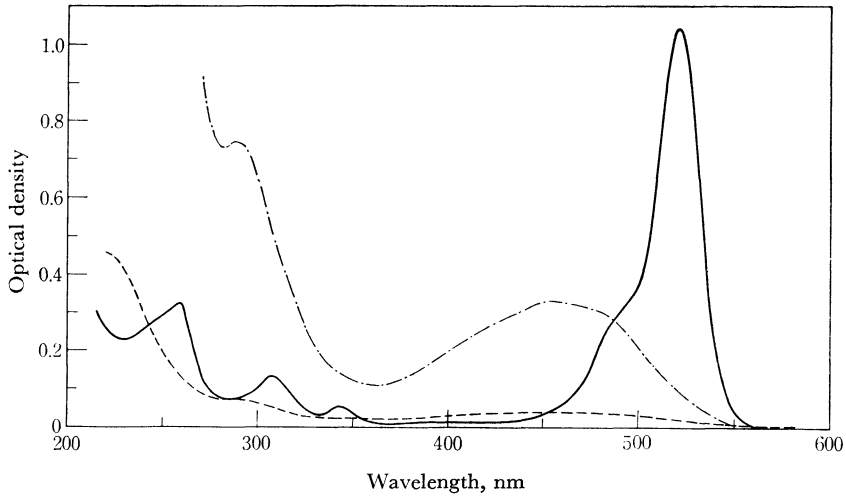



Fig. 2. Absorption spectra of $9.29\times10^{-5}$M eosin solution in MeOH before and after $\gamma$-irradiation.
—— before irradiation ($l$=1 mm), ------ afterirradiation $4.8\times10^{18}$ eV/m$l$ ($l$=1 mm), -·-·- ($l$=10 mm).

Similar absorption spectra were obtained with uranin solutions containing $2\times10^{-3}$M NaOH. Absorption peaks appear at 395, 280, 255, and $<$ 230 nm. The absorption at 395 nm probably corresponds to that for eosin at 455 nm; the latter was scarcely affected by light, however.

The radiation chemical yields for the disappearance of eosin can be obtained from the decrease in the optical density at 521 nm. The decrease in the optical density at 521 nm showed a good linear relationship with the radiation does. A typical example is shown in Fig. 3. The yields ($G$, the number of eosin molecules disappearing per 100 eV of energy absorbed) are plotted in Fig. 4 as a function of the initial concentration of eosin. The $G$(−eosin) rapidly increases to about 2 with an increase in the concentration from the initial 0 through $10^{-5}$M; then it increases rather slowly. The scattered plots in the higher concentration range may be partly due to the effect of stray light, despite the meticulous care, and partly to some contamination in the eosin.

In Fig. 4 are also shown the yields for the solutions containing $N_2O$ ($\sim10^{-2}$M), which is a solvated-electron scavenger. No substantial effect of $N_2O$ on the $G$-value is observed within the range of

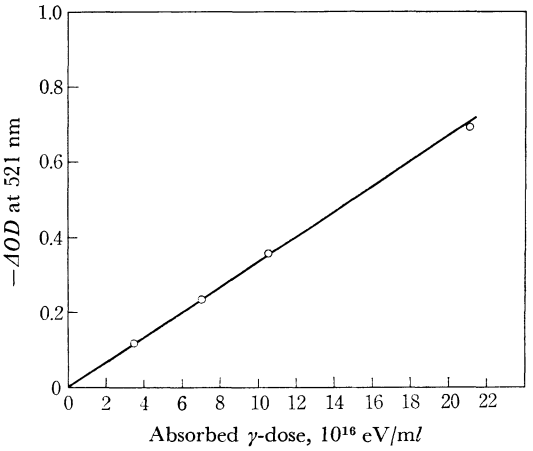



Fig. 3. A plot of $-\Delta OD$ at 521 nm *vs.* absorbed dose. Eosin $8.88\times10^{-6}$M in MeOH.

experimental error. The absorption spectra and the effects of light and oxygen on them were quite the same as in the case without $N_2O$.

**Alkaline Solutions.** Eosin disappears in an alkaline solution with $G(-\text{eosin})=4.0\pm0.3$ at $1.3\times10^{-5}$M eosin and 0.1—0.2M NaOH; this $G(-\text{eosin})$ is twice as large as in a neutral solution at the same concentration.

The absorption spectra of the irradiated eosin ($10^{-5}$—$10^{-3}$M) solutions in methanol containing 2 vol% $H_2O$ and alkali hydroxide (NaOH or KOH 0.1—0.3M) were quite different from those of neutral solutions. The absorption spectrum obtained with a $1.29\times10^{-5}$M eosin solution containing about 0.2M NaOH is shown in Fig. 5. Figure 5 shows that a product with its absorption maximum at 408 nm forms as the eosin disappears. A similar absorption spectrum was obtained with uranin under the same conditions, the peak wavelength being 394 nm.

This product is considerably stable *in vacuo* and in the dark, but upon the addition of oxygen it converts rapidly and almost completely into the original eosin or uranin. Assuming a full conversion of the product into eosin or uranin by the addition of oxygen, which is reasonable when the amount of bleached dye is small, one can obtain the molar extinction coefficients for the products as $\varepsilon(408\text{ nm})=(6.28\pm0.05)\times10^4\text{M}^{-1}\text{cm}^{-1}$ for eosin and $\varepsilon(394\text{ nm})=(3.95\pm0.03)\times10^4\text{M}^{-1}\text{cm}^{-1}$ for uranin both in 98 vol% methanol containing 0.1M NaOH.

By comparing these results with the previous photochemical results,[6,9,11,18,19] one can assign this product to the basic form of the semireduced eosin or uranin. The absorption spectra of semireduced eosin and uranin produced by the prolonged $\gamma$-irradiation of eosin and uranin solutions containing NaOH are shown in Figs. 6 and 7 respectively. The ESR spectra could in fact be obtained as shown in Figs. 8 and 9 for semireduced eosin and uranin respectively.

Interestingly, upon excitation with 408 nm light, the absorption at 408 nm of the irradiated eosin solution showed a hypsochromic shift as its intensity was decreased. Consequently, the ESR spectrum grew more complex, until ultimately it was superimposable on the corresponding spectrum obtained with an irradiated uranin solution. This result has

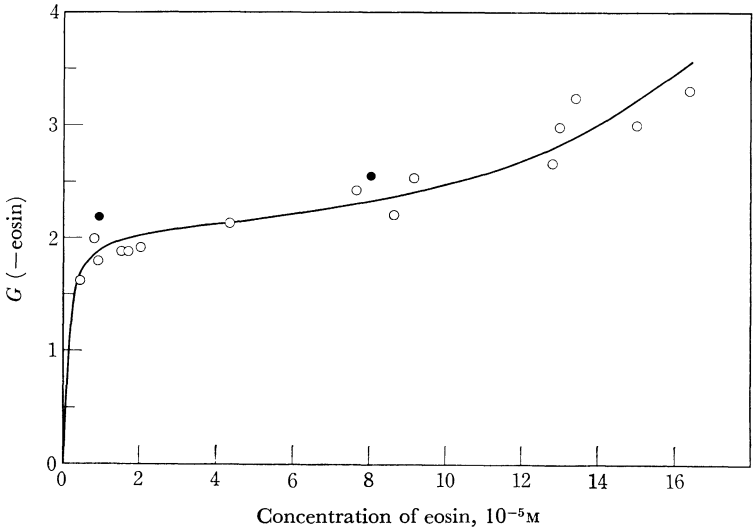



Fig. 4. $G(-\text{eosin})$ as a function of [eosin]. ○ in pure MeOH, ● in MeOH containing $N_2O$ ($1.6-1.9\times10^{-2}$M).

18) A. Kira and S. Kato, *Sci, Rep., Tohoku Univ.*, **48**, 142 (1965).

19) T. Ohno, S. Kato and M. Koizumi, This Bulletin, **39**, 232 (1966).

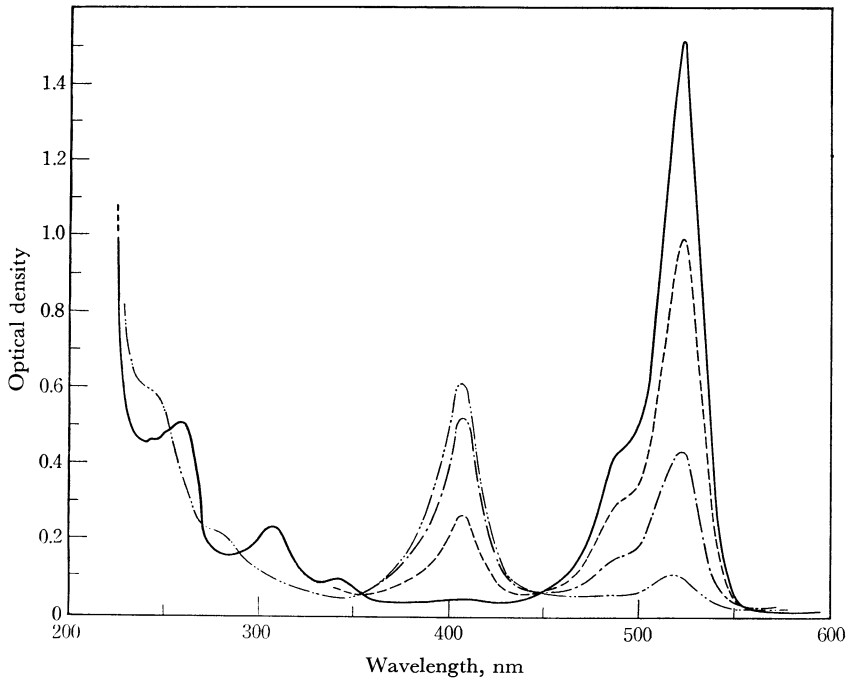



Fig. 5. Absorption spectra of $1.29\times10^{-5}$M eosin solution in 98% MeOH containing 0.2M NaOH before and after $\gamma$-irradiation ($l=10$ mm).
—— before irradiation, ····· after irradiation $7.5\times10^{16}$ eV/m$l$,
-·-·- $1.5\times10^{17}$ eV/m$l$, -··-··- $2.25\times10^{17}$ eV/m$l$.

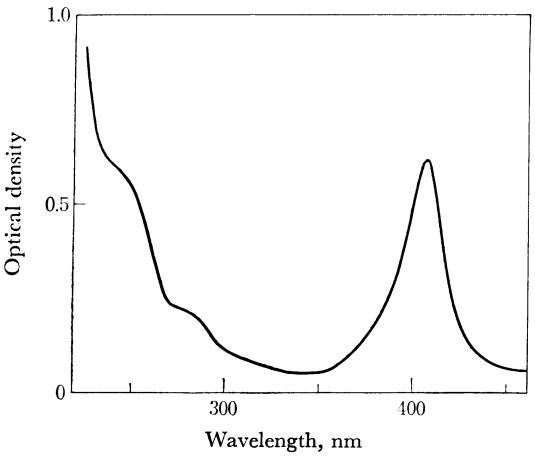



Fig. 6. Absorption spectrum of semireduced eosin prepared by prolonged $\gamma$-irradiation ($2.8\times10^{17}$ eV/m$l$) of $1.4\times10^{-5}$M eosin solution in 98% MeOH containing 0.3M NaOH.

been briefly communicated elsewhere;[20] a full account will be reported in the succeeding paper.

## Discussion

**Reactive Species in Aqueous and Methanolic Solutions.** The radiolysis of methanol yields several primary free radicals:

$$CH_3OH \rightsquigarrow e^-_{solv}, \cdot H, \cdot CH_3O, \cdot CH_2OH, \cdot CH_3, \cdot OH, \text{ and molecular products} \quad (1)$$

In the radiolysis of a methanolic solution of eosin in the absence of oxygen, eosin will be reduced with some of these radicals. In an aqueous solution eosin is assumed to react with radicals by the following fast processes:[12,14]

$$D + e^-_{aq}(H) \rightarrow \cdot S \quad (2)$$

$$D + \cdot OH \rightarrow \cdot X + OH^- \quad (3)$$

$$D + \cdot OH \rightarrow \cdot DOH \quad (4)$$

where D, S, X, and DOH are eosin, semireduced eosin, semioxidized eosin, and OH adduct respectively. The rate constants of Reactions (2) and (3) have been determined by the pulse-radiolysis technique to be $k_2 \geq 5\times10^9$M$^{-1}$ sec$^{-1}$ and $k_3=(1.4\pm0.4)\times10^{10}$M$^{-1}$ sec$^{-1}$.[12]

In the $\gamma$-radiolysis of methanol, solvated electrons ($G=2.0$[21]) decay with a rate constant of the order of $10^5$ sec$^{-1}$:[22,23]

20) K. Kimura, T. Miwa and M. Imamura, *Chem. Commun.*, **1968**, 1619.

21) H. Seki and M. Imamura, *J. Phys. Chem.*, **71**, 870 (1967).

22) H. Seki and M. Imamura, to be published.

23) a) I. A. Taub, D. A. Harter, M. C. Sauer, Jr., and L. M. Dorfman, *J. Chem. Phys.*, **41**, 979 (1964). b) F. S. Dainton, J. P. Keene, T. J. Kemp, G. A. Salmon and J. Teply, *Proc. Chem. Soc.*, **1964**, 265. c) K. N. Jha and G. R. Freeman, *J. Phys. Chem.*, **48**, 5480 (1968).

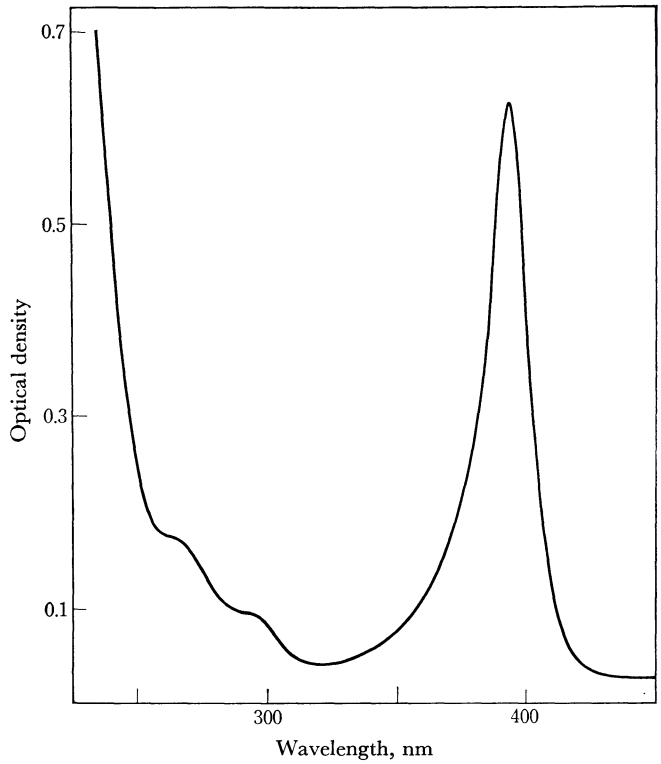



Fig. 7. Absorption spectrum of semireduced uranin prepared by prolonged $\gamma$-irradiation ($1.4\times10^{16}$ eV/m*l*) of $1.47\times10^{-5}$M uranin solution in 98% MeOH cantaining 0.1M KOH.

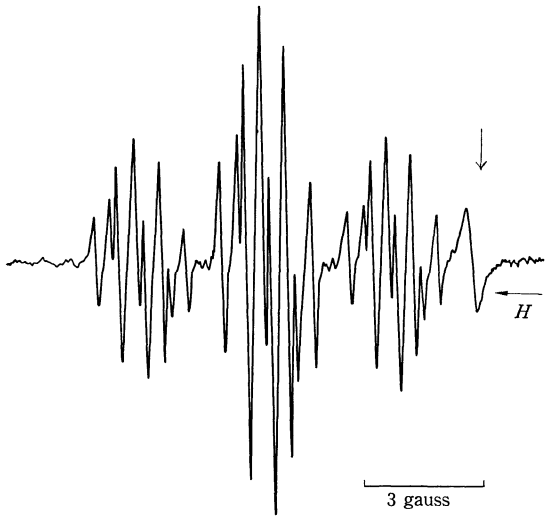



Fig. 8. ESR spectrum of semireduced eosin prepared by $\gamma$-irradiation ($10^{18}$ eV/m*l*) of $1\times10^{-4}$M eosin solution in 70% MeOH cantaining 0.3M NaOH. The arrow indicates the standard signal of $K_2(SO_3)_2NO$.

$$e^-_{solv} \rightarrow CH_3O^- + \cdot H \qquad (5)$$

and H atoms ($G=1.7$[21]) react with methanol with $k_6=1.6\times10^6$M$^{-1}$ sec$^{-1}$ [24] to produce hydrogen and $CH_2OH$ radicals:

$$\cdot H + CH_3OH \rightarrow H_2 + \cdot CH_2OH \qquad (6)$$

The other radicals produced by Reaction (1) also yield $CH_2OH$ radicals by reactions with methanol, the total radical yield in the $\gamma$-radiolysis of methanol being determined to be 6.2.[25,26]

**Reduction of Eosin in Methanolic Solutions.** If the rate constant between the solvated electron and eosin in methanol is assumed to be the same as that in the aqueous solution,[27] $k_2[D]<k_5$ for the solution of $10^{-5}$—$10^{-4}$M eosin. Figure 4 shows that $G(-\text{eosin})$ is about 2 at eosin concentrations as low as $10^{-5}$M; above this point the rate of the increase in $G(-\text{eosin})$ is lower. This fact may indicate that eosin undergoes reduction principally by Reaction (7) to form semireduced eosin:

$$D + \cdot CH_2OH \rightarrow \cdot S + CH_2O \qquad (7)$$

24) P. Neta and L. M. Dorfman, *J. Phys. Chem.*, **73**, 413 (1969).
25) S. U. Choi and N. N. Lichtin, *J. Amer. Chem. Soc.*, **86**, 3948 (1964).
26) H. Seki, R. Nagai and M. Imamura, This Bulletin, **41**, 2877 (1968).
27) This assumption will be correct if reactions are diffusion-controlled.

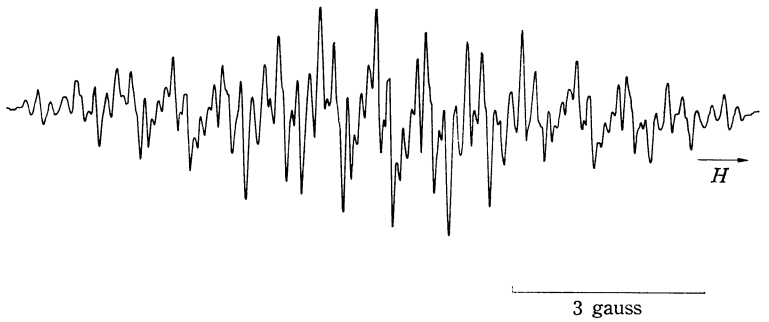



Fig. 9. ESR spectrum of semireduced uranin prepared by $\gamma$-irradiation ($10^{18}$ eV/m$l$) of $1.4\times10^{-4}$M uranin solution in 80% MeOH containing 0.4M NaOH.

A similar reaction between eosin and $C_2H_4OH$ radicals has been proved by Grossweiner *et al.* for aqueous eosin solutions containing 1 mM $H_2O_2$ and 10 mM $C_2H_5OH$; the rate constant of this reaction is $(1.1\pm0.2)\times10^{9}$M$^{-1}$ sec$^{-1}$.[12]

Hydroxymethyl radicals combine with each other to form ethylene glycol:

$$\cdot CH_2OH + \cdot CH_2OH \rightarrow C_2H_6O_2 \qquad (8)$$

Reaction (8) is the principal reaction pathway of $CH_2OH$ radicals in the radiolysis of methanol[26] and competes with Reaction (7) in the present systems. The rate constant of Reaction (8) has not been determined, but $2k=(1.4\pm0.4)\times10^{9}$M$^{-1}$ sec$^{-1}$ (23°) has been reported for $C_2H_4OH$ radicals.[28]

**Effect of Nitrous Oxide.** The argument for Reaction (7) is obtained from the results with eosin solutions containing $N_2O$. Nitrous oxide scavenges solvated electrons in methanol completely at less than $5\times10^{-3}$M to form equivalent amounts of $N_2$ and OH radicals:[21,23c]

$$e^-_{solv} + N_2O \rightarrow N_2 + O^- (\rightarrow \cdot OH) \qquad (9)$$

The hydroxyl radicals produced by Reaction (9) react rapidly with the neighboring methanol to form $CH_2OH$ radicals. If eosin is reduced with $CH_2OH$ radicals, as has been mentioned above, the addition of $N_2O$ to an eosin solution should not have any appreciable effect on $G(-\text{eosin})$; that is indeed the case, as may be seen from Fig. 4. As the concentration of eosin is increased, however, the reduction with solvated electrons may be of increasing importance.

**Leuco-Dye Formation in Neutral Solutions.** In deaerated aqueous solutions the reaction intermediates of eosin were assumed to decay predominantly by the back reaction:

$$\cdot S + \cdot X \rightarrow 2D \qquad (10)$$

accompanied by slower processes:

$$2X\cdot \rightarrow \text{products} \qquad (11)$$

$$2S\cdot \rightarrow D + L \qquad (12)$$

where L is the leuco-eosin.[14]

In dilute methanolic solutions, however, a principal intermediate is semireduced eosin, for the oxdizing radicals, $CH_3O$ and OH, will rapidly disappear to form $CH_2OH$ radicals by reactions with methanol. Thus, the semireduced eosin in methanol decays according solely to Reaction (12) to form leuco-eosin.

The absorption spectrum of the prolonged $\gamma$-irradiated eosin solution, Fig. 2, has three absorption peaks, at wavelengths of $<$230, 290 and 455 nm. For an irradiated uranin solution, four absorption peaks due to stable products were observed at $<$230, 255, 280, and 395 nm. The absorption spectrum of leuco-eosin has not been determined. However, Uchida *et al.*[29] have reported the absorption spectrum of leuco-uranin, which has two peaks at 210 and 280 nm ($\varepsilon=5\times10^{3}$M$^{-1}$cm$^{-1}$). We re-determined the absorption spectrum with leuco-uranin prepared chemically by a method similar to that employed by Uchida *et al.* and confirmed the existence of three absorption peaks, at $<$230, 255, and 280 nm. (We did not succeed in preparing leuco-eosin). Comparing the absorption spectra for the irradiated eosin and uranin with that for the chemically-prepared leuco-uranin, one may reasonably ascribe two of the three absorption peaks for eosin and three of the four for uranin to the formation of leuco-eosin and leuco-uranin by $\gamma$-irradiation.

**Absorption at 455 nm Produced by $\gamma$-Irradiation.** An absorption band at 455 nm produced in the $\gamma$-irradiated neutral eosin solution is sensitive to light, but is not affected by oxygen. A similar absorption appears in the uranin solution at 395 nm, but it is not sensitive at all to either light or oxygen. A free-radical nature can, therefore, be ruled out. The resumption of a complex between leuco-dye and one of the radiolysis products of methanol (say, formaldehyde) can also be ruled out, for no such absorption was observed for the leuco-dye solution containing formaldehyde. The experi-

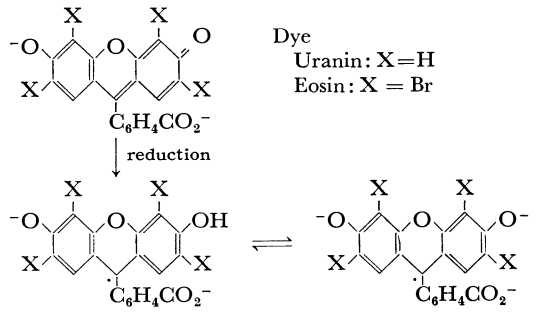
28) L. M. Dorfman and I. A. Taub, *J. Amer. Chem. Soc.*, **85**, 2370 (1963).

29) K. Uchida, S. Kato and M. Koizumi, This Bulletin, **35**, 16 (1962).

mental facts that, upon light absorption, a simultaneous decrease in the 455 nm band and increase in the 521 nm band, with slight shifts to shorter wavelengths, were observed, and that no similar change was observed for uranin solutions, may indicate that the 455 nm absorption is due to an unidentified product which undergoes photo-debromination (see below).

**Intermediate in Alkaline Solutions.** The reaction intermediate produced in alkaline eosin solutions ($\lambda_{max}=408$ nm) can be identified with semireduced eosin. The semireduced eosin formation in aqueous media has been confirmed by the flash-photolysis technique;[9,11,18,19] the molar extinction coefficient at the peak wavelength has been reported as $\varepsilon=4\times10^4$ (405 nm),[9] $3.8\times10^4$ (405 nm),[11] and $2.5\times10^4$ M$^{-1}$ cm$^{-1}$ (408 nm).[19] For uranin $\varepsilon=5\times10^4$ M$^{-1}$ cm$^{-1}$ (394 nm) in an aqueous medium has been reported as well.[8] Imamura found that the semireduced uranin is produced photochemically in alcoholic solutions and that it is stable in the dark in the absence of oxygen.[6] Several semireduced quinones have been known to be stable in alkaline solutions as a result of the formation of symmetrical anions. In alkaline solutions $G(-\text{eosin})$ is about twice that for neutral solutions; this indicates that the semireduced eosin is stable and does not undergo Reaction (11).

Semireduced xanthene dyes may be represented as follows:




**ESR Spectra of Simireduced Eosin and Uranin.** The ESR spectrum for an irradiated eosin solution, Fig. 8, consists of three groups of lines with the intensity ratio of 1 : 2 : 1 and with the coupling constant of 3.16 gauss. Each group has hfs with the ratio of 1 : 1 : 3 : 3 : 3 : 1 : 1. The fact that the ESR spectrum for the uranin solution (Fig. 9) is more complex than for the eosin solution is apparent from the fact that four bromine atoms are replaced by hydrogen atoms for uranin. This result is in agreement with, and is evidence for, the conclusion that the product is the semireduced eosin. An ESR study of the semireduced uranin has been carried out by Okuda *et al.*;[30] the analysis of the ESR spectra for semireduced forms of xanthene dyes will be described in detail elsewhere.

30) M. Okuda, Y. Momose, S. Niizuma and M. Koizumi, *ibid.*, **40**, 1332 (1967).