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Polarographic Investigations of Reactions in Aqueous Solutions Containing Copper and Cysteine (Cystine). I. Cuprous Copper and Cysteine in Ammoniacal Medium. The Dissociation Constant of Cuprous Cysteinate

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Cuprous copper and cysteine in ammoniacal medium react to give slightly dissociated cuprous cysteinate (RSCu). The addition of an excess of cuprous copper to a solution of cuprous cysteinate results in a marked decrease of the diffusion currents of both the cathodic cuprous copper and RSCu waves and in a shift of the half wave potential of the RSCu wave to more negative values. This indicates the formation of complex compounds between cuprous cysteinate with cuprous copper. The RSCu wave observed with a solution containing equimolar amounts of cysteine and cuprous copper is not ideally reversible. In the presence of a sufficient excess of cysteine the RSCu wave becomes reversible. No indication of complex formation between RSCu and cysteine has been obtained. From polarographic data the dissociation constant of RSCu (RSCu → $RS^- + Cu^+$) has been calculated to be 6.5×10^{-20} at 25° and the free energy of formation −26.2 kcal.

In studies to be reported later on the reaction between cupric copper and cysteine in ammoniacal medium, cuprous cysteinate was found to be one of the reaction products.

No data are found in the literature on the properties of cuprous cysteinate denoted below as RSCu. Preliminary experiments gave evidence that it is a slightly dissociated compound and more detailed information on the apparent dissociation of RSCu appeared desirable. The present paper deals with a polarographic study of the system cysteine (RSH–cuprous copper in ammoniacal medium.

Materials

Cysteine, which was used in the form of its hydrochloride, was a C.P. product from Eimer and Amend.

A standard cupric copper solution was prepared by dissolving an accurately weighed amount of C.P. copper (Eimer and Amend) in 6 *N* nitric acid. After dissolution and evaporation with sufficient sulfuric acid to remove all nitric acid, the nearly dry residue was diluted to the mark in a volumetric flask. The copper titer was checked iodometrically.

Cuprous copper solutions were prepared in two ways: (1) A stock solution was made up which was 10^{-2} *M* in cupric copper, 2.10^{-2} *M* in glucose (Mallinckrodt, C.P.) and 1 *M* in ammonia. Before use a portion of this solution was put into a test-tube and heated to near boiling. The solution became colorless within 1 minute. In order to minimize escape of vapor from the solution, a conical tube (centrifuge tube) filled with cold water was put into the open end of the test-tube during heating. The colorless solution which was 10^{-2} *M* in cuprous copper was allowed to cool under nitrogen. (2) A given volume of standard cupric copper solution was placed into a volumetric flask and a one molar sodium sulfite solution was added until the precipitate formed in the beginning dissolved in excess of sodium sulfite. The mixture was filled up with air-free distilled water to the mark of the flask. The resulting solution was 0.01 *M* in cuprous copper and 0.2 *M* in sodium sulfite. The solution was light green immediately after mixing and became colorless after a few minutes standing. Stock solutions prepared in this way contain dithionate.

Copper amalgam was prepared by electrolysis of a copper sulfate solution using a weighed amount of mercury as cathode and a pure copper rod as anode. The amalgam obtained in this way was 0.25% in copper. After washing with water and alcohol and drying in vacuum the concentrated amalgam was diluted with pure mercury to the desired composition (0.005% in copper).

Experimental Methods

Current voltage curves were measured at 25.0° with the manual apparatus and circuit described by Lingane and Kolthoff[1] and automatically with a Heyrovsky self-recording polarograph. All potentials were measured against the saturated calomel electrode (S.C.E.). Oxygen was removed from the solutions in the cell with a stream of nitrogen which was passed through an ammonia buffer of the same composition as that used in the test solution. During an experiment an atmosphere of nitrogen was maintained over the solution. Gelatin in a concentration of 0.005% was used as a maximum suppressor. Corrections were made for the residual current.

The characteristics of the capillary used were: $m = 1.804$ mg. sec.$^{-1}$, $m^{2/3}t^{1/6} = 1.89$ mg.$^{2/3}$ sec.$^{-1/2}$; the height of the mercury column was 76.1 cm. unless otherwise stated.

The *p*H of all solutions was measured with a Beckman *p*H meter, laboratory model G.

Results and Discussion

An air-free 10^{-3} *M* cysteine solution in ammoniacal medium was titrated with 0.01 *M* cuprous copper. Polarograms were taken after various additions of copper. As an illustration, a few examples are reproduced in Fig. 1; a complete summary of the (corrected) diffusion currents of all constituents during the titration are given in Fig. 4.

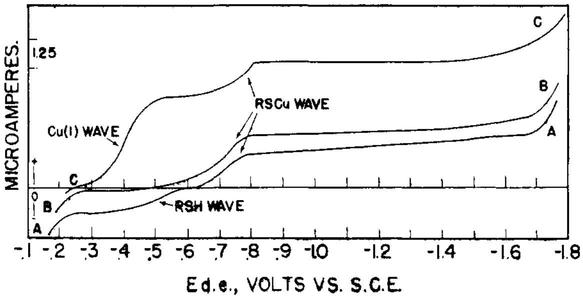

Fig. 1.—Polarograms taken during a titration of 50 ml. 10^{-3} *M* cysteine (0.1 *M* NH_3, 0.1 *M* NH_4Cl, 0.005% gelatin) with a 10^{-2} *M* cuprous copper solution. After addition of (A) 3 ml., (B) 5 ml. and (C) 11 ml. 10^{-2} *M* Cu(I) solution.

Upon addition of cuprous copper to cysteine a reaction occurs

$$RSH + Cu(I) \longrightarrow RSCu + H^+$$

Polarogram A in Fig. 1 was obtained after addition of 60% of the equivalent amount of copper to the cysteine solution. The anodic wave is that of the excess of cysteine, which has the characteristics described in a previous paper.[2] The cathodic wave starting at about −0.6 volt corresponds to the reduction of RSCu to copper amalgam When the amounts of RSH and Cu(I) are equivalent, only the RSCu wave is found (Fig. 1B). With an excess of cuprous copper the first cathodic wave corresponds to the reduction of the cuprous ammino ion (denoted here as Cu(I)) to copper amalgam while the

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[1] J. J. Lingane and I. M. Kolthoff, THIS JOURNAL, **61**, 825 (1939).

[2] I. M. Kolthoff and C. Barnum, *ibid.*, **62**, 3061 (1940).

second wave is that of RSCu (Fig. 1C). Evidently the RSCu is reduced at a much more negative potential than the cuprous ammino ion. The RSCu wave exhibits a maximum which is eliminated by the presence of 0.005% gelatin. This amount of gelatin has no effect on the height of the waves considered in this paper.

The height of the RSCu wave is considerably smaller than that of equivalent concentrations of cuprous ammino ion or of cysteine. In an ammonia buffer which is 1 M in ammonia, 1 M in ammonium chloride, 0.04 M in sodium sulfite and 0.005% in gelatin, the diffusion coefficient of cuprous cysteinate as calculated from the Ilkovic equation was found to be 4.1×10^{-6} cm.^{2} sec.$^{-1}$ at 25°, while those of cysteine and of the cuprous ammino complex in the same ammonia buffer were found to be 7.4×10^{-6} cm.^{2} sec.$^{-1}$ and 1.5×10^{-5} cm.^{2} sec.$^{-1}$, respectively. The diffusion coefficient of the RSCu in the ammoniacal buffer has about the same value as that of silver cysteinate for which a value of 3.8×10^{-6} cm.^{2} sec.$^{-1}$ was calculated.[3]

It is of interest to mention that the sulfite cuprous complex also has a much lower diffusion coefficient than the cuprous ammino ion. The addition of increasing amounts of sodium sulfite to an ammoniacal cuprous copper solution not only causes a shift of the half wave potential to more negative values, but also results in a decrease of the diffusion current of the cathodic cuprous copper wave. Thus a 10^{-3} M cuprous copper solution which was 0.1 M in ammonium chloride, 0.5 M in ammonia, 0.05 M in sodium sulfite and 0.005% in gelatin gave a wave with a diffusion current of 4.03 μA. In the presence of 0.5 M sodium sulfite under otherwise identical conditions, the diffusion current of the cuprous copper wave was found to be 2.58 μA. A study of these sulfito cuprous copper complexes is planned.

The low diffusion coefficient of cuprous cysteinate is at great variance with the covalent complexes of cupric copper with glycinate, alaninate and aspartate ions. In these compounds the diffusion coefficient of the complex is practically equal to the diffusion coefficient of the aquo cupric copper ion.[4,5,6]

Upon addition of cuprous copper to an ammoniacal solution of RSCu the values of i_d/c for both the ammino cuprous copper and the RSCu become less than calculated from the over-all composition of the mixture. At the same time the halfwave potential of the RSCu wave is shifted to somewhat more negative values. These phenomena indicate that ammino cuprous copper reacts with cuprous cysteinate in ammoniacal medium to form complexes of the type $Cu^+ + xRSCu = Cu(RSCu)_x^+$. The formation of these complexes seems to proceed slowly. The polarogram of a solution which was 2.10^{-3} M in RSCu, 3.10^{-3} M in cuprous copper, 0.1 M in ammonia, 0.1 M in ammonium chloride, 0.15 M in sodium sulfite and 0.005% in gelatin and which was allowed to stand at 25° in an atmosphere of nitrogen for 3 hours is represented in Fig. 2. Immediately after mixing, i_d/c of RSCu and Cu(I) were 2.12 and 2.26 and after 3 hours these values were 1.68 and 2.11, respectively. At the same time a new ill-defined wave preceding the RSCu wave appears upon standing, indicating the presence of more than one complex.

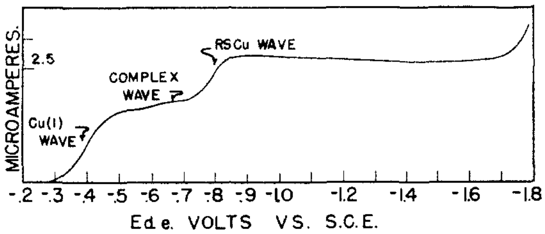

Fig. 2.—Polarogram of 2×10^{-3} M RSCu, 3×10^{-3} M Cu(I), 0.1 M NH_3, 0.1 M NH_4Cl, 0.15 M Na_2SO_3, 0.005% gelatin after 3 hours standing, sens. 1/100.

The change of the diffusion currents of the constituents in solutions of varying mole ratio of RSCu and cuprous copper can be followed polarographically by adding small increments of cuprous copper to a cuprous cysteinate solution.

Such experiments were carried out in electrolyte solutions of different ammonia concentrations (0.05, 0.1 and 0.5 M, respectively) and in the absence and presence of sodium sulfite (0.0, 0.05 and 0.15 M, respectively). The time intervals between each addition of cuprous copper were at least 20 minutes. Complete current–voltage curves were taken after each addition of cuprous copper. The results are given in Table I. The diffusion currents given in this table are corrected for change in volume by the added cuprous copper solution.

It is seen that the decrease in height of the RSCu wave upon the addition of cuprous copper is not greatly affected by ammonia or sulfite concentration. On the other hand, the reduction in the height of the cuprous copper wave by RSCu becomes greater with decreasing ammonia (0.05 M) and increasing sulfite concentrations (0.15 M). In solutions containing RSCu and Cu(I) in the mole ratio 1:2 and of sulfite concentrations of 0.15 M, 0.05 M and 0.0, respectively, the reduction of the diffusion current of the cuprous copper wave was found to be 18, 14 and 7.8%, respectively, as compared to cuprous copper solutions of the same composition, but without RSCu.

In runs A and B cuprous copper was prepared by method 2. In run C cuprous copper was prepared by method 1. The cuprous copper used in the runs A and B of Table I contained dithionate, while that in runs C was free of sulfite. The i_d/c values of the RSCu waves are practically the same in the three sets, A, B and C. This shows that dithionate or glucose have no effect on the RSCu wave under the conditions of the experiments.

If the dissociation of RSCu is given by the equation

$$RSCu \rightleftarrows RS^- + Cu^+ \qquad (1)$$

and the electrode reaction

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

were reversible, the equation of the RSCu wave in

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

(4) R. M. Keefer, *ibid.*, **68**, 2329 (1946).

(5) H. A. Laitinen, E. I. Onstott, J. C. Bailar, Jr., and S. Swanson, Jr., *ibid.*, **71**, 1550 (1949).

(6) N. C. Li and E. Doody, *ibid.*, **72**, 1891 (1950).

TABLE I

CATHODIC WAVES OF MIXTURES OF CUPROUS CYSTEINATE AND CUPROUS COPPER

Concn. of Cu(I) copper, M	Mole ratio RSCu: Cu(I)	A. Electrolyte: $5 \cdot 10^{-4}$ M RSCu, 0.05 M NH_4Cl, 0.05 M NH_3, 0.15 M Na_2SO_3			B. Electrolyte: $5 \cdot 10^{-4}$ M RSCu, 0.1 M NH_4Cl, 0.5 M NH_3, 0.04 M Na_2SO_3			C. Electrolyte: 10^{-3} M RSCu, 0.1 M NH_4Cl, 0.1 M NH_3, no Na_2SO_3 Concn. of Cu(I) copper, M	Mole-ratio RSCu: Cu(I)			
		RSCu-wave i_d/c	RSCu-wave $E_{1/2}$	Cu(I) wave[a] i_d/c	RSCu-wave i_d/c	RSCu-wave $E_{1/2}$	Cu(I) wave[b] i_d/c			RSCu-wave i_d/c	RSCu-wave $E_{1/2}$	Cu(I) wave[c] i_d/c
10^{-3}	No RSCu	..	.....	3.17	...	.....	4.03	10^{-3}	No RSCu	..	.....	3.5
None	...	2.42	−0.676	..	2.36	−0.694	..	None	...	2.34	−0.700	..
1.5×10^{-4}	1:0.3	2.64	− .675	0.80	2.46[d]	− .70	1.56	4×10^{-4}	1:0.4	2.04	− .73	3.13
2.5×10^{-4}	1:0.5	2.42	− .685	1.42	2.30	− .72	2.40	8×10^{-4}	1:0.8	1.89	− .75	3.60
5×10^{-4}	1:1	1.92	− .70	2.11	1.95	− .73	3.04	1.2×10^{-3}	1:1.2	1.71	− .765	3.93
7.5×10^{-4}	1:1.5	1.64	− .71	2.38	1.64	− .74	3.34	2×10^{-3}	1:2	1.43	− .775	4.15
10^{-3}	1:2	1.28	− .71	2.60	1.56	− .745	3.47					

$E_{1/2}$ in this medium was: [a] −0.370 volt in all experiments. [b] −0.450 volt in all experiments. [c] −0.390 volt in all experiments. [d] Concentration of Cu(I) copper was 10^{-4} M.

solutions containing equimolar amounts of Cu(I) and RSH should be

$$E = \text{const.} + \frac{RT}{F} \ln \frac{(i_d - i)}{i^2} \qquad (3)$$

In Fig. 3 is given the plot of log $(i_d - i)/i^2$ *versus* the potential. The plot is a straight line up to about the half wave potential with a slope of 0.07 instead of the theoretical value of 0.059. The plot of log $(i_d - i)/i$ *versus* potential which is also given in Fig. 3 is a curve in the entire potential range investigated.

According to equation (3) the half wave potential of RSCu is not constant, but becomes more negative with increasing concentrations. Actually, it was found that in a given ammonia buffer and supporting electrolyte the half wave potential changes from −0.676 volt for a 5×10^{-4} M RSCu solution to −0.730 volt for a 2×10^{-3} M solution. The difference is 0.054 volt as compared to the calculated value of 0.036 volt (equation (3)). At the same RSCu concentration and ionic strength, the half wave potential of the RSCu wave is shifted to more negative values with increasing *p*H.

Upon addition of cysteine to an ammoniacal cuprous cysteinate solution in a mole ratio RSH: RSCu of 1:1 or 2:1 the diffusion current and half wave potential of the RSCu wave are practically unchanged, but the waves approach more closely a reversible reaction (equation (3)).

The anodic diffusion current of cysteine with small concentrations of RSH in the presence of an excess of RSCu is ill defined and smaller than in the absence of RSCu. This decrease may be due to the fact that the anodic cysteine wave corresponds to the formation of mercury cysteinate: this reaction at the electrode may be affected by the presence of cuprous cysteinate.

The lowering of the anodic cysteine wave in the presence of cuprous cysteinate can be followed by a titration of cysteine with cuprous copper.

For this purpose, a slow titration of a 10^{-3} M air-free cysteine solution in an ammonia buffer (0.1 M in ammonia, 0.1 M in ammonium chloride, and 0.005% in gelatin) was carried out with a 10^{-2} M cuprous copper solution (prepared with glucose). The time intervals between each addition of cuprous copper were about 20 minutes. Complete current-voltage curves were taken after each addition of cuprous copper and from the data Fig. 4 was constructed which gives the diffusion currents of RSH, RSCu and Cu(I) as a function of the increments of cuprous copper added. The dotted lines give the ideal titration lines; the full lines correspond to the experimental data, corrected for the volume effect. The decrease of the diffusion current of RSCu in the presence of an excess of Cu(I) and that of Cu(I) by RSCu (as mentioned in a previous paragraph) is also clearly noticeable in Fig. 4).

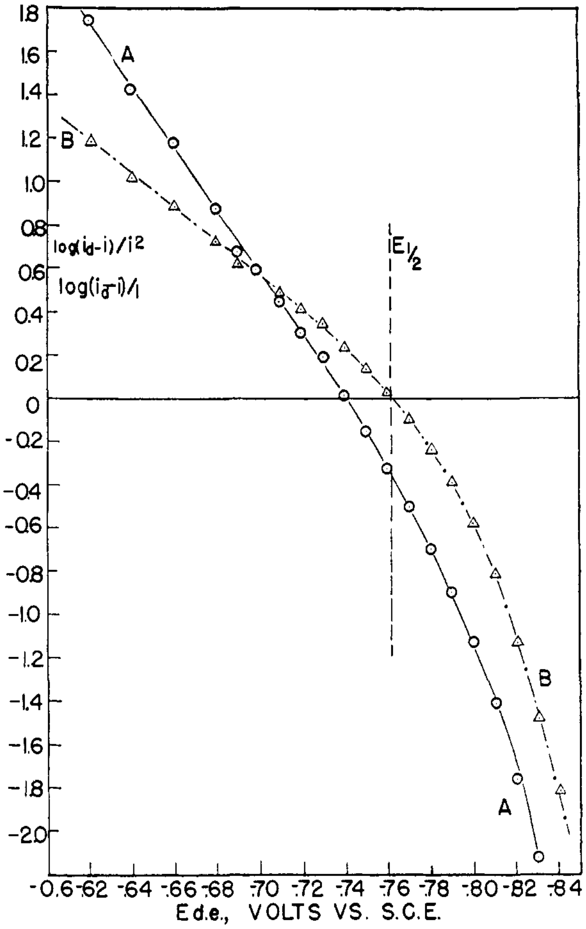

Fig. 3.—Plots of (A), log $(i_d - i)/i^2$ and (B), log $(i_d - i)/i$ *versus* potential of 2×10^{-3} M RSCu, 0.5 M NH_3, 0.1 M NH_4Cl, 0.04 M Na_2SO_3, 0.005% gelatin.

The addition of relatively large amounts of cysteine (1.5×10^{-2} M RSH and greater) to an ammoniacal cuprous cysteinate solution (2×10^{-3}

TABLE II

RSCu WAVES IN THE PRESENCE OF EXCESS OF CYSTEINE, $[Na_2SO_3] = 0.04\ M$ AND $[NH_3] = 1\ M$

Cu(I), *M*	Composition of electrolyte RSH, *M*	Composition of electrolyte NH_4Cl, *M*	Mole ratio Cu(I): RSH	RSCu wave i_d/c	RSCu wave $E_{1/2}$	*p*H	Slope	K_{RSCu} × (times) 10^{20}	No. of expt.
2×10^{-3}	None	1.0	...	4.4^a	0.503^a	9.3	...	...	1
2×10^{-3}	4×10^{-2}	1.0	1:20	2.54	.835	9.03	0.060	3.9	2
2×10^{-3}	4×10^{-2}	0.05 *M* NH_4Cl .95 *M* $NaNO_3$	1:20	2.42	.868	9.96	.055	7.6	3
2×10^{-3}	1.5×10^{-2}	1.0	1:7.5	2.32	.813	9.37	.059	6.4	4
5×10^{-4}	1.5×10^{-2}	1.0	1:30	2.50	.809	9.37	.062	8.8	5
5×10^{-4}	4×10^{-2}	1.0	1:80	2.50	.836	9.21	.058	5.8	6
								Average *K* 6.5×10^{-20}	

[a] Values of cuprous ammino ion.

M to 5×10^{-4} *M* RSCu) makes the RSCu wave completely reversible. Thus, when the concentrations of cysteinate ion in the bulk of the solution and at the electrode interface are practically the same equilibrium at the dropping mercury electrode is established rapidly. When the excess of RS^- is large enough the equation of the wave becomes

$$E = \text{const.} + \frac{RT}{F}\ln\frac{(i_d - i)}{i} \qquad (4)$$

The reversibility of the electrode reaction was tested by determining the slopes of the plots log $i/(i_d - i)$ against $E_{d.e.}$ and by measuring the values of $(E_{3/4} - E_{1/4})$. Moreover, the dissociation constant of RSCu could be calculated from the following experiments.

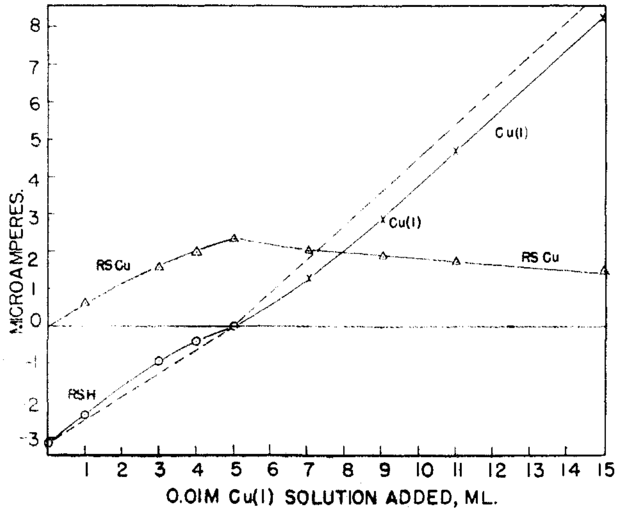

Fig. 4.—Slow titration of 50 ml. 10^{-3} *M* cysteine (0.1 *M* NH_3, 0.1 *M* NH_4Cl, 0.005% gelatin) with 10^{-2} *M* cuprous copper solution. Plot of diffusion currents *versus* ml. of Cu(I) solution added: ------ is the calculated titration line assuming RSCu to be the only reaction product.

Ammoniacal cuprous copper solutions in the presence of various amounts of cysteine and at various *p*H were polarographed and the diffusion currents and half wave potentials of the RSCu waves were measured. The values for the half wave potentials were taken from the plots log $i/(i_d - i)$ *versus* the potential. The results of the polarographic measurements are given in Table II. The slopes of the plots log $i/(i_d - i)$ *versus* *E* (called "slope" in Table II) indicate a one electron reduction. If the electrode reaction (equation 2) is reversible the following relationship[7]) should hold at 25°

$$\Delta E_{1/2}/\Delta \log C_x f_x = -p\ 0.0591/n$$

where C_x is the concentration of the cysteinate ion, f_x, its activity coefficient and *p* the number of cysteinate ions which combine with one cuprous copper. For our purposes activity coefficients are taken equal to unity. Since "*n*" is one, *p* can be determined. The value for $\Delta E_{1/2}/\Delta \log C_x$ is found to be −0.047 from experiments 2 and 4 and −0.062 from experiments 5 and 6, indicating that one cysteinate ion combines with one cuprous copper.

The dissociation constant

$$K_{RSCu} = ((Cu^+)(RS^-))/(RSCu) \qquad (5)$$

can be estimated since the dissociation constant of the cuprous ammino complex is known.[8] Bjerrum found that cuprous copper is present only as diammine in solutions which are 0.01 *M* in ammonia. Even at greater ammonia concentrations higher ammines are not formed by cuprous copper. The dissociation constant of this ammine is 1.35×10^{-11} according to Bjerrum. The half wave potential of RSCu ($n = 1$, $p = 1$) in excess of cysteinate at 25° can be expressed by the equation

$$E_{1/2}(\text{RSCu}) = \epsilon + 0.059 \log K_{RSCu} - 0.059 \log (k_{RSCu}/k_a) - 0.059 \log C_{RS^-} \qquad (6)$$

where ϵ is equal to $E^0_a + 0.059 \log a_{Hg}$. E^0_a is the standard potential of the amalgam and a_{Hg} is the activity of mercury in the amalgam. Since the amalgam formed at the electrode is very dilute a_{Hg} is practically equal to the activity of pure mercury and can be considered as constant. Therefore, ϵ is constant. The values of k_{RSCu} and k_a are proportional to the square roots of the diffusion coefficients of the cuprous cysteinate and the copper in the amalgam, respectively. C_{RS^-} is the concentration of the free cysteinate ion which can be calculated from the concentration of the free cysteine, *p*H of the solution and pK_S of cysteine by means of the relation

$$\log C_{RS^-} = \log K_{RSH} + \log C_{RSH} + pH = \log C_{RSH} - pK_{RSH} + pH \qquad (7)$$

Substituting in (6), the equation is obtained.

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(7) J. J. Lingane, *Chem. Revs.*, **29**, 1 (1941).

(8) J. Bjerrum, *Kgl. Danske Videnskab. Selskab. Math.fys. Medd.*, **11**, 5 (1931); **11**, 10 (1932); **12**, 15 (1934).

$$E_{1/2}\,(\text{RSCu}) = \epsilon + 0.059 \log K_{\text{RSCu}} - 0.059 \log \frac{k_{\text{RSCu}}}{k_a} - 0.059\,(p\text{H} - pK_{\text{RSH}}) - 0.059 \log C_{\text{RSH}} \quad (6a)$$

C_{RSH} is the analytical concentration of the free cysteine and is obtained by subtracting the concentration of cysteine combined with cuprous copper (on the base RSH:Cu(I) = 1:1) from the total cysteine concentration in the solution. pK_3(SH) of cysteine is reported to be 10.28.[9] For our purpose it is considered to correspond to the acid constant of the SH-group, although this is an over-simplification.[10] pK_1 (COOH) is equal to 1.96 and can be neglected at the pH range investigated. Since the thiol group and not the amino group is involved in the reaction between cysteine and cuprous copper, ($pK_2(\text{NH}_3^+)$) is not taken into account. The cysteinate ion is denoted as RS^-, disregarding the amino and carboxyl group.

The half wave potential of the cuprous ammino complex ($n = 1, p = 2$) is expressed by

$$E_{1/2}\,(\text{Cu(NH}_3)_2{}^+) = \epsilon + 0.059 \log K_{\text{Cu(NH}_3)_2{}^+} - 0.059 \log \frac{k_{\text{Cu(NH}_3)_2{}^+}}{k_a} - 2 \times 0.059 \log C_{\text{NH}_3} \quad (8)$$

Since the ammonia concentration is unity in all the experiments listed in Table II, the last term cancels out. The half wave potential and diffusion current of the cuprous ammino ion were determined separately and the values are given in Table II.

Combining equations (6a) and (8), the dissociation constant K_{RSCu} can be calculated from the relation

$$E_{1/2}\,(\text{RScu}) - E^{1/2}\,(\text{CuI}) = 0.059 \log K_{\text{RSCu}} + 0.059 \log \frac{k_{\text{Cu(I)}}}{k_{\text{RSCu}}} - 0.059\,(p\text{H} - pK_{\text{RSH}}) - 0.059 \log C_{\text{RSH}} - 0.059 \log K_{\text{Cu(NH}_3)_2{}^+} \quad (9)$$

The ratio $k_{\text{Cu(I)}}/k_{\text{RSCu}}$ is calculated from the ratio of the diffusion currents of cuprous ammino ion and cuprous cysteinate at the same concentration and in the same medium. The average value of the dissociation constant K_{RSCu} is found to be 6.5×10^{-20}. The free energy of formation of the cuprous cysteinate, $\Delta F = RT \ln K$, is -26.2 kcal.

It is seen from experiments 2, 6 and 4, 5 in Table II that the half wave potential of cuprous cysteinate in the presence of sufficient excess of cysteine is independent of the RSCu concentration as required by equation (6a) which does not involve the concentration of the cuprous cysteinate. This result, combined with the slope of 0.059 for the line log $i/(i_d - i)$ *versus* E substantiate the conclusion that the polarographic reduction of RSCu is reversible in the presence of a large excess of cysteinate.

The reversibility of the RSCu wave in the presence of a large excess of cysteine was further tested qualitatively by allowing the electrode reaction ($\text{RSCu} + e + \text{Hg} \rightleftarrows \text{Cu(Hg)} + \text{RS}^-$) to proceed in the reverse direction by using dropping copper amalgam instead of mercury as electrode material.

A solution which was 8.8×10^{-2} M in RSH, 1.8×10^{-3} M in RSCu, 0.9 M in both ammonia and ammonium chloride, 0.02 M in sodium sulfite and 0.005% in gelatin was electrolyzed at a dropping amalgam electrode. The amalgam was 0.005% in copper (approximately 0.01 M). It is seen from Fig. 5A that the RSCu waves resemble a typical composite wave[11]); Curve B in Fig. 5 gives the current–voltage curve observed in the medium in the absence of copper and cysteine. The apparent diffusion current of the anodic wave is much smaller than corresponds to the true diffusion current of cysteine. The diffusion current in 10^{-3} M RSH solution is only 0.13 μa. with either 0.005 or 0.0017% copper amalgam, whereas the true diffusion current observed with pure mercury is 3.1 μa.

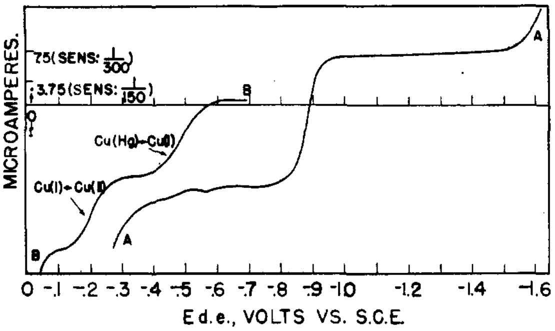

Fig. 5.—Polarograms with the dropping copper amalgam electrode (0.005% copper amalgam, h = 80 cm.): (A) electrolyte: 1.8×10^{-3} M RSCu, 8.8×10^{-2} M RSH, 0.9 M NH_3, 0.9 M NH_4Cl, 0.02 M Na_2SO_3, 0.005% gelatin, sens. 1/150. (B) electrolyte: M NH_3, M NH_4Cl, 0.005% gelatin, sens. 1/300.

Acknowledgment.—This investigation was supported by a research grant from the National Cancer Institute, United States Public Health Service.

MINNEAPOLIS, MINN. RECEIVED SEPTEMBER 23, 1950

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