teine, glutathione and thioglycolic acid are practically the same.

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

The Polarographic Prewaves of Cystine (RSSR) and Dithiodiglycolic Acid (TSST) and the Oxidation Potentials of the Systems RSSR–RSH and TSST–TSH

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The two-step reduction wave of cystine has been reinterpreted. Evidence is given that the prewave is determined by the over-all reversible reaction $RSSR + 2e^- + 2H^+ \rightleftarrows 2RSH$ where RSSR and RSH are cystine and cysteine. The prewave is found to be kinetic in nature. The electrode reaction is catalyzed by mercury which reacts with cystine to form mercury cysteinate, the latter being reduced rapidly to mercury and cysteine. The potential of the cystine–cysteine system at the dropping mercury electrode was found to correspond to the true oxidation potential. The same was found true for the thioglycolic–dithiodiglycolic acid couple but not for the glutathione system.

No satisfactory explanation has been offered for the two-step waves observed with cystine at the dropping mercury electrode in the *p*H range between 3 and 9.2.[2] The first and steeper part of the double wave, called the "prewave," has been attributed[2] to a mixed-potential effect. In the present paper it is shown that this interpretation is inadequate. Conclusive evidence is presented that the potential of the cystine–cysteine system measured at the dropping electrode after correction for the residual current corresponds to that of the couple

$$RSSR + 2H^+ + 2e^- \rightleftarrows 2RSH \qquad (1)$$

in which RSSR denotes cystine and RSH cysteine. At a platinum electrode this reaction is highly irreversible because of the slow rate of electroreduction of cystine. This reaction is catalyzed at the mercury surface which reacts rapidly with cystine according to

$$RSSR + Hg \rightleftarrows Hg(RS)_2 \qquad (2)$$

This reaction may occur in steps, *e.g.*, as

$$RSSR + Hg \rightleftarrows HgRS + RS\cdot \longrightarrow Hg(RS)_2 \qquad (2a)$$

but the experimental results do not allow further insight into the mechanism of this reaction. The mercuric cysteinate formed is rapidly reduced at the electrode

$$Hg(RS)_2 + 2e + 2H^+ \rightleftarrows Hg + 2RSH \qquad (3)$$

Thus equation 1 represents the over-all reaction which is the sum of equations 2 and 3. According to this interpretation the prewave of cystine should be kinetic in nature and the analysis of the wave should correspond to that of the reversible reaction (1). The effect of various factors upon the height of the prewave and the characteristics of the entire cystine wave are described in the experimental part.

The conclusion is arrived at that the potential of the cystine–cysteine system measured at the dropping mercury electrode corresponds to the oxidation potential of the system. In this connection the potentials of the thioglycolic–dithiodiglycolic acid and reduced and oxidized glutathione systems are briefly discussed.

Materials.—Thioglycolic acid and dithiodiglycolic acid were prepared and purified by methods described previously.[3] All other chemicals were C.P. reagent grade products.

Experimental

Current-voltage curves were measured with the manual apparatus and circuit described by Lingane and Kolthoff[4] and automatically with a Heyrovsky type self-recording Sargent polarograph, Model XII. All potentials were measured against the saturated calomel electrode (S.C.E.). Oxygen was removed from the solution in the cell with 99.99% pure Linde nitrogen. The characteristics of the capillary were $m = 2.53$ mg. sec.$^{-1}$, $t = 3.51$ sec. (open circuit) at a height of the mercury column of 60 cm. The *p*H was measured with a Beckman Model H-2 *p*H meter.

Results

Characteristics of the Cystine Wave. Effect of *p*H.

—The effect of *p*H on the current–voltage curves of 5×10^{-4} *M* cystine solutions is illustrated in Fig. 1. From the curves which were taken between *p*H 3 and 9 it is seen that the potentials at which the waves are shifted to more negative values with increasing *p*H, the shift for the prewave being approximately 60 millivolt per *p*H unit. The height of the prewave is little affected by *p*H and attains a maximum value at *p*H 8 while the height of the total wave (diffusion current) is constant over the *p*H range investigated.

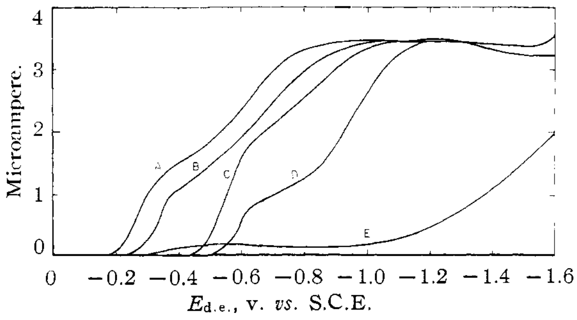

Fig. 1.—Current–voltage curves of 5×10^{-4} *M* RSSR at *p*H (A) 3.3, (B) 4.7, (C) 8.0, (D) 9.2 and of (E) 5×10^{-4} *M* TSST at *p*H 5.0.

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Effect of Cystine Concentration.—Polarograms were run over a concentration range from 5×10^{-5} to 8×10^{-3} *M* in buffered solutions at *p*H 9.2; the results are listed in Table I. The plateau of the prewave becomes ill defined at large cystine concentrations (larger than 3×10^{-3} *M* RSSR).

Table I

Prewave (i_p) and Total Wave (i_d) of Cystine at Various Cystine Concentrations at *p*H 9.2; *h* = 58 cm.

Buffer	Concn. cystine, *M* × 10³	i_p (μa.) at −0.7 v.	i_p/C	i_d at −1.3 v.	i_d/C
0.05 *M* borax–0.1 *M* KCl	0.50	1.01	2.03	3.21	6.42
.05 *M* borax– .1 *M* KCl	.167	0.361	2.16	1.10	6.59
.05 *M* borax– .1 *M* KCl	.067	0.16	2.40	0.448	6.69
.1 *M* NH_3–0.1 *M* NH_4Cl	8.7	(8.8)[a]	(1.01)	57.1[b]	6.60
.1 *M* NH_3– .1 *M* NH_4Cl	5.00	6.80[a]	1.36	33.65	6.73
.1 *M* NH_3– .1 *M* NH_4Cl	2.50	3.60	1.40	16.88	6.75
.1 *M* NH_3– .1 *M* NH_4Cl	0.91	1.26	1.40	6.18	6.80
.1 *M* NH_3– .1 *M* NH_4Cl	.48	0.74	1.50	3.29	6.85
.1 *M* NH_3– .1 *M* NH_4Cl	.246	.37	1.50	1.70_5	6.9
.1 *M* NH_3– .1 *M* NH_4Cl	.10	.15	1.50	0.69_6	7.0
.1 *M* NH_3– .1 *M* NH_4Cl	.050	.074	1.50	0.35_4	7.0

[a] Prewave poorly defined. [b] Main wave is preceded by a maximum.

Considering that the prewave quite generally is not sharply defined, it may be concluded from the results in Table I that the height of the prewave as well as the diffusion current are proportional to the cystine concentration at a given *p*H. However, the height of the prewave appears to be markedly affected by the composition of the buffer; in an ammonia buffer it is some 25% smaller than in a borax buffer of the same *p*H.

Effect of Temperature.—Polarograms obtained with a 5×10^{-4} *M* cystine solution in a borax buffer (*p*H 9.2) at 25, 35 and 45° gave temperature coefficients of 1 and 1.6% per degree for the heights of the prewave and total wave, respectively.

Effect of Height of Mercury Column.—The change in height of the prewave in a 5×10^{-4} *M* RSSR solution (0.05 *M* borax, 0.1 *M* KCl) with height *h* of the mercury column is listed in Table II. As expected the diffusion current is found to be inversely proportional to the square root of the height of the mercury while the prewave increases only 10% when *h* is increased 2.5 times. Experiments carried out in 5×10^{-3} *M* and 5×10^{-5} *M* cystine solutions in an ammonia buffer of *p*H 9.2 yielded results similar to those in Table II.

Table II

Cystine Waves of 5×10^{-4} *M* Cystine in 0.05 *M* Borax, 0.1 *M* KCl (*p*H 9.2) at Various Heights *h* of Mercury Column

	Prewave (at −0.7 v.)		Total wave (at −1.5 v.)	
h, cm.[a]	*i*, μa.	$1/\sqrt{h}$	*i*, μa.	$i/\sqrt{h}$
28	0.91	0.162	2.29	0.43
38	0.96	.156	2.67	.43
48	1.00	.144	2.90	.42
58	1.01	.133	3.21	.42
68	1.02	.126	3.62	.44

[a] *h* is corrected for back pressure.

Effect of Cysteine.—Capillary active substances like gelatin or thymol give rise to a marked reduction[2] in the height of the prewave but have no effect on the potential at which this wave occurs. Cysteine, which is also capillary active, was found to have no effect on the height of the prewave but to shift it to more negative potentials. The main wave of cystine was hardly affected by the presence of cysteine. Values of the oxidation potential of the system cystine–cysteine and also of TSST–TSH as measured at the dropping electrode are given in Table III.

Table III

Oxidation Potentials E_0 of the Cystine–Cysteine (RSSR–RSH) and of the Thioglycolic Acid Systems (TSST–TSH) Calculated from Zero-Potentials E_z at the Dropping Electrode

Buffer	*p*H	Concn. RSSR, *M* × 10³	Concn. RSH, *M* × 10³	E_z (RSSR), v. *vs* S.C.E.	E_0 (RSSR), v. *vs.* N.H.E.
Acetate[a]	4.98	0.50	1.0	−0.380	+0.076
Acetate	4.98	.50	5.0	− .420	+ .077
Acetate	4.88	.52	10.0	− .433	+ .076
Acetate	4.98	.80	1.0	− .375	+ .075
Acetate	4.96	.81	5.0	− .415	+ .075
Acetate	4.85	.82	10.0	− .426	+ .075
Borax[b]	9.2	.50	1.02	− .620	+ .067
Borax	9.2	.50	5.0	− .658	+ .070
				Av.	+0.074
		Concn. TSST, *M* × 10³	Concn. TSH, *M* × 10³	E_z (TSST)	E_0 (TSST)
Acetate[a]	4.82	5.00	4.93	−0.382	+0.075
Acetate	4.85	5.00	1.97	− .359	+ .077
Acetate	4.88	2.50	4.93	− .393	+ .078
Borax[b]	9.00	5.00	1.97	− .618	+ .063
				Av.	+0.073

[a] 0.06 *M* CH_3COOH, 0.14 *M* CH_3COONa. [b] 0.05 *M* borax, 0.1 *M* KCl.

Some Characteristics of the Wave of Dithiodiglycolic Acid.—Current–voltage curves of dithiodiglycolic acid have been described previously.[3] The polarogram of a 5×10^{-4} *M* TSST solution at *p*H 5 is shown in Fig. 1E. It is seen that the prewave is much smaller than that of cystine under the same conditions and that the main wave is more drawn out than that of cystine. The height of the prewave was found to be markedly affected by the *p*H. Thus the prewave of 10^{-3} *M* TSST in an acetate buffer (*p*H 5) was 0.50 μa. and in a borax buffer (*p*H 9.2) 0.14 μa. The height of the prewave was practically proportional to the concentration of TSST. When the height of the mercury column was increased 2.5 times the height of the prewave of 5×10^{-3} *M* TSST in acetate *p*H 5 increased by about 25%. The temperature coefficient between 25 and 5° was found to be about 2% per degree. Gelatin at a concentration of 0.0025% in 5×10^{-3} *M* TSST (acetate *p*H 5) was found to reduce the height of the prewave by about 7%. It appears that in many respects the characteristics of the prewave of TSST are similar to those of RSSR. This was also found to be true for mixtures of TSST and TSH.

Discussion

The Cystine Wave.—Although both the prewave and the main wave of cystine correspond to a reduction of cystine to cysteine the mechanisms of both electroreductions are different for the two waves. Most of the experimental results indicate that the prewave of cystine cannot be accounted for quantitatively by a mixed potential effect.[2] Neither is there any indication (see effect of *p*H) of the existence of two forms of cystine, an "active" and a "passive" form as suggested by Kaeppel.[5] The facts that the prewave is proportional to the cystine concentration and almost independent of the height of the mercury reveal that the wave is kinetic in nature, the over-all reaction being given by equation 1.

If reaction (1) were reversible at the dropping electrode, the potential at any point of the reduction wave at 25° would be expressed by

$$E = E_0 + 0.059 \log [\mathrm{H}^+] + 0.0295 \log \frac{i_d - i}{i^2} + 0.0295 \log \frac{f_{\mathrm{RSH}}{}^2}{f_{\mathrm{RSSR}}} \quad (4)$$

where E_0 is the standard potential of reaction (1) and f_{RSH} and f_{RSSR} are quantities which, according to the Ilkovic equation, are determined by the characteristics of the capillary and by the diffusion coefficients of cysteine and cystine, respectively. An example of a plot of log $(i_d - i)/i^2$ *vs.* $E_{\mathrm{d.e.}}$ for the prewave of 5×10^{-4} *M* RSSR in an acetate buffer of *p*H 4.7 is illustrated in Fig. 2. The plot is a straight line with a slope of 0.041 as compared to the theoretical value of 0.0295.

Comparing at different concentrations of cystine the potential E_α at a current $i = \alpha i_d$ where $\alpha < i_p/i_d$ (i_p height of prewave), we find from equation 4

$$E_\alpha = E_0 + 0.059 \log [\mathrm{H}^+] + 0.0295 \log \frac{1}{i_d} + 0.0295 \log \frac{1 - \alpha}{\alpha^2} + 0.0295 \log \frac{f_{\mathrm{RSH}}{}^2}{f_{\mathrm{RSSR}}} \quad (5)$$

A plot of log $1/i_d$ *vs.* E_α for varying concentrations of cystine for $\alpha = 0.05$ (see Fig. 2B) yielded a straight line with a slope of 0.036 in satisfactory agreement with the value 0.0295 required by equation 5. The same result was obtained from a plot for $\alpha = 0.1$.

From the known characteristics of the capillary and the diffusion coefficients of cystine ($D = 5.3 \times 10^{-6}$ cm.^{2} sec.$^{-1}$)[2] and of cystine ($D = 7.0 \times 10^{-6}$ cm.^{2} sec.$^{-1}$)[6] equation 5 allows the calculation of the standard oxidation potential E_0 of the cystine–cysteine system. The average value of E_0 for $\alpha = 0.1$ and 0.05 in the *p*H range between 3.3 and 6.8 and at RSSR concentrations of 5×10^{-4} *M* and 1.67×10^{-4} *M* was found to be +0.076 v. *vs.* N.H.E. The systems used were poorly poised with regard to cysteine. For this reason E_0 was also calculated from the potential E_z at zero current (after correction for the residual current) of mixtures of cystine and cysteine in buffered media, taking into account the known intrinsic dissociation constants of cystine and cysteine[7]

$$E_z = E_0 + 0.059 \log [\mathrm{H}^+] + 0.0295 \log \frac{{}^{\pm}\mathrm{RSSR}^{\pm}}{\mathrm{RSH}^{\pm 2}} \quad (6)$$

Experimental data and results are given in Table III. The average value of E_0 (+0.074 v.) is in excellent agreement with that obtained at a pretreated macro mercury electrode (+0.07 v.)[8] and that calculated from the equilibria in the iron–cysteine–cystine system (0.08 v.).[9] Potentials of the cystine–cysteine system measured at the dropping mercury electrode can therefore be considered to correspond to the thermodynamically reversible oxidation potentials of this system. In the above conclusive evidence has been given that the relation between current and potential of the prewave is determined by the over-all reversible equilibrium in equation 1, even though the drawn-out main wave of cystine corresponds to a typically irreversible reaction.

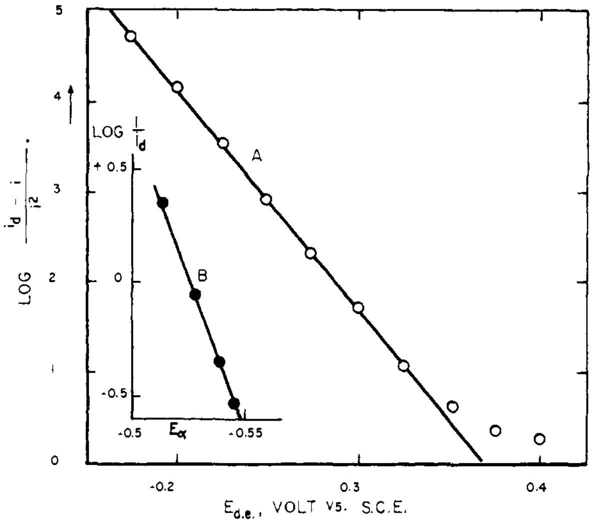

Fig. 2.—(A), plot of log $(i_d - i)/i^2$ *vs.* E of 5×10^{-4} *M* RSSR at *p*H 4.7 (acetate); (B), plot of log i/i_d *vs.* $E\alpha$ of RSSR (6.7×10^{-5} to 5.0×10^{-4} *M*) at *p*H 9.2 (borax) for $\alpha = 0.05$.

The reversible and kinetic nature of the prewave is accounted for by a "catalytic" effect of mercury which reacts rapidly with cystine according to equation 2. Actually it has been shown[10] that cystine in contact with mercury reacts with the formation of mercuric cysteinate. Adsorption of the reaction product may account for the slow establishment of equilibrium under Barnum's experimental conditions. At the dropping electrode the mercuric cysteinate formed is reduced instantaneously and the reaction can proceed rapidly. Assuming that the reaction is reversible, the equilibrium constant $K = [\mathrm{Hg(RS)_2}]/[\mathrm{RSSR}]$ of reaction (2) can be calculated from the known standard potential of the RSSR–RSH system ($E_{0\mathrm{RSSR}} = 0.08$ v. *vs.* N.H.E.) and from the standard potential $E_{0\mathrm{Hg(RS)_2}}$ of reaction (3). The potential of reaction (3) can be expressed by

$$E = E_{0\mathrm{Hg(RS)_2}} + \frac{RT}{F} \ln [\mathrm{H}^+] + \frac{RT}{2F} \ln \frac{\mathrm{RSSR}}{\mathrm{RSH}^2} + \frac{RT}{2F} \ln K \quad (7)$$

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while $E_{0\mathrm{RSSR}}$ can be expressed by the equation

$$E_{0\mathrm{RSSR}} = E_{0\mathrm{Hg(RS)_2}} + \frac{RT}{2F}\ln K \qquad (8)$$

From the known dissociation constants of mercury cysteinate[11] $E_{0\mathrm{Hg(RS)_2}}$ is calculated to be +0.175 v. *vs.* N.H.E. from which value the constant K is found to be 6.0×10^{-4}. This means that in a 5×10^{-4} M RSSR solution the concentration of $\mathrm{Hg(RS)_2}$ at the surface of the electrode would be of the order of 3×10^{-7} M.

The rate of formation of $\mathrm{Hg(RS)_2}$ at the electrode is given by

$$\frac{\mathrm{d}[\mathrm{Hg(RS)_2}]}{\mathrm{d}t} = k_1[\mathrm{RSSR}]^0 - k_2[\mathrm{Hg(RS)_2}]^0 \qquad (9)$$

where k_1 and k_2, the rate constants for the forward and backward reactions of equation 2, are given by $K = k_1/k_2$. The second term of the right side of equation 9 is negligible as compared to the first term, and the height of the prewave should be determined by the rate of the forward reaction. Thus in extreme cases when the rate of this reaction is very large only one steep wave and no prewave should be observed while if the rate is small a very small prewave and a drawn-out main wave is to be expected. Actually the height of the prewave was found almost independent of *p*H in the *p*H range 3 to 9. However, at *p*H of 1 or less cystine yields one steep wave[2] indicating a much larger rate of reaction (2) than at higher *p*H, whereas at *p*H 13 the prewave is very small and the main wave extremely drawn-out. The different behavior at the extreme ends of the *p*H scale may be due in part to differences in reaction rates of various charge types of cystine and to differences in adsorption and orientation of the cystine on the mercury surface.

Surface active substances like gelatin or thymol suppress the prewave. Since the diffusion currents of neither mercuric cysteinate nor RSSR are affected by these substances, the decrease in height of the prewave must be attributed to a decrease in the rate of formation of mercuric cysteinate in the presence of surface active compounds. In agreement with this interpretation is the observation[2] that the steep wave at *p*H 1 can be split into two waves on the addition of a proper amount of thymol, the resulting current–voltage curve consisting of a "prewave" and a main wave. In the presence of thymol the rate of reaction (2) is markedly decreased and thus only the initial part of the wave (prewave) is determined by this reaction while at more negative potentials cystine is slowly reduced according to equation 1.

All the observed facts are accounted for by the assumption that the prewave is kinetic in nature, with the exception of the effect of temperature upon the height of the wave. The temperature coefficient of this wave was found about equal to that of an ordinary diffusion current. Considering that adsorption of cystine is required before it reacts with mercury it appears possible that the increasing rate of reaction with temperature is compensated by decreasing rate of adsorption or orientation.

The TSST Wave.—The small height of the TSST prewave makes the analysis of this wave rather ambiguous. However, the zero potentials of mixtures of TSST and TSH could be measured with reasonable accuracy. The evaluation of these potentials according to equation 6 (see Table III) yields a value of +0.073 v. for the oxidation potential of the TSST–TSH system which is in good agreement with the value of +0.095 v. *vs.* N.H.E. as obtained from solubility data of cystine in the presence of thioglycolic acid.[12] Our experimental results thus indicate that the oxidation potential of TSST–TSH is also determined by over-all reaction (1).

Oxidized glutathione gives only one reversible diffusion controlled wave which has been interpreted as a one electron reduction involving a free radical mechanism.[13] In agreement with this interpretation the zero potential of the oxidized–reduced glutathione system measured at the dropping mercury electrode does not correspond to the oxidation potential of the system.

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