#### Discussion

Although the authors believe that the proposed theory is a general one capable of explaining the cause of minima in the diffusion plateaus of polarographic waves of highly oxidizing compounds, there are certain other "minima" that do not fall into this category and consequently must have some other mechanism.

A case very similar to the present one, although not involving oxidation of mercury, was reported by Laitinen and Ziegler.<sup>30</sup> The polarographic reduction of tungsten (VI) in the presence of perchloric acid produces a very large catalytic current at potentials corresponding to the reduction of W(VI) to W(V). The catalytic current is due to the perchloric acid which oxidizes W(V) back to the hexavalent state which again is electroreduced to W(V) and so forth. At somewhat more negative potential the current falls off producing a "minimum." Here the electroreduction of W(V) to W(III) starts

(30) H. A. Laitinen and W. A. Ziegler, THIS JOURNAL, 75, 3045 (1953).

to interfere with the chemical oxidation of the pentavalent form and the catalytic cycle drops off. The principle is thus identical with the one presented in this paper.

Other cases of polarographic minima do not necessarily fall into a similar category. For example, a minimum in the reduction of hexamminecobalt-(III) ion has been attributed to the formation of a film of cobalt(II) hydroxide.<sup>31</sup> The examples given by Pecsok and Juvet,<sup>11</sup> Kolthoff and Lingane,<sup>3</sup> Konopik and Berger,<sup>10</sup> Filipovic, *et al.*,<sup>12</sup> and Parry and Yakubik<sup>13</sup> merit further investigation.

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(31) H. A. Laitinen, A. J. Frank and P. Kivalo, *ibid.*, **75**, 2865 (1953).

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

#### The Anodic Waves of Cysteine at the Convection and Dropping Mercury Electrodes

#### By I. M. Kolthoff, Walter Stricks and Nobuyuki Tanaka<sup>1</sup>

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Current-voltage curves of cysteine at the convection mercury electrode (C.M.E.) were studied over the pH range between 1 and 13 at cysteine concentrations from  $10^{-4}$  to  $10^{-3}$  M and compared with polarograms observed at the dropping mercury electrode (D.M.E.). Polarograms run from negative to positive potentials at the C.M.E. in cysteine solutions more acid and more alkaline than pH 9 exhibit a prewave which is attributed to the formation of a film of mercurous cysteinate on the electrode. The film breaks down at more positive potentials when the "normal" wave corresponding to the formation of mercurous cysteinate at the electrode appears. From the area of cathodic dissolution patterns in polarograms recorded from positive to negative potentials the thickness of the film is calculated to correspond to that of a monomolecular layer of mercurous cysteinate. The film formed at the D.M.E. in solutions of pH 5 and of cysteine concentrations larger than 0.00025 M was also found to be of monomolecular dimension. Film formation at both electrodes is eliminated at 80° and also at 25° in the presence of sufficient surface active disulfides like dithiodiglycolic acid.

Under proper conditions<sup>2</sup> cysteine gives an anodic wave at the dropping mercury electrode (D.-M.E.) which corresponds to a reversible reaction

$$RSH + Hg \longrightarrow HgRS + H^+ + e^- \qquad (1)$$

In solutions with pH between 2 and 9 a prewave is observed at cysteine concentrations greater than 0.00025 *M*, the false diffusion current being almost independent of the cysteine concentration. Grubner<sup>3</sup> reports normal waves without a prewave at a temperature of 90°. The false diffusion current at room temperature has been attributed<sup>2,3</sup> to the formation of a film of mercurous cysteinate (HgRS) on the surface of the drop which prevents further electrode reaction with mercury until the potential is made positive enough to cause a breakdown of the film.

In a recent communication<sup>4</sup> the convection mercury electrode(C.M.E.) was found well suited for a study of electro-active films on mercury surfaces.

In order to obtain more insight into the properties

and formation of the mercurous cysteinate film the anodic waves of cysteine at the convection mercury electrode were studied in detail. Current-voltage curves observed at the convection mercury electrode in cysteine solutions of pH lower than 9 and also in 0.1 M sodium hydroxide exhibit similar characteristics as those found at the dropping mercury electrode as indicator electrode. Current-voltage curves were recorded from positive to negative potentials and vice versa. The polarograms indicate that the resistance exhibited by the film is greater when the potential is varied from negative to positive values than vice versa.

From the area of the cathodic dissolution patterns observed in polarograms recorded from positive to negative potentials the thickness of the film was found to be independent of the cysteine concentration, length of time of anodic polarization and to have the dimensions of a monomolecular layer. A film of monomolecular thickness was also postulated to be formed at the dropping mercury electrode.

#### Experimental

The experimental setup of the convection electrode was the same as described previously.<sup>5</sup> The effective area of

(5) I. M. Kolthoff, J. Jordan and S. Prager, ibid., 76, 5221 (1954).

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 $<sup>(2)\,</sup>$  I. M. Kolthoff and C. Barnum, This Journal, **62**, 306 (1940).

<sup>(3)</sup> O. Grubner, Collection Czechoslov. Chem. Communs., 19, 444 (1954).

<sup>(4)</sup> I. M. Kolthoff and J. Jordan, THIS JOURNAL, 77, 3215 (1955).

# TABLE I APPARENT HALF-WAVE POTENTIALS, $E_{1/2}$ AND LIMITING CURRENTS i OF CYSTEINE AT C.M.E. AT 25° — Recorded from positive to negative potentials. — Recorded from negative to negative potentials. — Recorded from negative to positive potentials.

|               | Concn.              | $\xrightarrow{E_{1/2}}$ v. | $E_{1/2}^{a}$ v. |                         |       |               |       |
|---------------|---------------------|----------------------------|------------------|-------------------------|-------|---------------|-------|
| ٥H            | $RSH \times 10^4 M$ | vs.<br>S.C.E.              | vs.<br>SCE       |                         | -> b  | ×             | the h |
| 19.0          | 1.67                | -0.36                      | -0.20            | $r_{1} \mu a$           | 10.9  | $r_{i} \mu a$ | 90.9  |
| 10.0          | 1.07                | -0.50                      | -0.20            | 1.70                    | 10.4  | 3 4           | ک، 0ک |
| 13 0          | 9.80                | -0.42                      | -0.20            | at $\rightarrow 0.33$ v | 15.5  | noorly        |       |
| 2010          | 0100                | 011                        | 0.20             | 15.2                    | 101.0 | defined       |       |
| 9.2           | 5.0                 | -0.51                      | -0.51            | at -0.35 v.             | 16.0  | at $-0.35$ v. | 16.2  |
|               |                     |                            |                  | 8.0                     |       | 8.1           |       |
| 7.0           | 1.67                | -0.15                      | -0.11            | at -0.1 v.              | 16.2  | at $-0.05$ v. | 17.9  |
|               |                     |                            |                  | 2.7                     |       | 3.0           |       |
| 5.2           | 1.67                | -0.12                      | -0.03            | at $-0.02$ v.           | 20.4  | at 0.0 v.     | 18.6  |
|               |                     |                            |                  | 3.4                     |       | 3.1           |       |
| $5.2^\circ$   | 1.65                | -0.14                      | 0.04             | at $-0.03$ v.           | 19.4  | at 0.0 v.     | 19.4  |
|               |                     |                            |                  | 3.2                     |       | 3.2           |       |
| $5.0^d$       | 1.65                | -0.23                      |                  | at $-0.1$ v.            | 21.8  |               |       |
|               |                     |                            |                  | 3.6                     |       |               |       |
| 5.2           | 5.00                | -0.15                      | -0.04            | at $-0.02$ v.           | 20.8  | at 0.0 v.     | 18.0  |
|               |                     |                            |                  | 10.4                    |       | 9.0           |       |
| $5.2^{e}$     | 1.67                | -0.24                      | -0.24            | at $-0.05$ v.           | 68.0  | at $-0.05$ v. | 65.0  |
|               |                     |                            |                  | 11.3                    |       | 10.8          |       |
| $5.2^{\circ}$ | 9.70                | · • •                      | -0.24            |                         |       | 61.0          | 63.0  |
| 1.0           | 1.68                | -0.01                      | +0.14            | at +0.1 v.              | 16.7  | at $+0.15$ v. | 17.8  |
|               |                     |                            |                  | 2.8                     |       | 3.00          |       |
| 1.0           | 5.0                 | +0.015                     | f                | at $+0.1 v$ .           | 16.6  | f             |       |
|               |                     |                            |                  | 8.3                     |       |               |       |
| 1.0           | 9.8                 | -0.01                      | f                | at $+0.1$ v.            | 16.5  | f             |       |
|               |                     |                            |                  | 16.2                    |       |               |       |

<sup>a</sup> Reversible half-wave potentials at 25° at pH 9.2, 7, 5.2 and 1.0 are -0.52, -0.39, -0.28 and -0.036 volt vs. S.C.E., respectively. <sup>b</sup> i/c is current of  $10^{-3}$  M cysteine solution. <sup>c</sup> Acetate buffer was 0.2 M in total acetate. <sup>d</sup> In the presence of  $1.63 \times 10^{-4}$  M cystine. <sup>e</sup> Temperature of solution was 80°. <sup>f</sup> No convection current plateau was observed.

the indicator electrode was 0.02 sq. cm. and the sensitivity<sup>4</sup> 21.0  $\mu$ a. meq./l. for thallous ion in 0.1 *M* potassium chloride at a rate of stirring of 900 r.p.m. Current-voltage curves were recorded with a Leeds and Northrup Chemograph Type E. The rate of change of applied potential,  $\Delta v/\Delta t$ , was 3.33 mv./sec. in all experiments. The capacity of the electrolysis cell was 250 ml. and the volume of the solution was 150 ml. in experiments with the C.M.E. After each recording of a polarogram the cuvet of the cell was cleaned with hot concentrated nitric acid and refilled with freshly distilled mercury.

The characteristics of the dropping mercury electrode were m = 2.02 mg. sec.<sup>-1</sup>, t = 3.83 sec. at open circuit. Current-voltage curves at the dropping electrode were recorded with a Heyrovsky Type Sargent polarograph, Model XII ( $\Delta v/\Delta t = 2.85$  mv./sec.).

The potentials were measured against the saturated calomel electrode (S.C.E.). All experiments were carried out at  $25 \pm 0.1^{\circ}$  unless stated otherwise.

Cysteine (RSH) stock solutions were prepared by dissolving cysteine hydrochloride (Pfanstiehl) in air-free distilled water and kept in bottles closed with caps which were provided with self-sealing rubber gaskets. The solution was withdrawn by means of a syringe. Thioglycolic acid (TSH), dithiodiglycolic acid (TSST) and oxidized glutathione were prepared in the same way as described previously.<sup>7,8</sup>

Were prepared in the same way as described previously.<sup>7,8</sup> Borax (0.05 *M* borax, 0.1 *M* KCl,  $\rho$ H 9.2), phosphate (0.1 *M* total phosphate, 0.1 *M* KCl,  $\rho$ H 7), acetate (0.1 *M* total acetate,  $\rho$ H 5.2) buffers, 0.1 *M* perchloric acid and 0.1 *M* sodium hydroxide were used as supporting electrolytes.

#### Experiments with the Convection Mercury Electrode

Current–voltage curves were run of solutions of varying cysteine concentrations (1.7 to  $9.8 \times 10^{-4} M$ ) in a *p*H range

(6) I. M. Kolthoff and J. Jordan, THIS JOURNAL, 76, 3893 (1954).

(7) D. L. Leussing and I. M. Kolthoff, J. Electrochem. Soc., 100, 334 (1953).

(8) W. Stricks and I. M. Kolthoff, THIS JOURNAL, 74, 4646 (1952).

of 1 to 13; some illustrations are given in Fig. 1. Apparent half-wave potentials and limiting currents are listed in Table I. All polarograms were recorded from positive to negative potentials and *vice versa*.

In a borax solution of pH 9.2 the shape of the anodic convection wave was similar to that observed in the same medium at the D.M.E.<sup>2</sup> The wave had the characteristics of a reversible one electron wave with a half-wave potential of -0.51 volt as compared to -0.52 volt at the D.M.E. The half-wave potential of the convection wave was found to be the same no matter whether the polarogram was run from positive to negative potentials or *vice versa* and the limiting current at a given potential was proportional to the cysteine concentration.

In media of  $p{\rm H}$  less than 9 and in 0.1 M sodium hydroxide the shape of the c-v curves at the C.M.E. exhibited irregularities and was different when recorded from positive to negative or vice versa. For example a  $1.7 \times 10^{-4} M$  cysteine solution at pH 1 gave a wave of normal appearance when recorded from positive to negative potentials while the polarogram of the same solution, recorded in the opposite direction exhibited a small prewave followed by a very steep wave at more positive potentials (compare curves A and A<sub>1</sub> in Fig. 1). In curve A the convection current remained constant over a potential range of 0.1 volt, while in curve  $A_1$  it extended over a range of only 0.03 volt. The potential region in which the convection current was constant became shorter with increasing cysteine concentration, but the convection current remained proportional to the concentration. The half-wave potential of wave A is about 0.026 volt more positive than the thermodynamic value. On the other hand the agrees satisfactorily with the theoretical value of -0.04 volt) volt. The prewave extends up to a potential of about +0.12 volt when the true convection current can develop. (h) = (h) + (h) +came ill defined, immaterial whether run from positive to negative potentials or vice versa and the half-wave potentials were considerably more positive than those corresponding to



Fig. 1.—Current-voltage curves at C.M.E. of  $1.67 \times 10^{-4} M$  cysteine in: A, A<sub>1</sub>, 0.1 M HClO<sub>4</sub> (pH 1); B, B<sub>1</sub>, E (dissolution pattern) acetate buffer (0.2 M total acetate, pH 5.2); C, C<sub>1</sub>, phosphate buffer (0.1 M total phosphate, pH 7.0); D, D<sub>1</sub>, 0.05 M borax, 0.1 M KCl (pH 9.2). Curves A, B, C, D, E are recorded from positive to negative potentials. Curves  $A_1$ ,  $B_1$ ,  $C_1$ ,  $D_1$ , are recorded from negative to positive potentials.

reversible reaction 1. Thus at pH 5 and 7 the potentials on curves B and C of Fig. 1 are 0.164 and 0.240 volt more positive than the true values. Some of the curves, like B and  $B_1$  showed a second ill-defined anodic wave of approximately the same height as that of the main wave. Similar waves also have been observed in polarograms at the D.M.E. both in solutions of cysteine and of glutathione.8 The second wave might be attributed to further oxidation of mercurous cysteinate to a compound to the type /S\_

$$\mathbb{R}$$

The behavior of cysteine in strongly alkaline solutions  $(0.1 \ M \ \text{NaOH})$  at the C.M.E. was similar to that observed in the  $\rho$ H range less than 9. From the data in Table I it is seen that the half-wave potential at  $\rho$ H 13 is considerably more positive than at  $\rho$ H 9.2, the shift to the positive side being graves are been from reporting the polarized from the positive side being graves are been from the polarized from the positive side being graves are been from the polarized from the positive side being graves are been from the positive side been from the positive s being greater in polarograms run from negative to positive potentials than vice versa.

Polarograms of cysteine at the D.M.E. at the extreme ends of the pH scale had a normal appearance<sup>2</sup> and thus differed markedly from those observed at the C.M.E. in the same media.

All polarograms at the C.M.E. at pH other than 9, when recorded from positive to negative potentials exhibited a small characteristic cathodic dissolution pattern. One such pattern is illustrated at great galvanometer sensitivity (recorded with a Sargent Model XII polarograph) in Fig. IE. Since the rate of change of applied potential was constant in our experiments, the abscissas in Fig. 1 may represent potential as well as time. Therefore, the quantity of electricity corresponding to the cathodic dissolution area could be calculated readily. The area of the dissolution patterns was measured for solutions at pH 5.2 at various cysteine concentrations after prepolarization at -0.1 volt for various periods of time. The data in Table II show that the area of the dissolution pattern is unaffected by the cysteine concentration and by the length of time of electrolysis. At a temperature of 80° cysteine solutions of pH 5 gave

TABLE II QUANTITY OF ELECTRICITY CORRESPONDING TO AREA OF DISSOLUTION PATTERNS AT C.M.E. IN CYSTEINE SOLUTIONS AT bH 5.2 AFTER PREPOLARIZATION AT -0.1 Volt

| 11 p11 0.2 m115.   | ic i noi obmicibititon mi           | 0.1 1001                                |
|--|-------------------------------------|---|
| $\begin{array}{c} \text{Conen.} \\ \text{RSH} \times 10^4, \\ M \end{array}$ | Time of<br>prepolarization,<br>min. | Quantity of<br>electricity,<br>µcoulomb |
| 1.67   | 1                                   | 3.8                                     |
| 1.67   | 10                                  | 3.5                                     |
| 10.00  | 1                                   | 3.6                                     |
| 10.00  | 10                                  | 3.9                                     |
|  |                                     |   |
|  |                                     | 97                                      |

perfectly normal convection waves, no matter whether recorded from positive to negative potentials or vice versa. The temperature coefficient of the convection current was found to be about 4% per degree between 25 and 80°. The difference  $E_{3/4} - E_{1/4}$  at 80° for  $1.67 \times 10^{-4}$  and  $9.7 \times 10^{-4}$  M cysteine solutions was found to be 54 and 57 mv., respectively, after correction for the IR drop. These values compare favorably with the calculated value of 65.5 mv. for a reversible one electron transfer at 80°. No dissolution patterns were observed at 80°. The remarkable change in the characteristics of the convection wave at higher temperature is analogous to that reported for the diffusion controlled cysteine wave at the D.M.E. in an acetate buffer.3

The prewave and the small cathodic dissolution pattern are attributed to a film of mercury cysteinate. It appeared of interest to study the effect of capillary active substances on the film formation in cysteine solutions of pH 5. Among the substances studied cystine (RSSR) and dithiodiglycolic acid (TSST) were found to be effective in preventing film formation and to give rise to cysteine waves with a normal appearance and the expected half wave potentials (see Table I for an example of a cysteine-cystine mixture)

Not included in Table I are mixtures of cysteine and

TSST which gave normal cysteine waves with a well-defined convection current plateau. As an example, the anodic wave of a  $2 \times 10^{-4}$  M cysteine solution (pH 5),  $10^{-4}$  M in TSST was found to have a half-wave potential of -0.21volt and a limiting current of  $3.34 \,\mu a$ . The difference  $E_{3/4} - E_{1/4}$  for this wave was 0.070 volt which compares favorably with the theoretical value of 0.059 volt at 25°. No dissolution patterns were found in the presence of RSSR or TSST.

Considering that thioglycolic acid (TSH) in contrast to cysteine gives normal c-v curves throughout the entire pH range at the D.M.E.,' it was of interest to study the behavior of this acid at the convection mercury electrode. A polarogram of a solution of  $4 \times 10^{-4}$  M TSH in 0.2 M total acetate (pH 5.0), recorded from positive to negative potentials, consisted of a single wave with a half-wave potential (-0.25 volt) which was practically equal to the thermodynamic value. No dissolution pattern was observed. When recorded from negative to positive the wave, although of similar appearance, was shifted by about 0.05 volt to more positive potentials. The i/c value for both waves, measured at +0.13 volt, was found to be only 16.5.

Cystine itself, at  $\rho$ H 5 gives a drawn-out cathodic convection wave starting at about -0.9 volt and attaining a plateau at -1.4 volt. The cathodic convection current was found to be  $6.90 \ \mu a$ . for a  $1.63 \times 10^{-4} M$  cystine solution ( $1.65 \times 10^{-4} M$  in cysteine) corresponding to an i/c value of  $21.05 \ \mu a$ ./meq./l., which compares favorably with the value of 21.8 for the anodic current of cysteine in the presence of cystine (see Table I). Dithiodiglycolic acid did not give a reduction wave at the C.M.E.

#### Experiments with the Dropping Mercury Electrode

For a better comparison of the abnormalities of cysteine waves at the convection- and dropping electrodes, a few polarograms of cysteine at the D.M.E. are illustrated in Fig. 2. Curves A, B and C all clearly show the prewave. Curve A (pH 3) exhibits a peak which has the appearance of a polarographic maximum. Similar breaks in the curves are observed at pH 5 and 7 (see I in Fig. 2), but also a second break (indicated by II in Fig. 2) occurs. The relatively poorly defined current at this second break corresponds to the true diffusion current. From the average value of  $i_d/c$  in a concentration range between  $10^{-2}$  and  $10^{-4}$  M, a value of the diffusion coefficient of cysteine at pH 5 and 7 was calculated to be  $8.2 \times 10^{-6}$  cm.<sup>2</sup> sec.<sup>-1</sup> as compared to 7.0  $\times 10^{-6}$  in 0.1 M perchloric acid.<sup>2</sup>



Fig. 2.—Current-voltage curves at the D.M.E. of 9.17  $\times$  10<sup>-4</sup> M cysteine at: A,  $p\rm{H}$  2.83; B,  $p\rm{H}$  4.95; C,  $p\rm{H}$  6.95.

Since RSSR and TSST had been found able to eliminate the film formation at the C.M.E., the effect of these disulfides and also of oxidized glutathione was studied on *c*-*v* curves of cysteine at the D.M.E. Experiments with cystine in the *p*H range from 7 to 3 were limited to concentrations not higher than  $10^{-3}$  M RSSR which is approximately the solubility of cystine in this *p*H range. At *p*H 7 and 5 cystine had no effect on the height and appearance of the anodic cysteine wave in the solutions of  $10^{-4}$  to  $10^{-3}$  M cysteine. At pH 3 the sharp maximum of the cysteine wave became rounded and its height was found to attain the value of the true diffusion current in a  $5 \times 10^{-4} M$  cysteine solution in the presence of  $5 \times 10^{-4}$  to  $10^{-3} M$  cystine. Considering the TSST and oxidized glutathione are freely coluble at AU 2 to 7 these substances provide solutions.

Considering the TSST and oxidized glutathione are freely soluble at  $\rho$ H 3 to 7 these substances were conveniently used for studies of the effect of disulfides on the cysteine wave. The change in the appearance of waves upon the addition of increasing amounts of TSST to a cysteine solution of  $\rho$ H 5 is illustrated in Fig. 3. The current at break I increased markedly (compare curves A and B in Fig. 3), and the break disappeared when the concentration of TSST is  $5 \times 10^{-3} M$  (curve C). With further addition of TSST (see curve D, Fig. 3) the true diffusion current of cysteine was measured within a potential region of  $\pm 0.1$  to  $\pm 0.1$  volt in a concentration range of  $10^{-4}$  to  $10^{-3} M$  cysteine and the half-wave potentials of the waves were independent of the concentration ( $\pm 0.272$  volt) and equal to the thermodynamic value. Curve D has the characteristics of a wave corresponding to a one-electron reversible reaction. Oxidized glutathione had qualitatively the same effect on the RSH-wave as TSST but larger concentrations of the peptide were required in order to obtain a normal cysteine wave. For example, a  $4 \times 10^{-4} M$  RSH solution ( $\rho$ H 5)  $8 \times 10^{-3} M$  in TSST gave a fully developed wave with an  $i_d/c$  value of 3.4, while in the presence of the same concentration of the peptide the anodic cysteine wave was still poorly defined and gave an apparent  $i_d/c$  value of 2.9 as measured at a potential of -0.1 volt.



Fig. 3.—Current-voltage curves at the D.M.E. of 9.5  $\times$  10<sup>-4</sup> *M* cysteine at *p*H 4.9 (0.1 *M* total acetate) in the presence of dithiodiglycolic acid at concentrations of: A, zero; B, 10<sup>-3</sup> *M*; C, 4.8  $\times$  10<sup>-3</sup> *M*; D, 9.2  $\times$  10<sup>-3</sup> *M*.

Experiments with cysteine solutions at  $\rho H$  5 in the presence of  $10^{-3}$  M thioglycolic acid (TSH) revealed that the polarographic behavior of cysteine is entirely changed in the presence of TSH, the anodic wave being well-defined and its height proportional to the cysteine concentration in the range between  $2 \times 10^{-4}$  to  $1.6 \times 10^{-3}$  M ( $i_{\rm d}/c = 3.4$ ). The effect of thioglycolic acid is analogous to that of reduced glutathione reported previously<sup>9</sup> for mixtures with cysteine.

#### Discussion

A comparison of Figs. 1 and 2 reveals that prewaves of cysteine are observed both at the C.M.E. and D.M.E. in a certain pH region. The cathodic dissolution pattern observed at the C.M.E. (see curve E, Fig. 1) strongly suggests that the prewave must be due to a film of mercury cysteinate. This film prevents further electrode reaction according to

(9) W. Stricks and I. M. Kolthoff, Anal. Chem., 25, 1050 (1953).

equation 1. From the fact that the main wave yielding the normal limiting current appears at both types of electrode at considerably more positive potentials than the prewave, it is evident that the film must break down at these more positive potentials. It is of interest to note (Fig. 2) that this breakdown potential is more positive at  $\rho H$  5 than at pH 7, the reversible electrode reaction also occurring at more negative potentials with increasing pH. The breakdown potential, especially at pH less than 5, is more positive when the polarogram is run from negative to positive potentials than in the opposite direction indicating that the structure of the film is affected by conditions of its formation.<sup>4</sup> Cysteine and its mercury compounds are dipolar and oriented at the electrode. The adherence of the film to the mercury surface therefore may depend on the charge of the electrode and on the pH of the solution. Film formation is decreased by increasing temperature. No film formation and waves corresponding to a reversible one electron reaction are observed at 80° both at C.M.E. and the D.M.E. RSSR and TSST when present at high enough concentrations appear to be stronger adsorbed on mercury than HgRS and eliminate the prewave at room temperature. Apparently the adsorbed layer of disulfide is permeable to cysteine.

The thickness of the film of mercurous cysteinate at the C.M.E. can be found from the dissolution pattern. The number of HgRS molecules reduced (see equation 1) from right to left can be calculated from the quantity of electricity corresponding to the area of the dissolution patterns (Table II). From the known area of the electrode surface this number was calculated to be  $11.6 \times 10^{14}$  molecules HgRS per cm.<sup>2</sup> of electrode surface and the area occupied by one HgRS molecule is therefore 8.6 Å.<sup>2</sup>. From the density of mercury, the effective area of the electrode and the assumption of cubic structure of mercury the number of mercury atoms on the surface of the electrode at 25° is calculated to be also  $11.7 \times 10^{14}$  atoms per cm.<sup>2</sup>, *i.e.*, one atom occupies 8.5 Å.<sup>2</sup>. These results clearly indicate that the film at the C.M.E. is composed of a monomolecular layer of HgRS.

Attributing the prewave observed at the D.M.E. also to an adsorbed film of HgRS, its thickness can be calculated from the Brdicka<sup>10</sup> relation

 $i = nFZ\Delta q$ 

where *i* is the current determined by the amount of adsorbed substance, *F* the faraday, *Z* the maximum number of adsorbed moles per cm.<sup>2</sup> and  $\Delta q$  the average increase of the electrode surface per second. Expressing the characteristics of the dropping electrode *m* in g. per sec. and *t* in sec.,  $\Delta q$  is given by

$$\Delta q = 0.85m^{2}/3t^{-1}/3 \text{ cm}.^{2}/\text{sec}.$$

Taking for *i* the value of the false diffusion current  $(0.62 \ \mu a. at -0.2 \text{ volt}$ , see Fig. 2B) measured in a solution of a cysteine concentration greater than  $2.5 \times 10^{-4} M$  at pH 5.0 the value for Z was calculated to be  $7.4 \times 10^{-10}$  mole per cm.<sup>2</sup> or  $4.4 \times 10^{14}$  adsorbed molecules per cm.<sup>2</sup>, a value which is of the same order of magnitude as that (11  $\times 10^{14}$ ) calculated for the film at the C.M.E. The agreement between these figures is rather striking, considering that the hydrodynamic conditions around the mercury are so different with both types of electrodes. It is reasonable, then, to conclude that the film at both electrodes is composed of a monomolecular layer of mercurous cysteinate which becomes the seat of a high electrical resistance.

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(10) R. Brdicka, Z. Elektrochem., 48, 278 (1942).

MINNEAPOLIS, MINNESOTA

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

## Polarographic Reduction of Cystine and of Dithiodiglycolic Acid in the Presence of Iron

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In alkaline medium (pH 9.2) iron has a great effect on the polarographic reduction of cystine. In the presence of sufficient iron only one wave of cystine is observed at fairly positive potentials which yields the normal diffusion current while in the absence of iron a relatively small prewave followed by a drawn out reduction wave is found. The potentials of the iron-catalyzed wave correspond to a reversible one-electron reaction, although the over-all reduction involves two electrons. The catalysis is explained by the reaction: RSSR + Fe(II)  $\rightarrow$  Fe(III) + RS<sup>+</sup> + RS<sup>-</sup> which is rapid and is followed by polarographic reduction of RS<sup>\*</sup> and Fe(III), of which the iron reduction is potential determining. An alternate mechanism accounts equally for the experimental facts. The iron catalysis is eliminated by Versene and much pyrophosphate, but tartrate has no effect. The oxidation potential measured at the dropping mercury electrode of the cystine-cysteine system in the presence of iron does no longer correspond to the reaction RSSR + 2H<sup>+</sup> + 2e<sup>-</sup>  $\rightleftharpoons$  2RSH.

In studies of iron-cysteine-cystine systems<sup>2</sup> it was observed that iron increases the polarographic pre-

(2) N. Tanaka, I. M. Kolthoff and W. Stricks, THIS JOURNAL, 77, 1980, 1996 (1955).

wave of cystine. This effect has been studied in greater detail and the results are reported in the present paper. The first (prewave) of the double wave of cystine at the dropping mercury electrode in a pH range of 3 to 9 was found to be kinetic in

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