

in Tables II and III. The precipitate of aluminum hydroxide obtained in III-4 on neutralization, reacidification and dilution to volume, was allowed to settle and an aliquot withdrawn from the superna-

tant. This procedure was found more satisfactory than to attempt to separate the aluminum by filtration.

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Kinetic Studies of the Thionine-Iron System. I

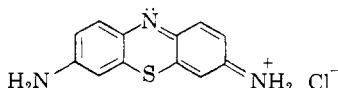
By R. HARDWICK

RECEIVED FEBRUARY 17, 1958

The kinetics of the photosensitive reaction thionine + ferrous ion = semithionine + ferric ion and of the second reduction step, semithionine + ferrous ion = leucothionine + ferric ion, is investigated. Conclusions made from photostationary state data are checked against initial rate data and reverse rate data with fair agreement. The rate constant for the reverse (color restoring reaction) is evaluated and the ratios of other constants are given. The long lived active thionine species produced by light absorption is reduced by ferrous ion. Most of the reduced dye seems to be present as the free radical semithionine, which reacts with ferric ions to produce again the unexcited purple thionine. Certain quantum yield results are explained on the basis of quenching by ferric ion. A reaction mechanism is proposed.

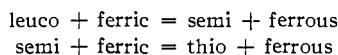
Introduction

Thionine (thio) is a purple dye of the thiazine class, probably having the structure



The dye can be reversibly reduced¹ to give two colorless species, the free-radical semithionine (semi) or the doubly reduced leuco form (leuco).

In aqueous solutions, one or more protons may be added by the dye and by its reduced form depending on the pH. The oxidation reduction potential of the system thio-leuco has been found² to be a linear function of pH in the range 1 to 4. The standard electrode potentials may be estimated for the two oxidation steps, leuco = semi + e⁻ and semi = thio + e⁻, to be about -0.53 and -0.39 v., respectively, at pH 1. (The estimation is made for pH 1 because the present experiments were carried out at that concentration.) The electrode potential for the reaction ferrous = ferric + e⁻ is -0.77 v. Accordingly, the two equilibria



lie entirely to the right (*i.e.*, ferric ions are much stronger oxidizing agents than either thio or semi). The equilibrium constant, [semi][ferrous]/[leuco][ferric], would be about 10⁴ given these estimated electrode potentials (*cf.* sec. c, Results). Determination of the potentials for the two single steps depends on use of the disproportionation constant and an estimation of the concentrations of semi and leuco in a half titrated solution at equilibrium. Since these concentrations cannot be conveniently measured, the E_0 values are approximate.

Thio molecules which have been excited by absorption in the band having a peak at about 5990 Å. are more easily reduced than unexcited molecules. This phenomenon may be demonstrated

by exposing a solution of thio and ferrous ion to sodium vapor radiation (or for that matter, to sunlight). The equilibrium between thio and its reduced forms is shifted to the extent that the purple solution loses nearly all its color. The rate of bleaching depends upon light intensity and the various concentrations, but under typical conditions the bleaching process takes five or ten seconds. As long as irradiation continues, the solution remains in this "photo-stationary" bleached state. When the light is removed, the equilibrium is restored to its former position within a few seconds, and the solution again becomes purple. In oxygen free solutions, the process may be repeated again and again, although the effect gradually weakens. Figure 1 is a plot of the light transmitted by such a solution against time with shutter actions noted.

A more complete preliminary discussion is given by Rabinowitch³ who made the first detailed photochemical investigation of the thio-iron system. The mechanism of the reaction was studied by determining the dependence of the position of the photostationary state on the concentration of the reactants and on the light intensity. Relations were given for these variables, but no rate study was attempted. A second paper by Rabinowitch⁴ discussed the photogalvanic properties of the system, a subject of considerable interest to workers in the solar energy conversion field. Michaelis and co-workers¹ and Clark and co-workers discuss the system from a non-photochemical point of view.

There have been several studies of the kinetics of the reaction between thio and organic reducing agents in non-reversible systems.⁵ Pestemer, in a rate study with thio and thiosinamine, showed that the initial step of the reaction is the transfer of an electron rather than a hydrogen atom. Pestemer also has discussed the role of long-lived excited states, presumably triplets, in the reaction, a subject which recently has received further attention from Oster and co-workers.⁶ In some of

(3) E. Rabinowitch, *J. Chem. Phys.*, **8**, 551 (1940).

(4) E. Rabinowitch, *ibid.*, **8**, 560 (1940).

(1) W. M. Clark, B. Cohen and H. D. Gibbs, *Public Health Reports*, **40**, 1131 (1925); W. M. Clark, "The Harvey Lectures," 1933, pp. 67-115; L. Michaelis, S. Granick and M. P. Schubert, *THIS JOURNAL*, **62**, 204 (1940); **63**, 351 (1941).

(2) W. M. Clark, *Cold Springs Harbor Symposia*, **VII**, 1 (1939).

(5) M. Pestemer, *Z. Elektrochem.*, **58**, 121 (1954); E. Matijevic, *Arhiv. Za. Kem.*, [XXI] **1**, (1949); E. Matijevic and K. Weber, *Rec. trav. chim.*, **70**, 481 (1951).

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

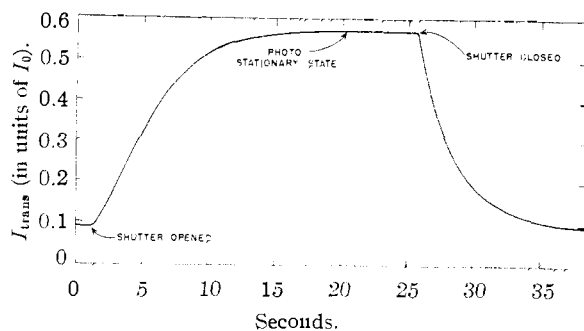


Fig. 1.—A typical reaction curve.

these investigations, quantum yields for the over-all reduction, discounting any reverse reaction, have been estimated.

The present work is a rate study of the reaction mechanism together with a somewhat different approach to the photon conversion problem. A second paper will discuss some microscopic aspects of the reduction reaction.

Experimental

Since the experimental part of the present study consisted largely of measuring the rate of appearance or disappearance of thionine, the apparatus used was a fairly standard photometer arrangement with provision for cross illumination.

The thio-ferrous system is quite sensitive to air oxidation, so that precautions were necessary to exclude air from the stored solutions, the measuring and mixing apparatus, the transfer lines and the photolysis cell. Stock solutions of dye, FeSO_4 , $\text{Fe}_2(\text{SO}_4)_3$ and H_2SO_4 were mixed individually, warmed to 40 or 50° and then boiled under reduced pressure for some time. Subsequently, nitrogen was bubbled through each liquid for several minutes, followed by rearming and further boiling. Finally the solutions were transferred under nitrogen into large storage flasks which had been sealed off, provided with stopcocks and pressurized with about 2 atmospheres of nitrogen. A flask of distilled water was prepared in a similar way.

A closed, pressurized (*i.e.* ~78 cm. of N_2) system was arranged such that none of the solutions was exposed to air until discarded. In this system, the stock solutions were measured by individual burets into a manifold which drained into a mixing chamber, the order of the burets being such that the water washed remaining traces of the stock solutions into the mixing chamber. Mixing was accomplished by bubbling nitrogen through the chamber, after which the solution was siphoned into the photolysis cell. The mixing chamber and the cell both could be emptied by aspirator lines.

The glass photolysis cell which was 6.1 cm. long, 1.8 cm. high and 0.35 cm. thick, was mounted on an optical bench such that the scanning beam traversed the long dimension and the perpendicular photolysis beam passed through the short way.

Water from a thermostat was circulated continually through the heavy metal frame of the cell, then through a water jacket surrounding a long, thin glass transfer tube from the mixing chamber, then through a 5 cm. water filter fronting the cell on the side having the photolysis lamp. The thermostat was kept at 25.0° except during the temperature dependence experiments. Tests with a thermocouple showed that the solutions reached the proper temperature almost immediately after being pumped through the heat exchanger into the cell.

A ribbon filament tungsten scanning lamp, stabilized by a Sola transformer, was mounted so that the beam passed in succession through a didymium filter, two collimating slits, the cell, a third collimating slit and a 546 $m\mu$ narrow band interference filter before striking the cathode of the phototube. The output of the 6292 photomultiplier, which was powered by a highly stabilized 1500 v. supply, was fed through a step-adjustable attenuator into a Varian G-10 recorder.

Frequent calibration was provided by an alternate beam which bypassed the cell through a Lucite rod. This beam, controlled by electrical shutters and a variable aperture, was adjusted so that it matched the scanning beam when pure water was in the cell. After every measurement, the machine was then recalibrated by this beam; after a series of experiments the calibration beam was again compared with distilled water. After warmup, it was found that the apparatus was stable within 1 or 2% for long periods. No measurable photolysis was caused by the scanning beam.

Photolyzing radiation was supplied by a 60-watt sodium vapor lamp stabilized by a Sola transformer. The beam was passed through Corning filters #3480 and #4600, through the water filter, then through the cell. A vacuum thermocouple, calibrated with NBS standard lamps, was placed in the emergent beam such that the transmitted light could be measured. Relative intensities of the incident radiation were determined by measuring the transmitted light when the cell was filled with distilled water.

Calibration of the scanning beam was made with solutions of thio whose concentrations were carefully controlled. Some deviation from Lambert-Beer absorption, possibly due to dimerization, was found at concentrations above 2×10^{-6} mole/liter; however, most of the results reported here are for lower concentrations.

Solutions of ferrous and ferric ion were made up at pH 3.5 from the reagent grade sulfates, with considerable care being taken to exclude as much as possible any ferric ion in the ferrous solution. Acid stock solution was prepared with reagent grade sulfuric acid. After preparation and storage, the solutions were titrated. The thio stock solution was prepared by dissolving a weighed amount of recrystallized thionine in warm water at pH 3.5, filtering, bubbling and storing as above. Because there was a small volume loss on boiling and because the recrystallized thionine might still have contained impurities, the absolute concentrations of thionine are known only within about 10% accuracy. Relative measurements, of course, are not affected by this uncertainty; therefore the dye concentrations are given to three significant figures.

Except for the few measurements involving pH dependence, all the data were taken at the same acid strength, equal amounts of acid being added to the solution for each run. The acid concentration was 0.1 *N*.

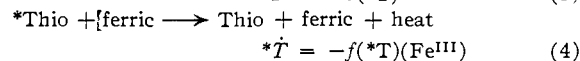
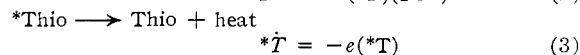
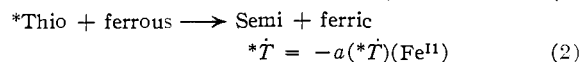
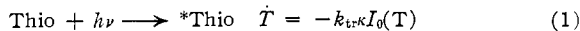
Since solutions of thio and iron together tend to change their characteristics with time, even in an oxygen-free atmosphere, a simple routine was followed on all the determinations, so that the time from mixing to measuring would be fairly constant.

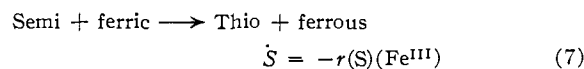
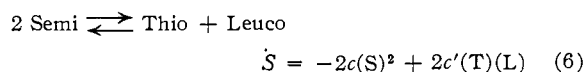
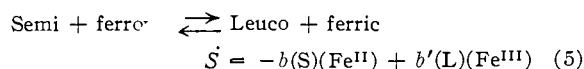
Results and Discussion

(a) **General.**—A few previous rate studies have been made⁵ of the mechanism of the reaction of thionine and similar dyes with organic oxidants, but a detailed rate study of the thionine-iron system has not been made. This section discusses a rate study as well as a series of steady-state measurements with their interpretations on a kinetic basis.

Of all the species present in the photostationary state, only the concentration of thio can be measured conveniently. Leuco and semi do not absorb appreciably in the visible, and the weak absorption of the iron ions is entirely obscured by the very strongly absorbing thio. The problem is simplified by the use of concentrations of iron so large as to remain practically constant, but the concentrations of the bleached dye species elude measurement.

A plausible set of reactions is





where (T) denotes the concentration of thionine, (S) the concentration of semithionine, (*T) the concentration of the excited reactive thionine species, etc. Rate equations are given for each reaction, the time derivatives of the concentrations are designated for example by \dot{T} , and the rate constants are defined by the equations. I_0 is the intensity of the incident photolyzing beam. Initial concentrations are designated by subscript zero, e.g., $(T)_0$.

(T), (S) and (L) each designate the total concentration of the particular oxidation state of the dye, although each of these states may add one or more protons to give several pH dependent species in equilibrium. The abbreviation may be used because the rate of acid-base equilibration is high compared to those of the oxidation-reduction reactions⁷ and because the data which follow were taken at constant pH. Since the electrode potentials for the two reduction steps are functions of the pH, the rate constants for the various reactions would, of course, be different at other hydrogen ion concentrations.

There is considerable reason to believe that the excited reactive species is the triplet thio,^{5,8} thus a constant should be included in the absorption term to account for the conversion to the triplet state. Since the experimental measurements show this constant to be approximately 1, it is omitted hereafter. (If ferric ion quenches the excited singlet thio, however, the present notation must be modified although the results would be approximately the same.)

The amount of light absorbed is given by $I_0 \kappa(T)$. This approximation holds only at low concentrations where absorption is small and roughly constant across the cell. At higher concentrations, not only is the approximation $(1 - e^{-\kappa(T)}) \sim \kappa(T)$ not valid, but the light intensity in the region of the solution scanned may be significantly less than I_0 because of absorption by the solution between the cell wall and the scanning beam. Most of the measurements taken in the present problem were for concentrations within this linear range.

It will be noted that eq. 2 is written as not being reversible but that the thio is restored in the *unexcited* state according to eq. 7. In an equilibrium, such a circular mechanism is not allowed by the principle of microscopic reversibility, but no equilibrium involving thio (except a photostationary state which is not a true equilibrium) is implied in any of the schemes suggested here.

The rate of appearance of each of the species is

$$*\dot{T} = \kappa I_0 T - a(*T)(\text{Fe}^{\text{II}}) - e(*T) - f(\text{Fe}^{\text{III}})(*T) \quad (8)$$

$$\dot{T} = -\kappa I_0(T) + e(*T) + f(\text{Fe}^{\text{III}})(*T) + c(S)^2 - c'(T)(L) + rS(\text{Fe}^{\text{III}}) \quad (9)$$

$$\dot{S} = a(*T)(\text{Fe}^{\text{II}}) - b(S)(\text{Fe}^{\text{II}}) + b'(L)(\text{Fe}^{\text{III}}) - 2c(S)^2 + 2c'(T)(L) - r(S)(\text{Fe}^{\text{III}}) \quad (10)$$

$$\dot{L} = b(S)(\text{Fe}^{\text{II}}) - b'(L)(\text{Fe}^{\text{III}}) + c(S)^2 - c'(L)(T) \quad (11)$$

If the steady-state assumption is made with regard to the excited thio, *T

$$\kappa I_0(T) - e(*T) - f(\text{Fe}^{\text{III}})(*T) = e(*T)(\text{Fe}^{\text{II}}) \quad (12)$$

Thus

$$\dot{T} = -I_0 \kappa \gamma(T) + c(S)^2 - c'(T)(L) + r(S)(\text{Fe}^{\text{III}}) \quad (13)$$

and

$$\dot{S} = I_0 \kappa \gamma(T) - b(S)(\text{Fe}^{\text{II}}) + b'(L)(\text{Fe}^{\text{III}}) - 2c(S)^2 + 2c'(T)(L) - r(S)(\text{Fe}^{\text{III}}) \quad (14)$$

where γ is the quantum yield for the reaction of thio with ferrous ion

$$\gamma = \frac{a(\text{Fe}^{\text{II}})}{a(\text{Fe}^{\text{II}}) + e + f(\text{Fe}^{\text{III}})} \quad (15)$$

(b) **Quantum Yield Measurements.**—Quantum yields were taken by measuring the initial reaction rate when the back reaction velocity was zero (*i.e.*, when no products had yet been formed). Since this involves measurement over *some* amount of reaction, however small, the data presented have been corrected by adding in the measured back reaction rate at the same concentrations. In all but extreme cases, this correction is negligible. Figures 2 and 3 show the reciprocal of the quantum

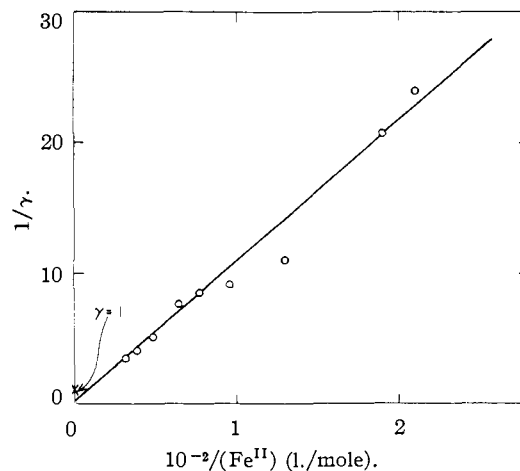
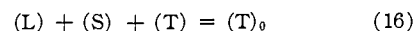


Fig. 2.—Effect of Fe^{II} on γ ; $(T)_0$, 1.68×10^{-5} mole/l.; (Fe^{III}) , 1.9×10^{-4} mole/l.; I_0 , 2.0×10^{-5} einstein/l. sec.

yield ($I_0 \kappa(T)$ /initial rate) plotted against $1/(\text{Fe}^{\text{II}})$ and against (Fe^{III}) , respectively. The plots are linear as required by eq. 15. From the data shown and other measurements, the ratios of the rate constants may be estimated as

$$e/a = 3.4 \times 10^{-3}, f/a \sim 20 \text{ to } 25$$

If all the rates of change of concentration could be measured, eq. 11, 13 and 14 and the stoichiometric equation



could be solved immediately for the rate constants; however, the purple thio is the only species whose concentration may be conveniently measured. This being the case, several kinds of experiments must be used in the study of the re-

(7) Th. Förster, *Z. Elektrochem.*, **54-1**, 42 (1950).

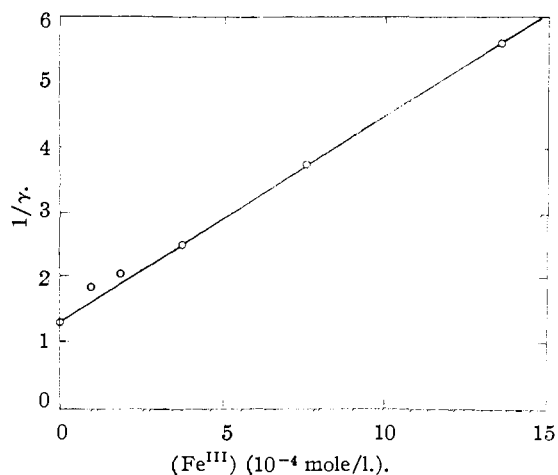


Fig. 3.—Effect of (Fe^{III}) on γ ; $(T)_0$, 1.68×10^{-5} mole/l.; (Fe^{II}) , 10.4×10^{-3} mole/l.; I_0 , 9.7×10^{-6} einstein/l. sec.

action mechanism. The types of measurements are: the quantum yield, the position of the photostationary state, the initial reaction rate and the rate of the color restoration (reverse) reaction, all of which are taken as functions of the concentrations and the light intensity. The conclusions drawn from one set can then be tested against the other data.

(c) **Photostationary State Results.**—Unless the rate of equilibration between semi and leuco is appreciably slower than the rate of bleaching,⁸ we may set eqs. 11, 13 and 14 each equal to zero at the photostationary state.

In principle, these equations with eq. 16 may now be solved explicitly, giving the concentrations of the three dye species as functions of the experimental variables and the rate constants. Actually, however, the solution is so complex as to be unwieldy, so that approximations must be made at the outset. Such approximations may correspond to the two limiting cases of (1) reduction of semi to leuco accomplished primarily by the reaction $\text{semi} + \text{ferrous} = \text{leuco} + \text{ferric}$ or (2) by the reaction $2 \text{ semi} = \text{leuco} + \text{thio}$. The second scheme leads to the dependence of (T_s) , the concentration of thio in the photostationary state, on I_0^2 , a possibility clearly ruled out by experimental results.

If the reduction is assumed to be by ferrous ion, however, the results are better. The relations are derived as follows.

Neglecting c and c' and solving eq. 14 for (S) leads to

$$(S_s) = \frac{I_0 \kappa \gamma (T_s)}{r(\text{Fe}^{\text{III}})} \quad (17)$$

and, similarly

$$L_s = \frac{b(S_s)(\text{Fe}^{\text{II}})}{b'(\text{Fe}^{\text{III}})} = \frac{b(\text{Fe}^{\text{II}})I_0 \kappa \gamma (T_s)}{b'r(\text{Fe}^{\text{III}})^2} \quad (18)$$

If these relations are substituted into the stoichiometric eq. 16 we find that

$$(T_s) + \frac{I_0 \kappa \gamma (T_s)}{r(\text{Fe}^{\text{III}})} + \frac{b(\text{Fe}^{\text{II}})I_0 \kappa \gamma (T_s)}{b'r(\text{Fe}^{\text{III}})^2} = (T)_0 \quad (19)$$

(8) If the LS equilibration rate were, say, only $1/1000$ of the bleaching rate, this would escape attention experimentally; and what is taken for a true photostationary state would in reality not yet be established.

or

$$\frac{(\text{Fe}^{\text{III}})(T_0) - (T_s)}{I_0 \kappa \gamma (T_s)} = \frac{1}{r} + \frac{b}{r b'} \frac{(\text{Fe}^{\text{II}})}{(\text{Fe}^{\text{III}})} \quad (20)$$

The experimental data from the photostationary state measurements may be plotted if the quantum yield, γ , is calculated for each point. There is considerable scatter, caused largely by the multiplication of already significant experimental error and by taking small differences of large numbers; however, the intercept may be read fairly accurately, giving a value for r of about 450 l./mole second. The slope is much less certain, giving a value for b'/b of about 450 as a rough minimum, although our generous experimental error for this value will accommodate even the estimated 10^4 given by the electrode potential $E_{\text{semi/leuco}}^0$.

The value of b'/b may be used to determine the ratio $(S)/(L)$, since

$$\frac{(S)}{(L)} = \frac{b'(\text{Fe}^{\text{II}})}{b(\text{Fe}^{\text{III}})}$$

The predominant reduced dye species, then, must be semi except at quite low values of $(\text{Fe}^{\text{III}})/(\text{Fe}^{\text{II}})$.

If this result is used to make a further approximation in eq. 19, *i.e.*, if the term for (L) is ignored, we have

$$(T_s) + \frac{I_0 \kappa \gamma (T_s)}{r(\text{Fe}^{\text{III}})} = (T)_0 \quad (21)$$

or

$$\left[\frac{(T_0) - (T_s)}{(T_s)} \right] r(\text{Fe}^{\text{III}}) = I_0 \kappa \gamma \quad (22)$$

Equation 21 predicts linearity of (T_s) in $(T)_0$ with intercept zero. Figure 4, a plot of observed (T_s) vs. $(T)_0$, is seen to meet these requirements.

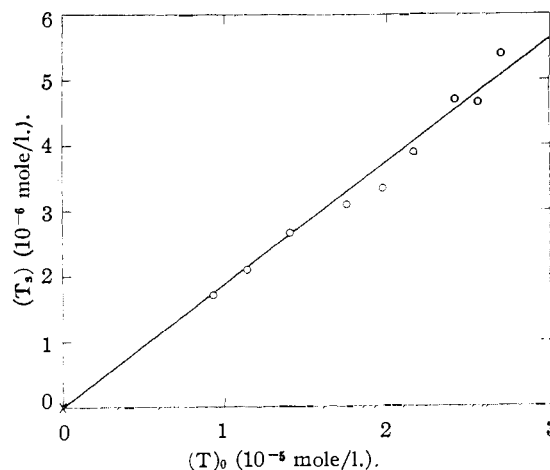


Fig. 4.—Effect of $(T)_0$ on (T_s) : (Fe^{II}) , 10.4×10^{-3} mole/l.; (Fe^{III}) , 1.9×10^{-4} mole/l.; I_0 , 1.7×10^{-6} einstein/l. sec.

Equation 22 predicts linearity of $((T)_0 - (T_s))/ (T_s)$ in I_0 with intercept zero. This equation is tested in Fig. 5, Plot b. There is a slight deviation from linearity at small I_0 , leading to a small intercept on the I_0 axis, otherwise the data meet the conditions of eq. 22.

If eq. 22 is inverted, the iron concentration data may be tested.

$$\frac{(T_s)}{((T)_0 - (T_s))} = \frac{r(\text{Fe}^{\text{III}})}{I_0 \kappa \gamma} = \frac{r(\text{Fe}^{\text{III}})}{I_0 \kappa} \left[\frac{a(\text{Fe}^{\text{II}}) + f(\text{Fe}^{\text{III}}) + e}{a(\text{Fe}^{\text{II}})} \right] \quad (23)$$

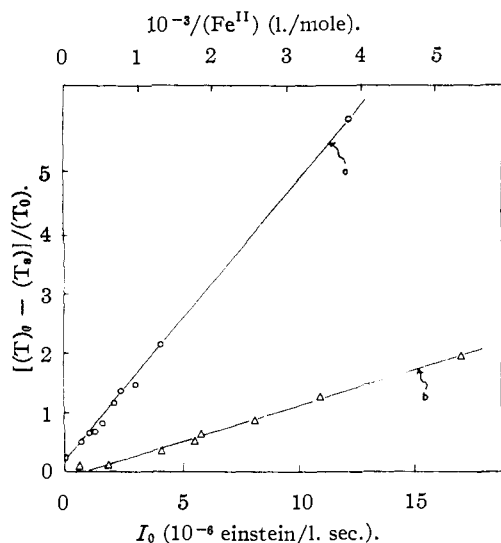


Fig. 5.—Photostationary state data: (a) effect of (Fe^{II}) ; $(T)_0$, 1.68×10^{-5} mole/l.; (Fe^{III}) , 1.9×10^{-4} mole/l.; I_0 , 2.0×10^{-5} einstein/l. sec.; (b) effect of I_0 ; $(T)_0$, 1.40×10^{-5} mole/l.; (Fe^{II}) , 1.04×10^{-3} mole/l.; (Fe^{III}) , 1.9×10^{-4} mole/l.

Equation 23 requires that $(T_s)/((T)_0 - (T_s))$ be linear in $1/(\text{Fe}^{\text{II}})$ with intercept $r(\text{Fe}^{\text{III}})/I_0\kappa$ and slope

$$\frac{r(\text{Fe}^{\text{III}})}{I_0\kappa} \left[\frac{f(\text{Fe}^{\text{III}})}{a} + \frac{c}{a} \right]$$

Figure 5, plot a, tests this requirement.⁹ The data are seen to be linear, and the intercept calculated by using $e/a = 3.4 \times 10^{-3}$ again falls on the curve. The slope gives a value for f/a of 20.

Equation 23 predicts linearity of $(1/(\text{Fe}^{\text{III}}))[(T_s)/((T)_0 - (T_s))]$ in Fe^{III} with intercept

$$\left[\frac{e}{a(\text{Fe}^{\text{II}})} + 1 \right] \frac{r}{I_0\kappa} \text{ and slope } \left[\frac{r}{I_0\kappa(\text{Fe}^{\text{II}})} \right] \frac{f}{a}$$

Figure 6 shows experimental data plotted according to eq. 23 with x designating the intercept calculated by using $e/a = 3.4 \times 10^{-3}$ given by the quantum yield measurements. The slope of Fig. 6 gives $f/a \sim 24$, compared with the value 20 to 25 given by quantum yield data. Many of the Fe^{III} runs yielded data with somewhat more scatter than Fig. 6, but all are linear within the (large) experimental error except at very low (Fe^{III}) . In these regions, the approximation of negligible L breaks down because of the low rate of the reaction leuco + ferric = semi + ferrous and the increasing relative importance of disproportionation. Also, at low concentrations of added ferric ion, the ferric ion created by thio + ferrous = semi + ferric becomes significant.

(d) **Initial Rate Results.**—The conditions on the initial rate measurements are discussed in Sec. b. Under initial conditions, eq. 13 may be written as

$$T_{\text{initial}} = I_0\kappa\gamma(T_0) \quad (24)$$

The dependence of the quantum yield on iron concentrations has been discussed. Equation 24 predicts linearity of T_{initial} in I_0 and (T_0) with in-

(9) In Fig. 5, the ordinate for curve a should be read as $T_s/(T_0 - T_s)$ instead of the inverse.

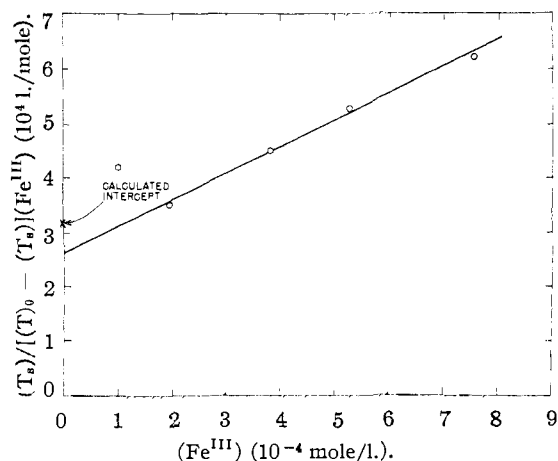


Fig. 6.—Effect of (Fe^{III}) on photostationary state: $(T)_0$, 1.65×10^{-5} mole/l.; (Fe^{II}) , 10.4×10^{-3} mole/l.; I_0 , 9.7×10^{-5} einstein/l. sec.

tercept zero. Figure 7 demonstrates that the initial rate data fit these requirements. If solutions of greater concentration are used in the (T_0) vs. T_{initial} measurements, the curve falls off somewhat because of the failure of the light absorption approximations (*cf.* last paragraph in Experimental section).

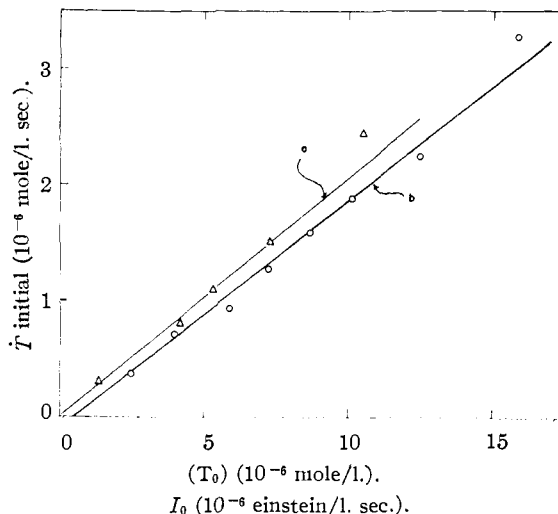


Fig. 7.—Initial rate data: (a) effect of I_0 ; (b), effect of $(T)_0$; (Fe^{II}) , 5.2×10^{-3} mole/l.; I_0 , 1.3×10^{-5} einstein/l. sec.; no Fe^{III} added.

(c) **Reverse Reaction Results.**—With the neglect of L and the reactions leading to the formation of leuco, eq. 13 becomes

$$T \dot{=} -I_0\kappa\gamma(T) + r(S)(\text{Fe}^{\text{III}}) \quad (25)$$

which with the photolyzing beam turned off simply is

$$T_{\text{rev}} = r(S_0) - (T)(\text{Fe}^{\text{III}}) \quad (26)$$

taking $(S) = (T_0) - (T)$. Equation 26 predicts linearity of T_{rev} in $(T_0) - (T)$ and in (Fe^{III}) with slope r and intercept zero. Figure 8 shows T_{rev} plotted against added Fe^{III} and $((T_0) - (T))$ (curves a and b, respectively). The data for $((T_0) - (T))$ dependence were taken as T_{rev} at different values of (T_0) and (T)

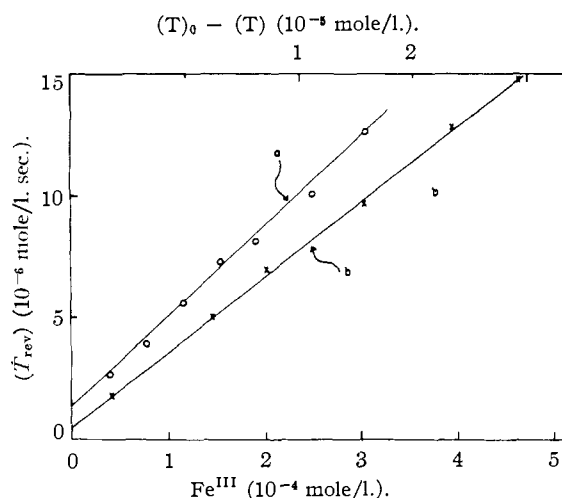


Fig. 8.—Reverse reaction data: (a) effect of Fe^{III} : (Fe^{II}), 2.6×10^{-3} mole/l.; (T), 1.7×10^{-6} mole/l.; ($T_0 - (T)$), 8.7×10^{-6} mole/l.; (b) effect of ($T_0 - (T)$): (Fe^{II}), 1.04×10^{-3} mole/l.; (Fe^{III}), 1.9×10^{-4} mole/l.

immediately after closing the shutter. Other sets taken at constant (T) but differing (T_0) show very similar results. The plots are linear and the intercept of curve b is very nearly zero. The intercept of curve a becomes acceptably small after correction for ferric contamination of the ferrous solution and for ferric ion created by reaction 2. The slope of curve b gives $r = 390$, while that of curve a Fig. 8 gives $r = 425$, both in fair agreement with $r = 450$ from the stationary state measurements.

Equation 26 predicts independence of \bar{T}_{rev} from (T) at constant ($(T_0 - (T))$), and several runs at different (T_0) demonstrate this independence. If all the requirements of eq. 26 are to be met, however, \bar{T}_{rev} must also be independent of (Fe^{II}). The experimental data taken at constant ($(T_0 - (T))$) and constant (Fe^{III}) show a small increase of \bar{T}_{rev} with (Fe^{II}) at small (Fe^{II}) and near independence at moderate and high (Fe^{II}) (there is very slight decline). This result is puzzling, since the increase seems somewhat too large to be accounted for by contamination of the ferrous solution.

The integrated form of eq. 26 is

$$2.30 \log \left[\frac{(T_0)}{(T_0 - (T))} \right] = r(\text{Fe}^{\text{III}})t \quad (27)$$

starting with a completely bleached solution at $t = 0$ (t designates time). The experimental plot of $\log (T_0)/(T_0 - (T))$ vs. t is linear, the slope giving a value for r of about 460 in agreement with earlier values.

(f) **Temperature Dependence Measurements.**—Preliminary temperature dependence measurements have been made, taking the rate, T_{rev} , as a function of temperature at constant (T_0), (T), (Fe^{III}) and (Fe^{II}). As one might expect, a plot of $\log \bar{T}_{\text{rev}}$ versus $1/\text{temperature}$ is linear within experimental error. Calculations made from the data give an activation energy for the reverse process of about 13 kcal.

The bleaching rate \dot{T} is not necessarily expected to be independent of temperature for the reason that the quenching rates of the excited thionine

may be functions of temperature. Further measurements are being made for the forward reaction; however, the present data show only that \dot{T} seems to decline with increasing temperature.

(g) **Discussion of Kinetic Results.**—The two equilibria, semi + ferric = thio + ferrous and leuco + ferric = semi + ferrous, lie entirely to the right in darkness. If a thio solution is titrated with a strong reducing agent, at the half titration point a significant amount of semi exists, but most of the reduced dye is in the leuco form. This is to be expected, in view of the relative electrode potentials for the two steps (actually, the potentials are estimated from data of this kind). If, however, the apparent electrode potential of thio is changed by light absorption, the second equilibrium is not necessarily shifted. It is reasonable, then, to expect that considerable amounts of semi might accumulate in the bleached solutions, provided that disproportionation did not play a significant part in diminishing the semi concentration. These expectations are supported by the experimental conclusion that semi is indeed the predominant reduced dye species in the bleached solutions. It is hoped that paramagnetic resonance measurements which are planned to begin soon will yield further information about the concentration of the free radical semi.

(h) **Identity of the Reactive Species.**—Quantum yields approaching unity may be attained in the thio-iron system with high enough (Fe^{II}) and low enough (Fe^{III}). Such efficiency might be expected either if the reactive thionine species were long lived or if the dye were complexed with ferrous ion so that the absorbed energy could cause merely the electron transfer within the complex.

Complexing may sometimes be detected by a change in the absorption spectrum of the substance involved. In the present experiments, two kinds of spectrophotometric measurements were made: (a) The absorption spectrum of the dye was found not to change its character as (Fe^{II}) was increased from zero to a value higher than those used in the bleaching experiments. The spectrum has a maximum at about 5970 Å. with a shoulder at about 5600 Å. which may be attributed to the dimer. (b) The extent of bleaching as a function of the wave length of the exciting light (*i.e.*, the action spectrum) was taken by using a large grating monochromator. The action spectrum was found to coincide with the absorption spectrum except that the action spectrum has no shoulder for the dimer.

These measurements, though by no means conclusive, indicate that thio may not be strongly complexed by ferrous ion; this in turn suggests that a long-lived, excited state is the reacting species. The latter is supported by the work of Oster and co-workers and Pestemer. Further investigation employing flash photolysis is beginning in this Laboratory.

(l) **Energy Conversion Considerations.**—Although photogalvanic cells employing the thionine-iron system have been widely discussed in connection with solar energy conversion, such cells are quite inefficient. It is of interest, then, to investigate the reasons for the energy loss.

Thionine absorbs quite strongly and, under the proper conditions, the quantum yield for bleaching approaches unity, so that most of the quanta falling on the system cause reaction. Past this point, however, one may see two possibilities of loss. Some of the energy of the single quantum is converted into the chemical energy of the semi-ferric pair, but it is not known how much of this is lost and how much is converted. (This question will be discussed more fully in the next paper of this series although at the present concentrations one may estimate a conversion of about 15% of the ~ 2 e.v. quantum.) A more likely possibility of loss may be found in the electrode process. Since the spontaneous back reaction in the body of the solu-

tion competes with the electrode process, it is a bit difficult to understand how *any* significant amount of electrical work may be taken from the system (indeed, this may be the situation; no power measurements have been published for photogalvanic cells). A means of separating the energetic species, of course, would eliminate most of this loss.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

The Interaction of Copper(II) with Bovine Serum Albumin¹

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Native bovine serum albumin (BSA) can be titrated amperometrically with copper(II) in an ammonia buffer using the rotated platinum wire or rotated dropping mercury electrode as indicator electrodes. At the end-point, one mole of copper is bound per mole of albumin. The reactive group is not sulfhydryl. Nickel(II) forms a stable complex under the same experimental conditions as copper(II) does. BSA denatured in 4 *M* guanidine hydrochloride contains the same reactive group as in the native state. However, an excess of copper oxidizes the sulfhydryl to disulfide whereas this reaction does not occur with native BSA. Sulfhydryl in native BSA can be oxidized by oxygen when one mole or more copper(II) per mole BSA is present. The titration with copper is proposed as a rapid, specific method for the determination of albumin in blood serum.

In work carried out in this Laboratory, it has been found that the sulfhydryl group in cysteine (RSH) is oxidized by copper(II) in ammoniacal medium to cystine (RSSR)³ according to the overall reaction $2RS^- + 2Cu^{(II)} \rightarrow RSSR + 2Cu^{(I)}$. In the presence of sulfite ion, the reaction is $2Cu^{(II)} + RS^- + SO_3^{2-} \rightarrow 2Cu^{(I)} + RSSO_3^-$. It was of interest to us to determine whether similar reactions occur with the sulfhydryl group in native and denatured bovine serum albumin (BSA).

The copper complexes of a number of proteins including BSA have been investigated by Klotz, *et al.*⁴ In particular, it was reported that copper is bound through the albumin sulfhydryl group under the experimental conditions chosen. The formation of complexes between cations and proteins recently has been reviewed by Gurd and Wilcox.⁵

Experimental

Materials.—The bovine serum albumin was obtained from Armour Laboratories, and the guanidine hydrochloride (GHCl) from Eastman. Details of the properties of the BSA, preparation of solutions and purification of the GHCl

have been described previously.⁶ γ -Globulin was a sample obtained from Professor John T. Edsall of the Harvard University Medical School. Conductivity water was used in the preparation of all solutions. Metal ion solutions were prepared from reagent grade commercial chemicals and analyzed by standard methods. Solutions were deaerated with Linde nitrogen of 99.9% purity. All other chemicals used were reagent grade.

Instrumentation.—Current-voltage curves were measured manually by a circuit similar to that described by Lingane and Kolthoff⁷ and automatically with a Leeds and Northrup type E Electrochemograph. Amperometric titrations were carried out using the manual circuit. The platinum electrode was rotated at a constant speed of 600 or 900 r.p.m. by a Bodine synchronous motor. The rotated dropping mercury electrode was of the type A described by Stricks and Kolthoff⁸; a rotation speed of 300 r.p.m. was used. All potentials were measured against the saturated calomel electrode. Measurements of pH were made with a Beckman Model H-2 pH meter.

Titration Procedure.—The titration vessels were 125-ml. beakers fitted with rubber stoppers with holes for the indicator electrode, nitrogen inlet and outlet, salt bridge and buret. The proper quantity of buffer (usually 25 ml. for R.p.e. titrations, 60 ml. for R.d.m.e) was placed in the titration cell and deaerated with a stream of nitrogen which had been passed previously through buffers of the same composition. The pH of the solution after completion of the titration was measured frequently and in no case was there any significant change. When deaeration was complete, the BSA solution (air-free) was introduced and the flow of nitrogen continued for several minutes. The native albumin solutions foamed considerably, and care was taken to avoid any loss of protein through the nitrogen outlet tube. On the other hand, the denatured protein solutions showed very little tendency to foam. In current-voltage curve measurements, the stream of nitrogen was diverted to pass over the surface of the solution, while in amperometric ti-

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