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

Polarographic Investigations of Reactions in Aqueous Solutions Containing Copper and Cysteine (Cystine). II. Reactions in Ammoniacal Medium in the Presence and Absence of Sulfite

BY I. M. KOLTHOFF AND W. STRICKS

The reactions between cysteine and cupric copper in ammoniacal medium have been studied polarographically. In the absence of sulfite, cupric copper reacts with cysteine to give cuprous cysteinate (RSCu) and cystine (RSSR): 2RSCu + 2Cu(II) $\rightleftarrows$ RSSR + 4Cu(I). This reaction is reversible. In the presence of sulfite cupric copper and cystine react quantitatively to give cysteine sulfonate and cuprous copper. On the addition of cupric copper to a cysteine solution the following over-all reaction is quantitative when the mole ratio of Cu(II) to RSH is 2:1. 2Cu(II) + RS^- + $SO_3^=$ = 2Cu(I) + $RSSO_3^-$. The reaction is rapid at low ammonia (0.05 *M*) and high sulfite (0.15 *M*) concentrations and under such conditions small amounts of air do not interfere. The reaction rate decreases at higher ammonia and smaller sulfite concentrations and under such conditions even traces of air cause deviations by catalyzed oxygen oxidation of cysteine.

The reaction between cysteine and cupric copper under various conditions is not described in the literature. In the present work it is found that cysteine (designated as RSH) in ammoniacal solution is oxidized to cystine (RSSR) upon addition of cupric copper with formation of cuprous cysteinate (RSCu). The nature of this reaction is markedly altered if sulfite is present in the reaction mixture.

In the presence of sulfite cysteine reacts quantitatively with cupric copper in ammoniacal medium to give cysteine sulfonate ($RSSO_3^-$) and cuprous copper as the final products. The cystine formed upon oxidation of cysteine with cupric copper reacts with sulfite: $RSSR + SO_3^= \rightleftarrows RSSO_3^- + RS^-$.

The present paper deals with a polarographic study of the reaction between cupric copper and cysteine in ammoniacal medium in the absence and mainly in the presence of sulfite.

Materials.—The cysteine and cystine solutions were prepared in the same way as described in a previous paper.[1]

The cuprous copper solutions were prepared by reduction of cupric copper solutions with sodium sulfite or with glucose as described previously.[2] Ammoniacal cuprous copper solutions free of sulfite or glucose were prepared by dissolving an accurately weighed amount of pure cuprous oxide in air-free ammonia solution. The cuprous oxide was made by boiling Benedict solution with glucose; the precipitate was collected; washed and dried *in vacuo* over magnesium perchlorate

A 2×10^{-2} *M* cysteine sulfonate ($RSSO_3^-$) solution was prepared by bubbling air through 250 ml. of a solution which was 10^{-2} *M* in cystine, 0.2 *M* in ammonia and 0.2 *M* in sodium sulfite. In order to maintain the ammonia concentration approximately constant, the air was passed through two wash bottles containing ammonia of the same concentration as the cystine solution. The progress of the reaction was followed polarographically. The air bubbling was continued until no cysteine and cystine could be detected. Toward the end of the reaction ammonia was driven out from the solution with purified air. Most of the excess of sulfite had been converted into sulfate. The solution was stored in a refrigerator. The reactions involved are

$$\begin{array}{l} 2RSSR + 2SO_3^= = 2RSSO_3^- + 2RS^- \\ 2RS^- + {}^1/_2O_2 + 2H^+ = RSSR + H_2O \\ \hline RSSR + 2SO_3^- + {}^1/_2O_2 + 2H^+ = 2RSSO_3^- + H_2O \end{array}$$

The reaction is greatly catalyzed by small amounts of cupric copper. We made no use of this catalyst because a solution, free of heavy metal ions, was desired.

Experimental Methods.—Polarographic current voltage (*c–v*) curves were obtained with a Heyrovsky self-recording polarograph. The potentials refer to the saturated calomel electrode (S.C.E.). The characteristics of the capillary are the same as given previously.[2] All measurements were carried out at 25°. The values reported for the diffusion currents of cysteine sulfonate, which is reduced at about −1.5 volts, are corrected for the $m^{2/3}t^{1/6}$ effect.[3]

The values i_d/c correspond to the diffusion currents of a 10^{-3} molar solution.

Results and Discussion

Cysteine and Cupric Copper in the Absence of Sulfite.—Upon addition of cysteine to an ammoniacal 0.05 *M* air-free cupric copper solution the blue color disappears immediately and a yellowish solution is obtained. This change in color indicates that the cupric copper is reduced by cysteine to univalent copper and this is substantiated polarographically. The addition of increasing amounts of cupric copper to an air-free ammoniacal cysteine solution results in a decrease in height of the anodic cysteine wave. At the same time the cuprous cysteinate (RSCu) wave and the second step of the cystine wave[4] appear and increase in height as more cupric copper is added.

In Fig. 1, polarograms are presented of mixtures of 50 ml. of 0.001 *M* ammoniacal cysteine solution with 1 and 3.5 ml. of 0.01 *M* cupric copper solution, respectively. Complete polarograms were taken in a titration of 0.001 *M* ammoniacal cysteine solution with cupric copper. Figure 2 gives the cupric, cuprous, RSH, RSCu and RSSR diffusion currents during the titration. At a molar ratio of RSH to Cu(II) of 2 to 1 the anodic cysteine wave disappears completely. Up to this point the reaction is given by the equation

$$\begin{array}{lr} 2RS^- + 2Cu^{++} = 2Cu^+ + RSSR & (1) \\ 2RS^- + 2Cu^+ = 2RSCu & (2) \\ \hline 4RS^- + 2Cu^{++} = 2RSCu + RSSR & (3) \end{array}$$

Upon further addition of cupric copper a cupric (ammino) and a cuprous (ammino) wave appear. The RSCu wave decreases with increasing excess of

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(1) I. M. Kolthoff and W. Stricks, THIS JOURNAL, **72**, 1952 (1950).

(2) W. Stricks and I. M. Kolthoff, *ibid.*, **73**, 1723 (1951).

(3) I. M. Kolthoff, *Ind. Eng. Chem., Anal. Ed.*, **14**, 195 (1942).

(4) I. M. Kolthoff and C. Barnum, THIS JOURNAL, **63**, 520 (1941).

TABLE I

POLAROGRAPHIC DATA OBTAINED WITH MIXTURES OF CYSTINE AND CUPRIC COPPER, 0.005% IN GELATIN, $[NH_3] = [NH_4Cl]$, i_d IS THE APPARENT DIFFUSION CURRENT

Concn. of RSSR, M	Concn. of Cu(II) copper, M	Mole ratio RSSR: Cu(II)	Concn. of NH_3, M	Cu(II) wave $i_d//c^a$	Cu(II) wave $E_{1/2}$	Cu(I) wave i_d/c^a	RSCu wave i_d	RSCu wave $E_{1/2}$	Cystine wave i_d/c^a	Expt.
None	10^{-3}	..	1.0	3.43	-0.217^b	3.43	..	.....	....	1
10^{-3}	10^{-3}	1:1	1.0	3.72	− .214	3.45	0.75	−0.725	4.05^c	2
2.5×10^{-4}	2.5×10^{-4}	1:1	0.1	3.80	− .126	1.44	.45	− .663	4.92	3
10^{-3}	5×10^{-4}	2:1	.5	3.82	− .175	2.66	.46	− .70	4.90	4
2.5×10^{-4}	1.25×10^{-4}	2:1	.1	3.52	− .148	None	.39	− .664	4.60	5
10^{-3}	2.5×10^{-4}	4:1 -	1.0	4.52	− .218	2.20	.81	− .717	4.72	6
5×10^{-3}	2.5×10^{-4}	20:1	1.0	4.20	− .224	1.44	2.25	− .762	4.67	7
5×10^{-3}	2.5×10^{-4}	20:1	0.5	4.00	− .193	1.08	2.59	− .758	5.04	8
10^{-3}	None	..	1.0	..	.....	..	..	.....	5.25	9

[a] i_d/c is the apparent diffusion current corresponding to a 10^{-3} molar solution. [b] The half wave potentials of Cu(II) copper (in the absence of cystine) in 0.5 M NH_3 and 0.1 M NH_3 are −0.178 and −0.084 volt, respectively. [c] The second step of the cystine wave is given in all experiments.

cupric copper. This decrease is much greater than can be accounted for by the effect of cuprous copper on the RSCu wave[2] and is partly due to an oxidation of RSCu by cupric copper to RSSR

$$2RSCu + 2Cu^{++} \rightleftarrows RSSR + 4Cu^+ \quad (4)$$

This interpretation is substantiated by the fact that the cuprous ammino wave increases to a greater extent than the cupric wave upon addition of increasing excess of cupric copper.

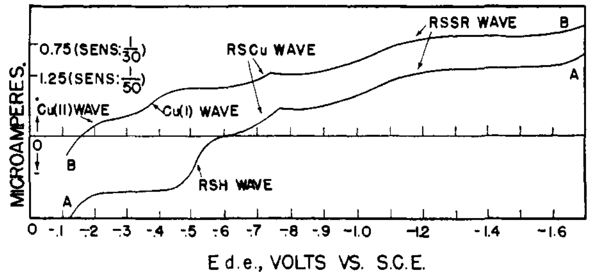

Fig. 1.—Polarograms of 50 ml. 10^{-3} M RSH, 0.1 M NH_3, 0.1 M NH_4Cl, 0.005% gelatin: A, after addition of 1 ml. 10^{-2} M cupric copper, RSH: Cu(II) = 5:1, sens. 1/30; B, after addition of 3.5 ml. 10^{-2} M cupric copper, RSH: Cu(II) = 5:3.5, sens. 1/50.

When polarograms are made of mixtures of cuprous copper and cystine in ammoniacal medium similar current voltage curves are found as shown in Fig. 1B. This shows that equation (4) is reversible.

According to equation (4) the RSSR wave should increase with increasing excess of cupric copper. Actually, the RSSR wave increases very slightly only and then decreases with increasing excess of cupric copper (Fig. 2). This may be due in part to a complex formation between cupric copper and RSSR, but the data in Table I show that this complex formation cannot be pronounced in ammoniacal medium. Polarographic studies of mixtures of cupric copper and cystine in ammoniacal medium show that complicated reactions occur at the surface of the dropping electrode, thus making it impossible to interpret quantitatively the apparent diffusion currents in Fig. 2 in the presence of an excess of cupric copper.

Cupric copper in ammoniacal medium gives a two-step reduction wave (Cu(II) → Cu(I) and Cu(I) → Cu(O)) both being of the same height. On the addition of an excess of cystine over copper the ammino cuprous wave (Cu(I) → Cu(O)) decreases markedly in height and may even disappear. Some of the results are summarized in Table I.

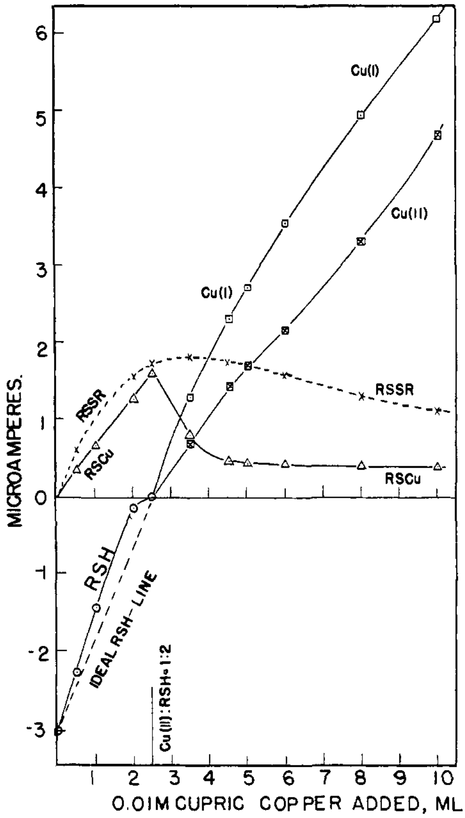

Fig. 2.—Titration of 50 ml. 10^{-3} M RSH (0.1 M NH_3, 0.1 M NH_4Cl, 0.005% gelatin) with 10^{-2} M cupric copper solution. Plot of diffusion currents *versus* ml. of cupric copper solution added.

It is seen that the diffusion current of the cupric wave (Cu(II) → Cu(I)) is either unaffected or *slightly* increased by the cystine, while its half wave potential is shifted to slightly more negative potentials. This shift, which is more pronounced at lower ammonia concentrations (see experiments 3 and 5), indicates some complex formation. The wave which starts at −0.7 volt has the characteristics of the RSCu wave, although no RSCu can be present in the cupric–RSSR mixture. The appearance of the RSCu wave can be interpreted only by assuming a reaction between cuprous copper (and/or copper amalgam) with RSSR (equation (4) from right to left) at the surface of the dropping electrode. This accounts for the fact that the cuprous ammino wave decreases with increasing concentrations of RSSR and even may disappear entirely. The above interpretation also accounts for the decrease of the RSSR wave in Fig. 2 with increasing excess of cupric copper.

The heights of the cuprous ammino and RSCu waves in mixtures of cystine and cupric copper are not only affected by the RSSR concentration, but also by the molarity of ammonia in solutions of the same *p*H. From Table I it appears that the effect is greater at smaller ammonia concentrations. The RSCu wave, measured in the above mixtures, is not or only partly diffusion controlled as could be inferred from measurements of its height *versus* the height of the mercury in the reservoir. In one instance the height of this wave was found to be 1.67, 2.66 and 3.66 μa. with mercury columns of 50, 76 and 100 cm., respectively. The corresponding quotients $i/\sqrt{h}$ are 0.237, 0.305 and 0.366. If the current were diffusion controlled, this quotient should be constant.

Polarographic experiments with ammoniacal cystine solutions and dropping copper amalgam (0.005% copper) as indicator electrode give conclusive evidence that under the conditions of the experiments the copper amalgam reacts with cystine to form RSCu

$$\mathrm{Cu(Hg)} + \mathrm{RSSR} \rightleftharpoons 2\mathrm{RSCu} + \mathrm{Hg} \qquad (5)$$

It is seen from Fig. 3, which presents polarograms obtained by electrolysis of an ammoniacal (10^{-3} *M*) cystine solution with dropping mercury and copper amalgam electrodes, that the prewave of cystine[4] disappears in the electrolysis with copper amalgam, while the RSCu wave is observed as a result of the reaction between copper amalgam and cystine.

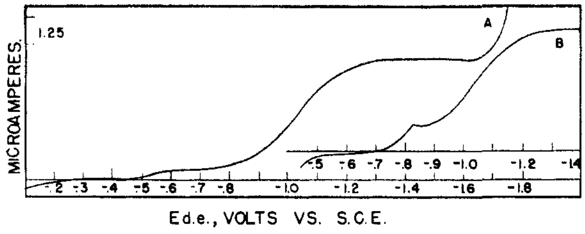

Fig. 3.—Polarograms of 10^{-3} *M* RSSR, 0.1 *M* NH_3, 0.1 *M* NH_4Cl, 0.005% gelatin, sens. 1/50: A, with the dropping mercury electrode, *h* = 76 cm.; B, with the dropping copper amalgam electrode (0.005% copper), *h* = 80 cm.

Cysteine and Cupric Copper in the Presence of Sulfite.—Cysteine in ammoniacal solution is oxidized by cupric copper to cysteine sulfonate ($RSSO_3^-$) in the presence of an excess of sulfite.

Cysteine sulfonate gives a reduction wave at the dropping mercury electrode. An example is given in Fig. 4A. The half wave potential and the diffusion current are affected by the *p*H. At *p*H 8.5 and 9.3 $E_{1/2}$ of an ammoniacal 5×10^{-4} cysteine sulfonate solution in the presence of 0.005% gelatin is −1.25 and −1.48 volts, respectively, and i_d/c is 6.0 and 5.2, respectively. The diffusion current is better defined at lower *p*H (8 to 8.5) in the presence of 0.0025 to 0.005% gelatin. The height of the wave corresponds to a two-electron reduction

$$\mathrm{RSSO_3^-} + 2\mathrm{e}^- \longrightarrow \mathrm{RS^-} + \mathrm{SO_3^=} \qquad (6)$$

In the absence of gelatin the shape of the wave varies with the concentration of cysteine sulfonate and the composition of the supporting electrolyte. Under certain conditions (see Fig. 4B) two waves may be found in the absence of gelatin. The concentrations of ammonia (0.5 to 0.08 *M*) and of sulfite (0.3 to 0.0 *M*) have no effect on the diffusion current of cysteine sulfonate in the presence of gelatin. Also cupric copper has hardly any effect on the $RSSO_3^-$ wave. This was found for a 10^{-3} *M* $RSSO_3^-$ solution which was 0.1 *M* in both ammonia and ammonium chloride, 0.2 *M* in sodium sulfite, 0.005% in gelatin and 5×10^{-4} to 1.5×10^{-3} *M* in cupric copper. Also the copper waves are not affected by the presence of cysteine sulfonate. Cuprous copper added to a 10^{-3} *M* cysteine sulfonate solution (0.1 *M* in ammonium chloride and ammonia, 0.2 *M* in sodium sulfite, 0.005% in gelatin) reduces the height of the $RSSO_3^-$ wave. Without cuprous copper i_d/c of the $RSSO_3^-$ wave is 5.20 in the above buffer. In the presence of 10^{-3} and 2.10^{-3} *M* cuprous copper the i_d/c values of the wave are 5.04 and 4.48, respectively. The waves of cuprous copper are unaffected by the presence of cysteine sulfonate in the solutions investigated. The interpretation of the peculiar effect of cuprous copper seems to be the following. In the reduction of cysteine sulfonate (equation (6)) cysteine is formed which diffuses away from the electrode and reacts with cuprous copper diffusing to the electrode. Since the diffusion coefficient of RSCu is considerably less than that of Cu(I) an apparent reduction of the $RSSO_3^-$ wave is observed. This is not found with cupric copper which reacts much more slowly with cysteine than cuprous copper does.

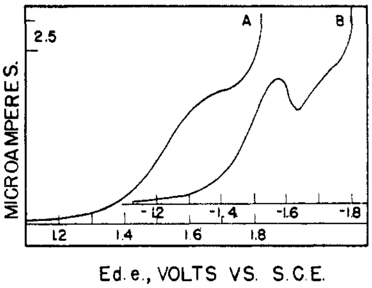

Fig. 4.—Polarograms of a 2×10^{-3} *M* cysteine sulfonate solution (0.5 *M* NH_3, 0.1 *M* NH_4Cl, 0.05 *M* Na_2SO_3) sens. 1/100: A, in the presence of 0.0025% gelatin; B, in the absence of gelatin.

In solutions containing a large excess of cupric or cuprous copper over cysteine sulfonate, no accurate values for the diffusion current of cysteine sulfonate are obtainable unless the copper waves are eliminated. This is done successfully by the addition of potassium cyanide (about 0.05 *M*) to the ammoniacal solution and adjustment of the *p*H with hydrochloric acid to about 9. A volume correction is made for the added cyanide and hydrochloric acid solution. The addition of gelatin is not required if cyanide is present in the solution. In fact, under these conditions better waves are obtained in the absence of gelatin.

Tests which were carried out with an ammoniacal cysteine sulfonate solution of known molarity in the presence of various amounts of cupric and cuprous copper and potassium cyanide showed that the $RSSO_3^-$ wave is not affected in height after elimination of the copper waves. Figure 5 illustrates the effect of cyanide in the solutions under consideration.

Polarograms obtained with mixtures of cuprous cysteinate and cysteine sulfonate showed that these two components do not affect each other's reduction wave.

We now return to the polarographic study of the reaction between cysteine–cupric copper–sulfite in ammoniacal medium. In one series of experiments increasing amounts of an air-free cysteine solution were added to an ammoniacal cupric copper solution containing sulfite. In another series, small increments of cupric copper solution were added to air-free cysteine solutions of various composition. The interval between each addition of an increment of the titrating agent was at least 20 minutes. Complete current–voltage curves were taken within these time intervals. The titration mixture was kept under an atmosphere of nitrogen. The diffusion currents are corrected for change in volume.

The addition of cysteine to an ammoniacal cupric copper solution containing sulfite (5×10^{-4} *M* Cu(II)), 0.05 *M* Na_2SO_3, 0.1 *M* NH_3, 0.1 *M* NH_4Cl, causes a reduction in the height of the cupric copper wave while the cuprous copper wave increases in height. The $RSSO_3^-$ wave appears. At the mole ratio Cu(II):RSH = 1:0.5 only the cuprous copper and $RSSO_3^-$ waves are found. The i_d/c and half wave potential of the ammino cuprous copper wave (Cu(I) → Cu(0)) in this solution are found to be 4.2 microampere and −0.38 volt, respectively. This is in exact agreement with the diffusion current and half wave potential of a cuprous copper solution prepared from cuprous oxide in the same buffer. The i_d/c of the cuprous wave in an ammoniacal cupric copper solution of the same composition is only 3.6. The difference between this value and the value of 4.2 must be accounted for by the difference in diffusion coefficient of the cupric and cuprous ammino ions. Thus, upon the conversion of cupric copper into an equimolar amount of cuprous copper in ammoniacal medium, the height of the cuprous wave increases.

From a quantitative analysis of the polarographic results the reaction between cupric copper and cysteine in the presence of sulfite can be represented by the equations

$$2Cu(II) + 2RS^- = RSSR + 2Cu(I) \qquad (7)$$

$$RSSR + SO_3^{=} = RS^- + RSSO_3^- \qquad (8)$$

$$2Cu(II) + RS^- + SO_3^{=} = 2Cu(I) + RSSO_3^- \qquad (9)$$

In agreement with the experimental results it is seen from equation (9) that all the cupric copper is reduced to the ammino cuprous ion after the addition of 1 mole of cysteine to 2 moles of cupric copper.

The addition of more cysteine to the above solution results in a reduction of the height of the cuprous wave and in the appearance of the cuprous cysteinate wave (equation (2)). The polarography of RSCu is discussed in a previous paper.[2] At the mol ratio Cu(II):RSH = 1:1.5 all the cuprous copper is found to be combined with cysteine and only the RSCu and the $RSSO_3^-$ waves are observed.

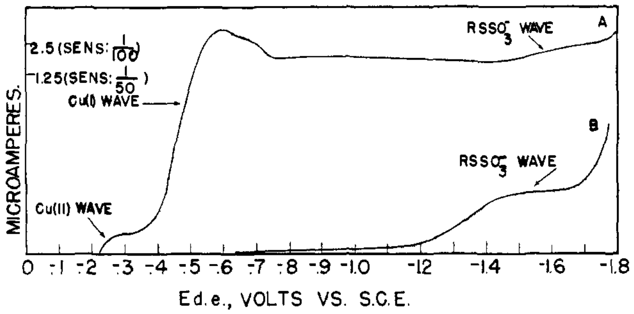

Fig. 5.—Polarograms of ammoniacal mixtures of cysteine sulfonate, cupric copper and cuprous copper before and after addition of potassium cyanide: A, 19.5 ml. of 5×10^{-4} *M* $RSSO_3$, 5×10^{-4} *M* Cu(II), 3×10^{-3} *M* Cu(I), 0.05 *M* Na_2SO_3, 0.5 *M* NH_3, 0.1 *M* NH_4Cl sens. 1/100; B, after addition of 1 ml. *M* KCN and 1 ml. 5 *M* HCl to solution A sens. 1/50.

Upon continued addition of cysteine the anodic cysteine wave appears.

The over-all reaction upon the addition of an excess of cysteine to an ammoniacal cupric copper solution containing sulfite is represented by

$$2Cu(II) + RS^- + SO_3^{=} = 2RSCu + RSSO_3^- \qquad (10)$$

Upon addition of cupric copper to an ammoniacal RSCu solution, which contains sulfite, oxidation takes place according to the over-all reaction (11). The partial reactions are

$$2RSCu + 2Cu(II) \rightleftarrows 4Cu(I) + RSSR \qquad (4)$$

$$RSSR + SO_3^{=} \rightleftarrows RSSO_3^- + RS^- \qquad (8)$$

$$RS^- + Cu(I) \longrightarrow RSCu \qquad (2)$$

$$RSCu + 2Cu(II) + SO_3^{=} \longrightarrow 3Cu(I) + RSSO_3^- \qquad (11)$$

That this reaction occurs quantitatively was proved polarographically with mixtures of RSCu, cupric copper and sulfite in ammoniacal medium and by titration of an ammoniacal cysteine solution with cupric copper in the presence of an excess of sulfite. The results in a titration of 50 ml. of a 5×10^{-4} *M* air-free cysteine solution (0.05 *M* NH_3, 0.05 *M* NH_4Cl, 0.15 *M* Na_2SO_3, 0.005% gelatin) with a 5×10^{-3} *M* cupric copper solution are discussed below. Some complete polarograms after various additions of cupric copper are presented in Fig. 6. In Fig. 7, the apparent diffusion currents of the components of the reaction mixture (RSH, RSCu, $RSSO_3^-$, Cu(I), Cu(II)) are plotted *versus* the amount (ml.) of cupric copper solution added. Since the i_d/c values of the components are known from separate experiments, it is possible to calcu-

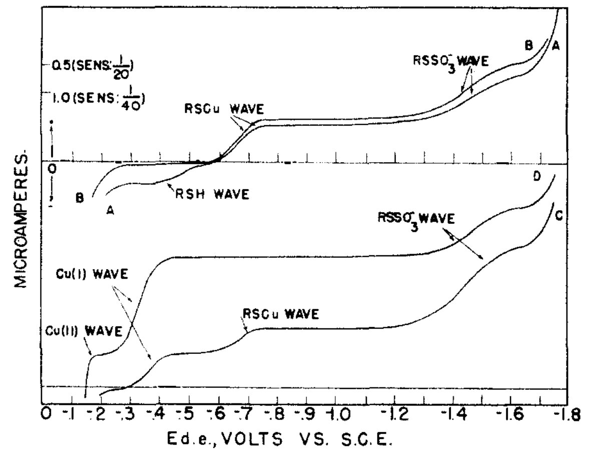

Fig. 6.—Polarograms in titration of 50 ml. 5 × 10^{-4} *M* RSH solution (0.05 *M* NH_3, 0.05 *M* NH_4Cl, 0.15 *M* Na_2SO_3, 0.005% gelatin) with a 5 × 10^{-3} *M* cupric copper solution: A, with 2.5 ml. Cu(II) solution, Cu(II):RSH = 0.5:1, sens. 1/20; B, with 3.3 ml. Cu(II) solution, Cu(II):RSH = 2:3, sens. 1/20; C, with 5.0 ml. Cu(II) solution, Cu(II):RSH = 1:1, sens. 1/20; D, with 15.0 ml. Cu(II) solution, Cu(II):RSH = 1:3, sens. 1/40.

late the molarities of each constituent from the diffusion currents.

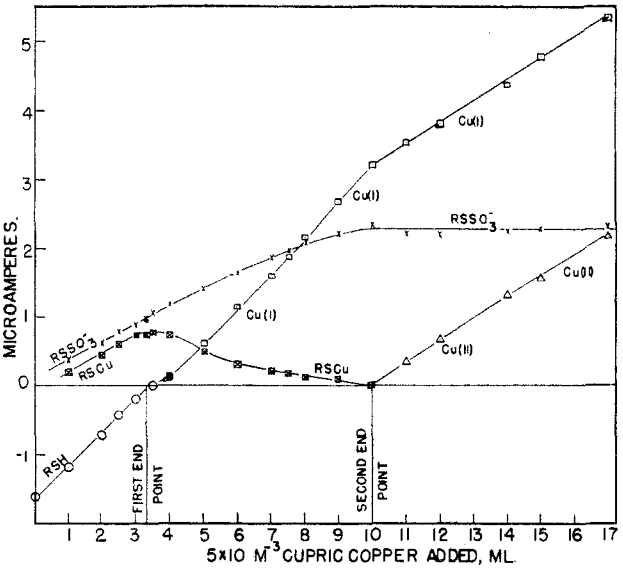

Fig. 7.—Titration of 50 ml. 5 × 10^{-4} *M* RSH (0.05 *M* NH_3, 0.05 *M* NH_4Cl, 0.15 *M* Na_2SO_3, 0.005% gelatin) with a 5 × 10^{-3} *M* cupric copper solution. Plot of apparent diffusion currents *versus* ml. of cupric copper solution added.

The anodic cysteine wave decreases as increasing amounts of cupric copper are added, while the cathodic waves of RSCu and $RSSO_3^-$ appear and increase in height. An end-point is found after copper has been added at a mole ratio Cu(II):RSH = 2:3. At this point all the cysteine in the solution is converted into RSCu and $RSSO_3^-$ according to equation (10). The diffusion current of RSCu at this point is 0.79 μa. The value of i_d/c for RSCu in the same electrolyte was found to be 2.42.[2] Therefore, the molarity of RSCu is 3.27 × 10^{-4} which is in good agreement with the theoretical value (3.33 × 10^{-4}) as required by equation (10). The molarity of the cysteine sulfonate is found to be 1.88 × 10^{-4} while the theoretical value is 1.67 × 10^{-4} at this end-point.

On further addition of cupric copper the cuprous wave appears and increases in height. The diffusion current of the RSCu wave decreases for two reasons. First, RSCu is oxidized according to equation (11). Secondly, cuprous amino ion decreases the RSCu wave.[2] Thus, the decrease of the RSCu diffusion current is found to be represented by a curve and not by a straight line. Similarly, the $RSSO_3^-$ wave does not increase along a straight line since it is somewhat affected by cuprous ammino ion as was shown in a previous section of this paper.

When the cuprous cysteinate is converted completely into cysteine sulfonate and cuprous copper, a second end-point is obtained. At this point the total amount of added cupric copper is twice the molarity of the cysteine. A combination of equations (10) and (11) gives the stoichiometry for the second end-point

$$2Cu(II) + RS^- + SO_3^= = 2Cu(I) + RSSO_3^- \quad (12)$$

The plot given in Fig. 7 shows that reaction (12) is quantitative at the second end-point. At this point the RSCu wave has disappeared, and the diffusion current of the $RSSO_3^-$ wave reaches a value of 2.27 μa. The i_d/c of $RSSO_3^-$ at this point is 4.48. This corresponds to a molarity of 5.06 × 10^{-4} for cysteine sulfonate which is in good agreement with the value of 5.00 × $10^{-4}M$ calculated from equation (12). This value is also obtained in separate experiments after elimination of the cuprous wave with cyanide. The value of i_d/c of cuprous ammino ion under the conditions of the experiment was determined separately and found to be 3.2. In agreement with equation (12) the same value is found for the diffusion current of Cu(I) at the second end-point.

The addition of cupric copper after the second end-point results in the appearance of the cupric copper wave. The decrease of the slope of the line representing the height of the cuprous copper wave after the end-point is explained again by the difference in diffusion coefficient of cupric and cuprous ammino ions.

Polarographic titrations of cysteine with cupric copper similar to the one graphed in Figs. 6 and 7 were carried out in solutions of varying ammonia and sulfite concentrations. The rate of the reaction between cupric copper and cysteine was found to decrease at higher ammonia (0.5 *M*) and lower sulfite (0.05 *M*) concentrations, the over-all reaction is given again by equation (12) when the titration mixture and the cupric reagent are completely free of oxygen. Titrations with air-saturated copper solutions of air-free mixtures of large ammonia and small sulfite concentration gave errors of −20 to −25% due to oxygen oxidations.

It is remarkable that the reaction in these titrations with air-containing copper solutions is found to be quantitative at the first end-point, according to equation (10) while only the second part of the titration (oxidation of RSCu to $RSSO_3^-$ and

Cu(I)) is affected by ammonia and sulfite concentration. The polarographic analysis of these titrations indicates that all the cysteine is completely converted into cysteine sulfonate at the second end-point. The low consumption of cupric copper in these titrations is due to the oxygen oxidation of the cuprous cysteinate after the first end-point. This oxidation is catalyzed by the cuprous copper formed in the reaction between cuprous cysteinate and cupric copper. The air oxidation of RSCu is almost negligible when the reaction between cuprous cysteinate, cupric copper and sulfite is fast, but it becomes pronounced when this reaction is slow. The reaction is rapid at low ammonia (0.05 *M*) and high sulfite (0.15 *M*) concentrations and under those conditions, stoichiometrically correct results with air-saturated cupric copper solutions are obtained (see Fig. 7).

In a subsequent paper it is shown that the reaction between cysteine and cupric copper in the presence of sulfite can be made the basis of an accurate and rapid amperometric titration of traces of cysteine and cystine, using the rotating platinum wire electrode as indicator electrode.

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The Reaction between Ferrous Iron and Peroxides. IV. Reaction with Potassium Persulfate[1a]

BY I. M. KOLTHOFF, A. I. MEDALIA[1b] AND HELEN PARKS RAAEN

Upon very slow addition of ferrous iron to an acid (0.5 *M* sulfuric acid), neutral or alkaline (0.1 *M* pyrophosphate) persulfate solution, the molar reaction ratio of ferrous iron to persulfate is found less than 2. Upon rapid mixing of the reactants, the stoichiometric reaction ratio of 2 is found in the absence of organic substances. Upon rapid mixing or slow addition of ferrous iron to ethanol containing persulfate solution in the absence of oxygen, Fenton reaction occurs, in which alcohol is oxidized to aldehyde. The reaction ratio is less than 2. Chloride and especially bromide suppress the induced alcohol oxidation, but acetic acid and acetone have no effect. Acrylonitrile suppresses the induced alcohol oxidation; the reaction ratio in the presence of acrylonitrile is close to 1, because the $SO_4^{\overline{\cdot}}$ radical-ions formed in the reaction between ferrous iron and persulfate are effectively consumed in the initiation of the acrylonitrile polymerization. In the presence of oxygen and organic compounds like ethanol, acetone, acetic acid, reaction ratios greater than 2 are found. The induced oxygen oxidation of ferrous iron is explained. Bromide is an effective suppressor of this induced reaction.

The peroxidisulfate ion, $S_2O_8^{=}$ (hereinafter referred to simply as the persulfate ion), is a symmetrically substituted derivative of hydrogen peroxide. The present study was carried out in order to establish whether and to what extent the phenomena which are characteristic of the reaction of ferrous iron with hydrogen peroxide[2,3] also occur with persulfate; *viz.*, the decomposition of the peroxide induced by the peroxide–ferrous iron reaction, the induced reaction between the peroxide and alcohols, the induced oxidation of ferrous iron by molecular oxygen in the presence of the peroxide and various organic substrata, and the effect of chloride ion and other compounds in suppressing these induced reactions.

Since the start of the present work, valuable papers by Merz and Waters[4,5] have been published. These authors have made a thorough quantitative study of the reaction ratios obtained in the oxidation of various organic compounds by hydrogen peroxide in the presence of ferrous iron (Fenton reaction), a few experiments also having been carried out with sodium persulfate.[4] By extrapolation of a series of experiments with increasing ratios of organic substratum to ferrous iron, values have been found for the relative reaction rates of the hydroxyl radical (formed in the hydrogen peroxide–ferrous iron reaction) with the substratum and with ferrous iron, respectively. Unfortunately, Merz and Waters were evidently unaware of the induced oxygen oxidation accompanying these reactions,[2] and although they worked in relatively concentrated solutions, our work indicates that this induced oxidation may have led to a very considerable error in the observed reaction ratios. This is particularly true in experiments with an initial large excess of organic substratum, since under these conditions the direct oxidation of ferrous iron by peroxide becomes so small as to be of comparable magnitude with the induced oxidation by dissolved air. On the other hand, at *low* initial concentrations of the organic substratum, considerable error may result from competition or suppression by the primary products, as recognized by Merz and Waters. For these reasons some uncertainty must be attached to the ratios reported by these authors (*cf.* Table II). The work of Merz and Waters has shown definitely, however, that the primary active intermediate in the persulfate–ferrous iron reaction is not the same as that formed in the hydrogen peroxide–ferrous iron reaction (contrary to the suggestion of Morgan[6] that the hydroxyl radical might be the active species in both reactions). Polymerization

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(1b) Brookhaven National Laboratory, Upton, Long Island, New York.

(2) I. M. Kolthoff and A. I. Medalia, THIS JOURNAL, **71**, 3777, 3784 (1949).

(3) A. I. Medalia and I. M. Kolthoff, *J. Polymer Sci.*, **4**, 377 (1949).

(4) J. H. Merz and W. A. Waters, *Faraday Soc. Discussion*, **2**, 179 (1947).

(5) J. H. Merz and W. A. Waters, *J. Chem. Soc.*, S-15 (1949).

(6) L. B. Morgan, *Trans. Faraday Soc.*, **42**, 169 (1946).