

298. *The Preparation of Stable Free Radicals in Solution by Means of Ionising Radiation.*

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Irradiation of a mixture of an easily reduced and an easily oxidised substance gives rise to a coupled oxidation-reduction. Methylene-blue has been irradiated in the presence of ethanol, and in strongly acid solution is reduced to the same stable semiquinone free radical as is produced by titanous chloride. Thionine also gives some free radical under these conditions, but attempts to prepare triphenylmethyl and diphenylpicrylhydrazyl by irradiation have not succeeded. Reasons are given.

A CHARACTERISTIC reaction can occur in good yield when an aqueous two-solute system is irradiated with X - or γ -rays, β -particles, or fast electrons: ¹ one of the solutes becomes reduced while simultaneously the other becomes oxidised. Often one of the solutes is oxygen. The reaction has been most studied for aqueous solutions, but it can also occur in organic solvents,² the solvent itself becoming oxidised. We have used this reaction to prepare a stable free radical from methylene-blue.

The oxidisable substance used in this work was 0.5M-ethanol, the concentration of the dye being 1.6×10^{-4} M. In order to obtain results which could be compared with those of Michaelis, Schubert, and Granick,³ methylene-blue was dissolved in 22.9N-sulphuric acid.

To obtain a control, 4 ml. of 1.6×10^{-4} M-methylene-blue were half-reduced under

¹ Proskurnin, Orekhov, and Barelko, *Doklady Akad. Nauk, S.S.S.R.*, 1955, **103**, 651; *Uspekhi Khim.*, 1955, **24**, 584.

² Piffault, Duhamel, and Longuet, *Acta Radiol.*, 1953, **39**, 64; Collinson, Dainton, and Thomson, see Collinson and Dainton, *Nature*, 1956, **177**, 1224.

³ Michaelis, Schubert, and Granick, *J. Amer. Chem. Soc.*, 1940, **62**, 204.

nitrogen by the addition of 0.0204 ml. of $3.14 \times 10^{-2}N$ -titanous chloride in 22.9N-sulphuric acid (standardised against a neutral methylene-blue solution). The absorption curve of the yellow solution is shown in Fig. 1 together with the curve of the original methylene-blue solution (green at this acidity). According to Michaelis *et al.*,³ 55% of the reduction product is in the semiquinone form.

4-ml. of $1.6 \times 10^{-4}M$ -methylene-blue solution which was also 0.5M in ethanol were irradiated in the absence of air with ^{60}Co γ -rays. The change in optical density with dose was followed at 400, 440, 500, 680, and 750 $m\mu$ and, when the values approximated closely to those shown in Fig. 1B, the complete absorption curve was taken (Fig. 2). Air was then admitted to the solution and a few small crystals of copper sulphate added to catalyse the oxidation of the product. Within a few hours the solution had regained its original green colour, the optical densities at 400 and 440 $m\mu$ being 0.11 and 0.04 below their original values, while the optical density at 500 $m\mu$ was 0.06 above its original value.

It is clear that irradiation and titanous chloride have produced the same reduced forms of methylene-blue. The irradiation method also appears to have produced ~10% of irreversible destruction, as was noted for neutral solutions by Day and Stein.⁴ From

FIG. 1. Absorption curve of (A) methylene-blue solution and (B) the chemically reduced product.

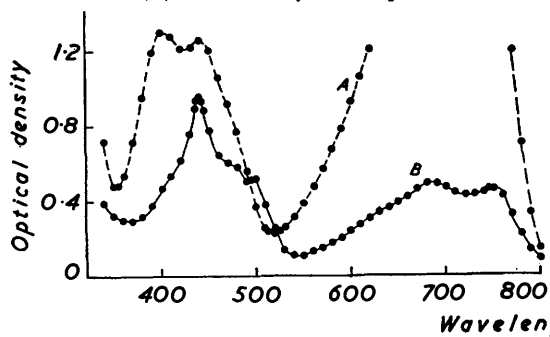
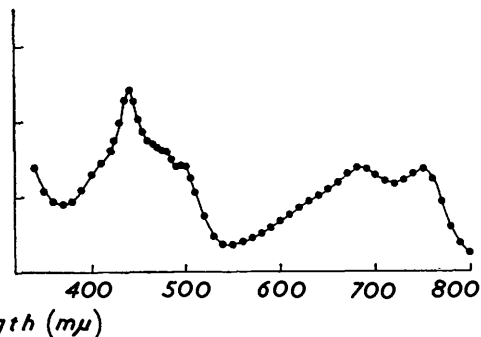


FIG. 2. Absorption curve of irradiated solution.



the dose required to produce the curve shown in Fig. 2, the overall yield of the irradiation reduction is $G = 3.4 \pm 0.4$ molecules of methylene-blue reduced per 100 ev absorbed. This value cannot be quoted more accurately because of difficulties in accurate titration with dilute titanous chloride.

It is suggested that oxidation or reduction induced by radiation could be applied in chemical analysis as a substitute for titration in certain cases. The general advantages of radiation as an agent are that any substance can be treated in any phase, in any solvent, at any pH, and at any temperature. Control by dose given would be exceptionally easy, and the "titration" could be carried out in a closed vessel.

Other Free Radicals.—Experiments were performed with $2.4 \times 10^{-4}M$ -thionine in 26.6N-sulphuric acid. The chemically prepared free radical showed absorption maxima at 440, 460, 480, 500, and 695 $m\mu$ but it was impossible to obtain an identical curve by irradiation in the presence of 0.5M-ethanol, a peak at 463 $m\mu$ being noted as well as peaks at 440, 500, and 695 $m\mu$. Although some free radical is present, as was also shown by the partial restoration of colour by air, there must also be other products. The situation is further complicated by the existence of an appreciable dark reaction of ethanol with thionine.

Air-free solutions of triphenylmethyl chloride and triphenylmethane in hexane have been irradiated with X-rays (about 2×10^5 rad) but no colour was produced. The triphenylmethane solution was given fast-electron doses up to 3×10^6 rad, but again

⁴ Day and Stein, *J. Chim. phys.*, 1955, **52**, 634.

without production of colour. Diphenylpicrylhydrazine has been X-irradiated in air-saturated ether, benzene, chloroform, and chloroform containing a little pyridine: although the solutions often became coloured, the colour never resembled the intense violet of diphenylpicrylhydrazyl.

To be able to make a stable free radical in the liquid phase by irradiation, it seems to be necessary that the reaction, Oxidised form + Reduced form \rightleftharpoons 2 stable free radicals, takes place, the position of equilibrium being to the right. This condition is satisfied for riboflavin,⁵ methylene-blue, and thionine in strongly acid solution. If this reaction does not occur, as for triphenylmethyl and diphenylpicrylhydrazyl, then any stable free radical formed would at once be irreversibly destroyed by reaction with free radicals produced from the solvent.

EXPERIMENTAL

Irradiation Arrangements.—X-Irradiations were with a Newton-Victor maximar X-ray therapy set operated at 190 kvp, 10 ma. The source of γ -rays was a 20-curie ⁶⁰Co irradiation unit. Fast-electron irradiations were with a 2 Mev Van de Graaff accelerator manufactured by the High Voltage Corporation, U.S.A.

The irradiation vessel for ⁶⁰Co irradiations was a modified version of the type described by Laser,⁶ permitting ultraviolet absorption curves of irradiated solutions to be measured without exposure to air. The spectrophotometer cuvette was shielded during irradiation.

Dosimetry was by the ferrous sulphate method, the yield of ferrous ions oxidised being taken as 15.5 ions/100 ev absorbed.⁷ To obtain the energy absorption in the strongly acid solutions used, the γ -ray dose as measured with the ferrous sulphate solution was multiplied by the density of the sulphuric acid.

Materials.—Methylene-blue and thionine were commercial samples, recrystallised from water. The moisture content was determined by drying *in vacuo* at 100° to constant weight. Other chemicals were of "AnalaR" quality.

Absorption Curves.—These were determined with 1 cm. cells in a Unicam S.P. 500 spectrophotometer, sulphuric acid being used as control. At the wavelengths used, there was no interference by titanous ions or ethanol.

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⁵ Swallow, *Nature*, 1955, **176**, 793.

⁶ Laser, *ibid.*, p. 361.

⁷ Hochanadel, *J. Phys. Chem.*, 1952, **56**, 587; Haybittle, Saunders, and Swallow, *J. Chem. Phys.*, 1956, **25**, 1213.