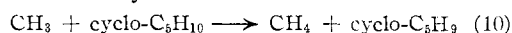
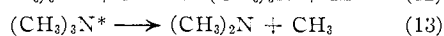
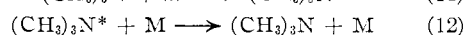
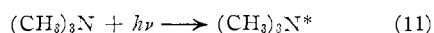


namely, an additional step for ethane formation at the high temperature. The disagreement between the intercept values may be due to strong absorption for trimethylamine (in comparison to acetone) and the consequent smaller effective volume. This also accounts for the small effects observed at low temperatures when the concentration of trimethylamine and the incident intensity were varied. Trimethylamine begins to absorb at about 2570 Å.⁸ with an extinction coefficient of about 0.15×10^6 cm.²/mole at 2500 Å, which increases to 1×10^6 cm.²/mole at 2330 Å, and 4×10^6 at 2000 Å. These high extinction coefficients imply transmittancy values much less than 0.05% in agreement with the above conclusion.

In the presence of cyclopentane, methane can also be formed by the reaction



It is possible to calculate⁴ a value of E_{10} (positive for the two highest temperatures only) of 14.4 kcal. This value is about 6 kcal. too high and signifies an incomplete mechanism. A reaction sequence utilizing an excited intermediate in the primary process can account for the results

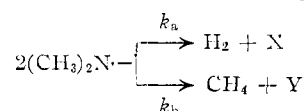


where M is a molecule or the wall. Such a se-

(8) E. Tannenbaum, E. M. Coffin and A. J. Harrison, *J. Chem. Phys.*, **21**, 311 (1953).

quence is consistent with the results at low temperature where a decrease in concentration of trimethylamine from 0.27×10^{18} mol./cm.³ to 0.11×10^{18} mol./cm.³ is accompanied by a slight increase in R_{CH_4} .

At low temperature, the results (Table II) show that $R_{\text{C}_2\text{H}_6}/(R_{\text{CH}_4} + R_{\text{H}_2})$ is approximately equal to unity (0.97 ± 0.06), and that $R_{\text{H}_2}/R_{\text{CH}_4}$ has an average value of 1.5 with a mean deviation of ± 0.24 . It is reasonable to assume that reaction 9 does not occur at the low temperature. Hence if all methyl radicals react to form ethane, then the methane and hydrogen are formed by the disproportionation of the dimethylamine radical. It is therefore proposed that at low temperatures, reactions 11, 12, 13 and 7 are followed by the reaction



where k_a/k_b is 1.5 and X and Y are the liquid products and perhaps dimethylamine. It is hoped that a detailed study of the reactions of the dimethylamine radical will further clarify the mechanism.

Acknowledgment.—The authors are indebted to Mr. George Ensell of the National Research Council of Canada for the construction of the low temperature cell.

WINNIPEG, CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Photoreduction of Methylene Blue by Ethylenediaminetetraacetic Acid^{1a,b}

BY GERALD OSTER AND NEIL WOTHERSPOON

RECEIVED APRIL 1, 1957

Methylene blue in the presence of ethylenediaminetetraacetic acid (EDTA) is reduced to the leuco dye on irradiation with red light. The rate of photoreduction depends upon pH in the same way as does the base titration of EDTA. EDTA is consumed in the reaction suggesting that it is oxidized although it does not normally function as a reducing agent. A number of nitrogen-containing chelating agents were tested but only those with secondary or tertiary nitrogens behaved as electron donors in the photochemical reaction. The photoreduction involves a long-lived excited state of the dye (10^9 times that of the first electronically excited state) and is retarded by small amounts of *p*-phenylenediamine. The rate of regeneration of the dye by near ultraviolet irradiation of the leuco form increases with increasing hydrogen ion concentration.

Introduction

Early workers in the development of photographic bleach-out processes noted that thiazine dyes are photoreduced readily in the presence of mild reducing agents such as allylthiourea, anethol, glyoxal² and ferrous sulfate,³ etc. When a thiazine dye is photoreduced by ferrous ion to give the leuco dye, the ferric ion thus produced oxidizes the leuco dye in the dark, and for intermediate illu-

mination levels, a steady state is achieved.⁴ Stannous chloride and ascorbic acid, on the other hand, do not allow the leuco dye to revert to its colored form, but under excitation with near ultraviolet light the dye is regenerated.⁵

Nickerson and Merkel⁶ in their studies on the photochemistry of riboflavin noted that methylene blue is photoreduced with ethylenediaminetetraacetic acid (EDTA). This is an unexpected result since EDTA does not normally function as a reducing agent. It is the purpose of the present paper to investigate the role of EDTA as an elec-

(1) (a) This paper represents a part of the dissertation to be submitted by Neil Wotherspoon to the faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (b) This research was supported by the United States Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command under Contract No. AF 18(600) 1182.

(2) M. Mudrovcic, *Z. wiss. Photo.*, **26**, 171 (1928).

(3) K. Weber, *Z. physik. Chem.*, **B15**, 18 (1931); G. Ilolst, *ibid.*, **B169** 9 (1934).

(4) J. Weiss, *Trans. Faraday Soc.*, **32**, 1331 (1936); **35**, 48 (1939); **42**, 133 (1946). See also E. Rabinowitch, "Photosynthesis," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1945, p. 76.

(5) G. Oster and N. Wotherspoon, *J. Chem. Phys.*, **22**, 157 (1954).

(6) J. R. Merkel and W. J. Nickerson, *Biochim. Biophys. Acta*, **14**, 303 (1954).

tron donor in the photoreduction of methylene blue.

Experimental

The rates of photobleaching and the determination of quantum yields employing monochromatic light of wave length 665 $m\mu$ were carried out in the same manner as previously described.⁷ The colorless product obtained upon photobleaching a solution of methylene blue (Merck, zinc free) with EDTA (Versene, analytical reagent) is the leuco form of the dye since it converts tetrazolium salts to their corresponding formazans. Oxygen readily oxidizes the leuco dye, hence the experiments were carried out under helium. In the absence of oxygen no appreciable recovery of the dye takes place. The rapid ultraviolet light induced recovery of the dye under anaerobic conditions⁸ also takes place in this system at pH 7 but not at pH 9 or higher.

The rate of photoreduction of methylene blue with EDTA is strongly pH dependent (Fig. 1). Under strongly alkaline conditions (pH 11 or higher) methylene blue is slowly converted into methylene violet by hydrolysis of a dimethylamino group⁸ and we have found that the reaction is greatly

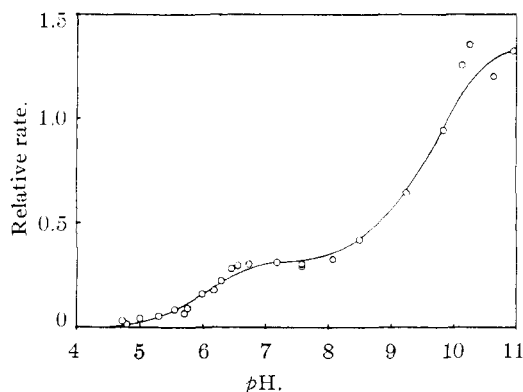


Fig. 1.—Rate of photoreduction as a function of pH . Methylene blue concentration $1 \times 10^{-5} M$, EDTA concentration $1 \times 10^{-3} M$.

accelerated by red light. The rate of photoreduction as a function of EDTA concentration is shown in Fig. 2 plotted as reciprocal quantities. At higher pH values the rates are greater than at pH 7 but extrapolate to the same limiting quantum yield, namely, 0.21. EDTA does not affect the absorption spectrum of methylene blue. The intensity of the feeble red fluorescence of the dye is not quenched by EDTA except above 0.1 M .

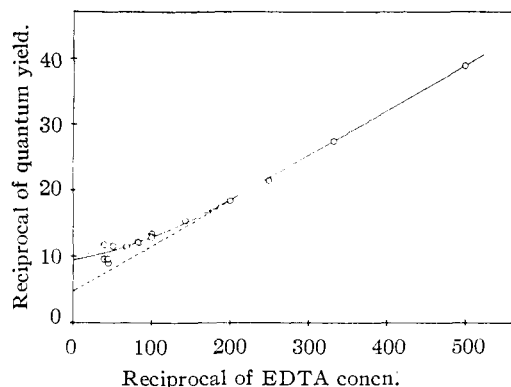


Fig. 2.—Variation of quantum yield with EDTA concentration (mole/l.) phosphate buffer (0.1 M) at pH 7.0.

A solution of EDTA with a concentration of $10^{-4} M$ at pH 9 was flushed with oxygen for 24 hours. At the end of

(7) N. Wotherspoon and G. Oster, *THIS JOURNAL*, **79**, 3992 (1957).

(8) H. J. Conn, "Biological Stains," Biotech Publications, Geneva, N. Y., Fifth Edition, 1946, p. 96.

this period the EDTA was just as effective for the photoreduction of methylene blue as was a solution not treated with oxygen. A solution of $5 \times 10^{-4} M$ dye containing EDTA of twice this concentration at pH 8 was placed between microscope slides spaced with cover slips about 0.1 mm. thick and irradiated. Then the leuco dye was oxidized in the dark by atmospheric oxygen which diffused into the cell. The irradiation was repeated and it was found that the dye faded slowly and incompletely. A further oxidation and irradiation resulted in no more fading. From this it is concluded that EDTA is consumed in the photochemical process.

The following nitrogen-containing chelating agents were found to be effective for the photoreduction of methylene blue at pH 8: EDTA, hydroxyethylethylenediaminetriacetic acid, diethylenetriaminepentaacetic acid, 1,2-diaminocyclohexanetetraacetic acid, hydroxyethyl tris-(hydroxypropyl)-ethylenediamine, bis-(hydroxyethyl)-glycine, ammonia-diacetic acid, methylaminodiacetic acid and *N*-phenylglycine. On the other hand, glycine, lysine, glutamic acid and tris-(hydroxymethyl)-aminomethane were ineffective.

The photoreduction of the dye in the presence of EDTA is retarded by small amounts of *p*-phenylenediamine (Fig. 3). Copper(II) salts and also hydroquinone are likewise retarders of the photobleaching.

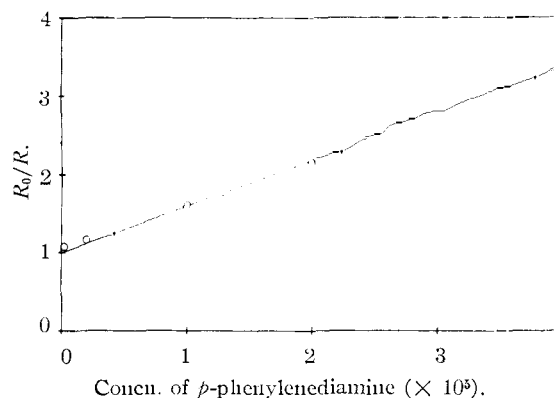


Fig. 3.—Retardation of the photoreduction of methylene blue ($1 \times 10^{-5} M$) as a function of concentration of *p*-phenylenediamine (mole/l.). EDTA concentration $1 \times 10^{-3} M$ at pH 7.0. R and R_0 are the rates with and without *p*-phenylenediamine, respectively.

Discussion

In order to undergo photoreduction the light excited methylene blue must obtain electrons from EDTA. EDTA is not, however, a reducing agent as evidenced by its resistance to oxidation under normal conditions. In the photoreduction of the dye EDTA is consumed and it would therefore appear that EDTA is oxidized in this reaction. A possible oxidation product of EDTA is an amine oxide. The close correspondence of the pH dependence of the rate (Fig. 1) with the base titration of EDTA suggests that the dye cation reacts with a negatively charged species whose charge increases with increasing pH .

Of all the nitrogen-containing chelating agents tested only those containing secondary or tertiary nitrogens were effective. Secondary and tertiary amines are known to form hydroxylamines and amine oxides, respectively, upon oxidation with hydrogen peroxide.⁹

The fact that EDTA is not a quencher of fluorescence of methylene blue except at very high con-

(9) N. V. Sidgwick, "Organic Chemistry of Nitrogen," Oxford University Press, New York, N. Y., 1937, Ch. 6.

centrations suggests that the first excited electronic state is not involved in the photoreduction (compare appendix of ref. 10). Since small amounts of *p*-phenylenediamine retard the reaction, a long-lived excited state of the dye must be the chemically reactive species. From the data of Fig. 3 and the theoretical frequency of encounters between diffusing molecules in solution (6.6×10^9 sec.⁻¹ liter mole⁻¹ in water at room temperature¹¹) we calculate that the life-time of this metastable excited species is equal to or greater than 8.7×10^{-5} sec., *i.e.*, about 10^5 times longer than the first excited electronic state. In all respects the kinetics of the photoreduction of methylene blue resemble that of eosin using conventional reducing agents.¹¹ The deviation from linearity at very

high EDTA concentrations can be attributed to direct deactivation of the first electronically excited state and is manifested in fluorescence quenching.

Photooxidation of the leuco dye by near ultraviolet involves excited states of the leuco dye molecule. The leuco dye absorbs in the near ultraviolet and exhibits a bright yellow fluorescence and in rigid media an intense greenish phosphorescence. The rate of photooxidation is *pH* dependent and increases with hydrogen ion concentration. For this reason photorecovery of leuco dye in the presence of EDTA is more rapid at *pH* 7 than at *pH* 9 although the opposite is the case for photobleaching. Leuco dye made with acidic reductants such as ascorbic acid or stannous chloride exhibit a greater quantum yield of photorecovery.⁵

BROOKLYN, N. Y.

(10) M. Pestemer, *Z. Elektrochem.*, **58**, 121 (1954).

(11) G. Oster and A. H. Adelman, *THIS JOURNAL*, **78**, 913 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA AND THE RAND CORPORATION]

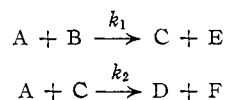
Determination of the Rate Constant Ratio in Competitive Consecutive Second-order Reactions

BY W. G. McMILLAN

RECEIVED APRIL 24, 1957

The ratio of the rate equations for a pair of competitive consecutive second-order reactions is solved in such a way as to yield the rate constant ratio as an implicit function of any two simultaneous concentrations.

Several detailed numerical procedures have been given for the experimental determination of the individual rate constants k_1 and k_2 in kinetic systems consisting of two competitive and consecutive irreversible second-order reactions



Interest in this system arose originally in connection with those reactions, such as the successive saponification of dibasic esters, wherein the concentration of component A is easily followed titrimetrically. Thus, the previous kinetic treatments given by Ingold,¹ Ritchie,² Westheimer, Jones and Lad,³ French,⁴ and most recently by Frost⁵⁻⁷ and co-workers, have aimed at extracting the values of k_1 and k_2 solely from a knowledge of the concentration $A(t)$ of component A as a function of time. Although considerable ingenuity has been shown in the solution of this rather difficult problem, the solution has been given in detail only for the case of equivalent initial reactant concentrations (*i.e.*, $A_0 = 2B_0$), or for a limited range of the extent of reaction.

It is the purpose of the present note to solve the

(1) C. K. Ingold, *J. Chem. Soc.*, 2170 (1931).

(2) M. Ritchie, *ibid.*, 3112 (1931).

(3) F. H. Westheimer, W. A. Jones and R. A. Lad, *J. Chem. Phys.*, **10**, 478 (1942).

(4) D. French, *THIS JOURNAL*, **72**, 4806 (1950).

(5) W. C. Schwemer and A. A. Frost, *ibid.*, **73**, 4541 (1951).

(6) A. A. Frost and W. C. Schwemer, *ibid.*, **74**, 1268 (1952).

(7) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, pp. 165ff.

much simpler problem of obtaining the ratio $\kappa = k_2/k_1$ of the two rate constants from a knowledge of the concentrations of two components at a single time. This case assumes greater relevance in view of the increasingly powerful analytical methods whereby one can often directly determine the concentration of products and reactants other than A. Moreover, the restriction to equivalent initial concentrations will not be necessary here. Although the procedure to be given is much simpler than in the general case, it also accomplishes less in providing only the ratio κ rather than the values of the individual rate constants k_1 and k_2 .

The rates of change of the concentrations B and C are

$$dB/dt = -k_1AB \quad (1)$$

and

$$dC/dt = k_1A(B - \kappa C) \quad (2)$$

In terms of reduced concentrations, defined by

$$\beta = B/B_0 \quad (3a)$$

$$\gamma = C/B_0 \quad (b)$$

the ratio of eq. 2 to 1 becomes

$$d\gamma/d\beta = (\kappa\gamma - \beta)/\beta \quad (4)$$

By changing to a new pair of variables ($\beta, \gamma/\beta$), this homogeneous differential equation is solved easily, after which the original variables are restored. Integration between the initial (1,0) and final (β, γ) values of the original variables then gives

$$\kappa \ln \beta = \ln[\beta + (1 - \kappa)\gamma] \quad (5)$$

OR

$$\gamma = \beta(1 - \beta^{\kappa-1})/(\kappa - 1) \quad (6)$$