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THE MECHANISM OF ELECTROREDUCTION OF CYSTINE AT THE MERCURY ELECTRODE

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INTRODUCTION

Polarography and potential sweep oscillopolarography or cyclic voltammetry are known to be useful methods for the quantitative determination of disulfide or sulfhydril groups in proteins or free sulfide-containing amino acids. The same methods are also powerful tools for investigation of electro-redox mechanisms. Knowledge of the electrode reactions of sulfide-containing amino acids in the presence of different ions contributes to the understanding of the possible effect of heavy ions on sulfide-containing protein molecules. The electrode reaction depends on the interactions of the sulfide groups with metallic and ionic mercury, and is greatly influenced by the presence of other heavy metal ions.

In the present study we used triangular wave oscillopolarography (cyclic voltammetry) for studying the mechanism of electro oxy-reduction of cystine and cysteine, as a function of concentration, pH and concentration of added Cu^{2+} and Cd^{2+}.

The reaction of cystine (RSSR) with metallic mercury may result in RSHg and $(RS)_2Hg$ which are then reduced on the electrode by the following mechanisms[1,2]:

$$\begin{aligned} RSHg + H^+ + e &\rightleftarrows Hg + RSH \\ (RS)_2Hg + 2H^+ + 2e &\rightleftarrows Hg + RSH \end{aligned} \qquad (A)$$

Evidence will be presented in this paper for the existence of the two forms of mercury cysteinate in the surface.

EXPERIMENTAL

Cystine and cysteine were purchased from Fluka Schweiz as puriss grade and were dissolved in water. The cysteine was used in its hydrochloride form. All the inorganic salts were of analytical grade and were used without further purification, except sodium nitrate which was recrystallized. Dissolved oxygen was removed by bubbling nitrogen into the solution for 10 min just before starting the measurements.

A dropping mercury electrode was used as working electrode and a silver chloride (1 *M* salt) electrode with mixed salt bridge as reference electrode. The po-

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tential was introduced between the mercury electrode and a third platinum wire electrode. The Chemtrix model S5P-1 (Beaverton-Oregon) polarographic analyzer system was used for the oxy-reduction investigation by the potential sweep method. The system comprises a Tektronix type 564 storage oscilloscope, Chemtrix type 300 polarographic operational amplifier plug-in unit and a Chemtrix type 200 polarographic time base plug-in unit. This system was used to measure the current arising from different sweep rates, at different mercury drop ages for single or repetitive sweeps.

RESULTS

1. *Formation of mercury cysteinate on the mercury electrode*

Lee[3] showed that the reduction of mercury cysteinate is reversible giving a sharp oscillographic peak, while the reduction of cystine is irreversible giving a spread out peak at a higher negative potential.

Figure 1 shows a first sweep polarogram of cystine in solution at pH 2, and another obtained after subjecting the electrode surface to a repeat oxidation reduction sweep for otherwise identical conditions. In the repetitive sweeps only that corresponding to the delay time of the single sweep polarogram is shown. In every case two peaks on the cathodic sweep are observed. The sharp peak at the more positive potential is of a reversible nature and corresponds to the reduction of mercury cysteinate, while the second, widely spread irreversible peak corresponds to the reduction of cystine. The irreversible peak appears in the region of the capacitance hump of the supporting electrolyte. The irreversible peak diminishes when the potential sweep cycle is repeated, and eventually vanishes until only the capacitance hump of the supporting electrolyte remains. The considerably higher peak observed on the repeat sweep clearly indicates, as was also shown by Lee[3], that the reoxidation of cysteine (RSH) obtained by the irreversibly reduced cystine gives mercury cysteinate which is then reduced reversibly at the more positive potential. If one starts with cysteine solution only the reversible peak is observed. In this case the peak observed on the

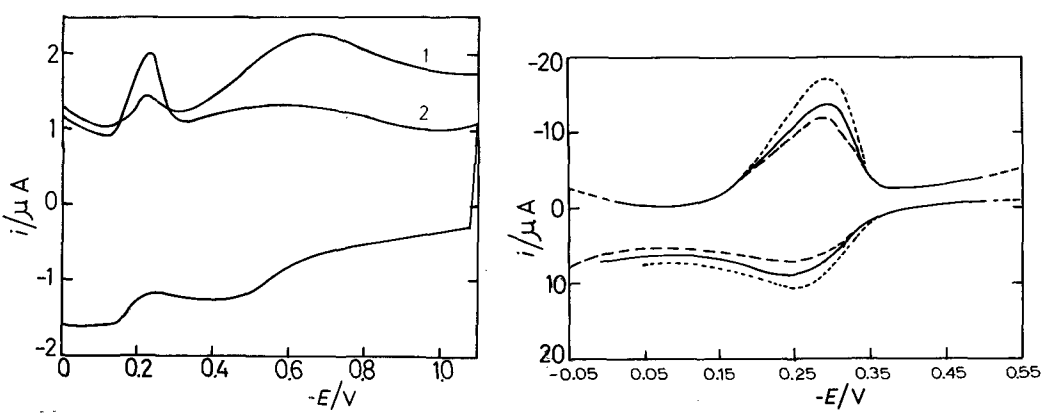


Fig. 1. Oscillopolarogram of 2.5×10^{-5} *M* cystine, 0.01 *M* HNO_3, 0.09 *M* $NaNO_3$ soln. (1) Single, (2) repetitive sweep. Span 1 V; sweep 2 V s^{-1}; starting potential 0 V.

Fig. 2. Oscillopolarograms of cystine at different starting potentials. 5×10^{-4} *M* cystine, 0.01 *M* HNO_3, 0.09 *M* $NaNO_3$; span 0.5 V; sweep rate 2.5 V s^{-1}. Starting potential: (.....) + 50, (——) 0, (------) 50 mV.

repeat cathodic sweep is somewhat lower than the first sweep since part of the cysteine diffuses away from the negatively charged surface. There is a close relation between the size of the reversible peak obtained from a cystine solution and the amount of cysteine produced by the irreversible reduction of cystine in the preceding sweep. This is evident from Fig. 2 where the size of the peak in the repeat sweep of a 0.5 V span is shown to increase with the negativity of the starting, and thus also the final, potential. Since there is no dependence on the starting potential if the span is 1 V and in every case a high enough potential for completion of the irreversible reduction peak of cystine is reached, it must be concluded that the increase in the peak obtained by repeating the sweep is directly related to the amount of cystine slowly reduced during the sweep.

Thus if mercury cysteinate is responsible for the reversible peak, as can be deduced from the oscillopolarogram, it is formed from cystine only slowly by the reactions:

$$\begin{aligned} &\mathrm{RSSR} + \mathrm{Hg} \rightleftarrows \mathrm{Hg(RS)_2} \\ &\mathrm{Hg(RS)_2} + \mathrm{Hg} \rightleftarrows 2\mathrm{HgRS} \end{aligned} \qquad \text{(B)}$$

and rapidly the electro-oxidation of cysteine (RSH in Scheme A).

Lee suggested that even in cystine solution, the mercury cysteinate in the surface at positive polarizations is formed from traces of cysteine from the solution. This assumption was checked by measuring the height of the reversible peak as a function of delay time (time that passes from the start of formation of the mercury drop until the triggering of the sweep). This is based on a further, but plausible, assumption that the insoluble mercury cysteinate remains adsorbed on the surface. The height of the peak is proportional to the amount of mercury cysteinate accumulated on the surfaces when the sweep is triggered, or rather at the appearance of the peak which at a fast sweep rate is practically the same. The formation of mercury cysteinate from cysteine, which is a fast process, should be diffusion controlled, while from cystine it should be controlled by the rate of the surface reaction. The surface concentration for diffusion controlled adsorption[4]:

$$\Gamma_t = 0{,}743\, c_b D^{\frac{1}{2}} t^{\frac{1}{2}} \qquad (1)$$

where c_b is the concentration of the substance (cysteine) to be absorbed in the bulk, D its diffusion coefficient and t the delay time. The total amount of adsorbed material is the surface concentration times the area which is proportional to $t^{\frac{2}{3}}$. Summarizing, we obtain for the peak current ($i_{t,p}$) brought about by the reversible reduction of RSHg accumulated in the surface by diffusion:

$$i_{t,p} \propto t^{7/6} \qquad (2)$$

If, however, the accumulation in the surface is controlled by the rate of the surface reaction (see Scheme B):

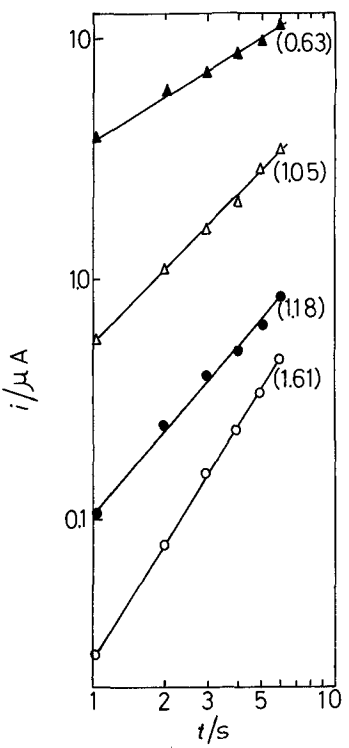
$$\mathrm{d}N_S/\mathrm{d}t = k A_t c_b = k' t^{\frac{2}{3}} c_b \qquad (3)$$

where N_S is the amount accumulated in the surface of the drop of area A_t, at delay time t, and k is the rate constant. After integration, since the peak current $i_{p,t}$ is proportional to N_S, we obtain:

$$i_{p,t} \propto t^{\frac{5}{3}} \qquad (4)$$

Hence the plot of log $i_{p,t}$ *vs.* log t should indicate whether the rate of accumulation of mercury cysteinate in the interface between positively charged mercury and a cystine solution is controlled by the diffusion of traces of cysteine, or by the rate of surface reaction of cystine. For comparison, the height of the reversible peak was measured as a function of the delay time in solutions of cystine as well as cysteine. The results are shown in Fig. 3 where log $i_{p,t}$ is plotted against log t. In every case straight lines were obtained. The slope with cystine in the aqueous solution is about 1.6 which is slightly less than the 1.67 expected for reaction rate controlled surface accumulation. In the case of the highly soluble cysteine, dependence of $i_{p,t}$ on t could be measured over a wider range of concentrations. At low concentrations the slope of log $i_{p,t}$ *vs.* log t is indeed 1.15 as expected. The slope decreases at higher concentrations of cysteine as surface saturation is approached.

Further evidence for the formation of mercury cysteinate from cystine, together with information about the dependence of the rate of formation on the potential and on pH, is obtained from the dependence of the peak current on the initial



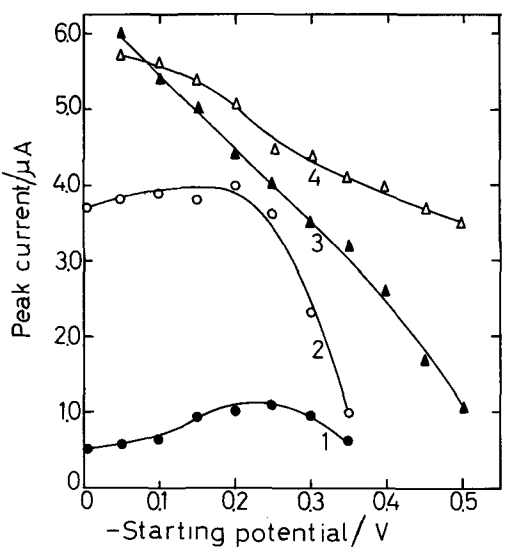


Fig. 3. Peak current (logarithmic scale) as function of drop age. 0.01 *M* HNO_3, 0.09 *M* $NaNO_3$; sweep rate 2 V s^{-1}; span 1 V. (○) 8.5×10^{-5} *M* cystine (●) 2×10^{-5} *M* cysteine HCl; (△) 8×10^{-5} *M* cystine HCl, (▲) 3.9×10^{-4} *M* cysteine HCl. Slopes given in parentheses.

Fig. 4. Peak current as function of starting potential. Span 1 V; sweep rate 2 V s^{-1}; delay 5 s. (1) 2.5×10^{-5} *M* Cystine, 0.25 *M* acetate buffer, pH = 4.95; (2) 5×10^{-5} *M* cysteine hydrochloride, 0.25 *M* acetate buffer, pH = 4.95; (3) 2.5×10^{-5} *M* cystine, 0.1 *M* ammonia + ammonium chloride buffer, pH = 10.45; (4) 5×10^{-5} *M* cysteine hydrochloride, 0.1 *M* ammonia + ammonium chloride buffer, pH = 10.45.

(resting) potential. These experiments were carried out at higher pH, in order to shift the peak to more negative potentials, allowing a larger variation in the initial potential.

As shown in Fig. 4, the peak current $i_{t,p}$ is a function of the initial potential of the cathodic sweep depending on pH and on ionic composition of the solution. The peak currents are proportional to the rates of formation of mercury cysteinate from cystine which depends on the initial potential. The concentration of mercury cysteinate formed from cysteine depnds only on the local concentration of the latter and an equilibrium coefficient characteristic of the given initial potential. Comparing the peak currents obtained from cystine solutions with those obtained from cysteine solutions for different initial potentials, it can be seen that at high pH, the rate of formation of mercury cysteinate from cystine increases and equilibrium values (identical with those obtained from cysteine) are approached. At low pH values the cystine/cysteine peak ratio (Fig. 4, curves 1 and 2) increases with increasing negativity of the starting potential.

2. *Mercury cysteinate and dicysteinate*

At this point it was not clear whether the mercury in the cysteinate was monovalent or divalent or whether both valencies coexisted with their relative abundance depending on the concentration of cysteine in the surface. To resolve this question potential sweep oscillopolarograms were made at different concentrations of cysteine (or cystine with repetitive sweeps). According to the reaction schemes (B) and (C) we would expect formation of $Hg(RS)_2$ at higher concentrations of cystine. With increasing surface concentration, the peak shifts toward negative polarizations and another reduction current peak appears at potentials 100 mV more negative (compare

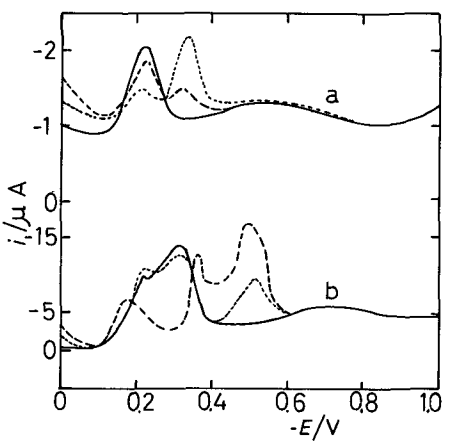


Fig. 5. Oscillopolarograms of cystine in presence of Cu^{2+}; repetitive sweep; span 1V; sweep rate 2 V s^{-1}; starting potential 0 V. (a) 2.5×10^{-5} *M* Cystine; (——) without copper, (.....) 3×10^{-5} *M* $Cu(NO_3)_2$. (b) 5×10^{-4} *M* Cystine; (——) without copper, (.....) 10^{-4} *M* $Cu(NO_3)_2$, (----) 4×10^{-4} *M* $Cu(NO_3)_2$.

the solid line curves of Figs. 5a and 5b). The relative size of the two peaks depends on surface concentrations. It is reasonable to assume that the more negative peak which appears at higher concentrations of cysteine corresponds to the reduction of $Hg(RS)_2$,

while the more positive peak corresponds to the reduction of HgRS. Thus the sizes of the two peaks represent the surface concentrations of HgRS and $Hg(RS)_2$ respectively.

Assuming the following reaction in the surface:

$$2\ HgRS \rightleftarrows Hg + Hg(RS)_2 \qquad (C)$$

the equilibrium constant $K = [Hg(RS)_2]/[HgRS]^2$ could be determined from the relative size of the two peaks if the transition $Hg(RS)_2 \rightarrow HgRS$ during the reduction of the latter did not obscure the picture. The order of magnitude of K can be estimated from the height of the hump at -0.200 V (corresponding to the reduction of RSHg) which is about half the peak at -0.300 V (corresponding to the reduction of $(RS)_2Hg$) when surface saturation is reached. Surface saturation is characterized by invariant area of the combined reversible peak with concentration. This corresponds to about 100 μC transferred charge per cm^2 or to formation of about 6×10^{14} cysteine molecules. In other words, at saturation the area per RS group associated with charged mercury on the electrode surface is about 16 $Å^2$. Below saturation concentration of the reversible peak, the irreversible spread out peak appears and continues growing after saturation has been reached. Evidently when the electrode surface is covered with mercury cysteinate, cysteine is oxidized to cystine which is then reduced irreversibly during the cathodic sweep. The formation of cystine may presumably be catalyzed by Hg^{2+}; the same effect as found[5] for Cu^{2+}.

3. *Interference of heavy ions (Cu^{2+}, Cd^{2+}) with the cystine–cysteine electrode redox process*

Adding Cu^{2+} to a solution of cysteine so that the molar ratio Cu^{2+}/cysteine < 1 causes a lowering of the original cathodic peak and the appearance of another peak at a more negative potential. The ratio of the new, more negative peak to the original increases with the ratio Cu^{2+}/cysteine until eventually at a more than two-fold excess of Cu^{2+} (at a sweep rate of 2 Vs^{-1}) the more positive peak vanishes. This behavior is seen at any pH or concentration (Fig. 5) and on single or repetitive cathodic sweeps. These results are in keeping[6] with the d.c. polarography of cystine in the presence of Cu^{2+}.

Kuta *et al.*[7] suggested that the interference by Cu^{2+} is through the formation of highly surface active monovalent copper cysteinate (CuRS) which, by adsorption, inhibits further reduction of cystine. However, this hypothesis in its simple form is untenable since interference by Cu^{2+} occurs already at too low a concentration to allow formation of an impermeable surface layer, even if it were controlled by diffusion kinetics. Moreover, experiments at high concentrations of cysteine indicate (Fig. 5b) reduction peaks of $(RS)_2Cu$ which by analogy to mercury cysteinates are at a more negative potential than the reduction peak of RSCu. The reduction peaks of RSCu reappear on increasing the concentration of added Cu^{2+}.

As shown in the previous paragraph, the formation of HgRS from a solution of cystine at low pH is slow and only about 25% of the total reduction takes place in the reversible peak of a single sweep. The addition of relatively low concentrations of added Cu^{2+} suffice to suppress this peak and to form a peak at a more negative potential.

For repetitive sweeps at low pH values and under any conditions at high pH

values, the double peak is obtained when up to two equivalents of Cu^{2+} with respect to cystine is added to the solution. The relative size of the two peaks depends on the initial potential. The more negative initial potentials give larger reduction peaks of copper cysteinate and cause a decrease in the peak of mercury cysteinate. The anodic sweep shows in the presence of Cu^{2+} at low concentrations of cystine or cysteine only one spread out peak of an irreversible type. Only at high concentrations of cysteine is an additional peak corresponding to the formation of $Cu(RS)_2$ observed.

The effect of Cd^{2+} on the reduction peaks is shown in Fig. 6 (curves a, b, c, d), to be similar to that of Cu^{2+}. The reduction peak of mercury cysteinate decreases gradually while a new peak appears and increases with increasing concentration of Cd^{2+}. For the starting potential of 0 V relative to the 1 *M* silver–silver chloride elec-

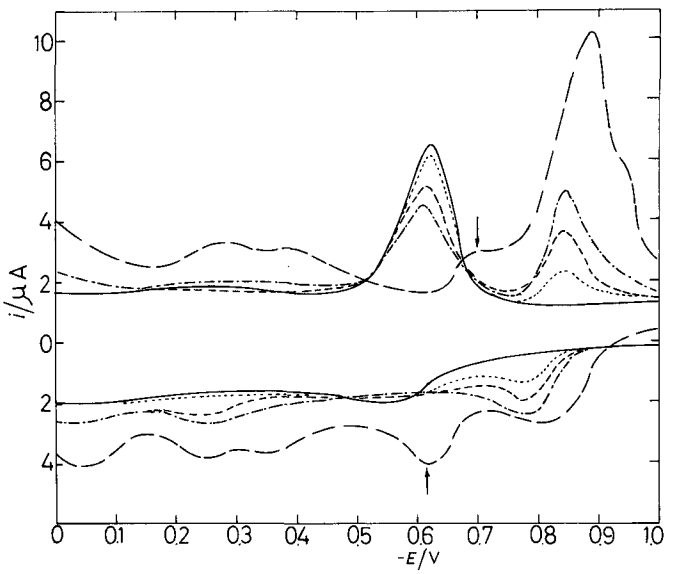


Fig. 6. Oscillopolarogram of cystine in presence of $Cd(NO_3)_2$; span 1 V; sweep rate 2 V/s; starting potential 0 V; 2.7×10^{-5} *M* cystine, 0.1 *M* $NaNO_3$; pH = 9 (without buffer). (a, ——) Without $Cd(NO_3)_2$, (b, ...) 10^{-5} *M* $Cd(NO_3)_2$, (c, - - - - -) 2×10^{-5} *M* $Cd(NO_3)_2$, (d, ·····) 3×10^{-5} *M* $Cd(NO_3)_2$, (e, ———) 10^{-4} *M* $Cd(NO_3)_2$ added.

trode the two reduction peaks are about equal at equivalent concentrations of cystine and Cd^{2+}. Presumably the metallic cadmium obtained by the re-reduction of Cd^{2+} displaces mercury from mercury cysteinate as the negativity of the potential increases. The cadmium cysteinate formed is then reduced at higher negative potential. An excess of Cd^{2+} with respect to cystine is required to get complete displacement of the mercury ion, and only then does the reduction peak of the unbound Cd^{2+} appear (arrow on curve e, Fig. 6). Under these conditions the ionic Cd^{2+} displaces mercury ions from the mercury cysteinate and these are then reduced giving rise to two spread out reduction peaks at potentials less negative than either that of Cd^{2+} or of the cysteinate.

As shown in Fig. 7 (cystine in ammonia and NH_4Cl as supporting electrolyte) the ratio of the reduction peaks of Hg-cysteinate and of Cd-cysteinate depends, just as in the presence of Cu^{2+}, on the starting potential. The effect of Cd^{2+} on the reduc-

tion peaks is smaller in a solution of cysteine which binds Cd^{2+} in the bulk than in a solution containing cystine. However, qualitatively the behavior is similar under all conditions investigated and supports the intuitive expectation that the equilibrium ratio of mercury cysteinate to cadmium cysteinate on mercury surfaces is higher the more positive its polarization.

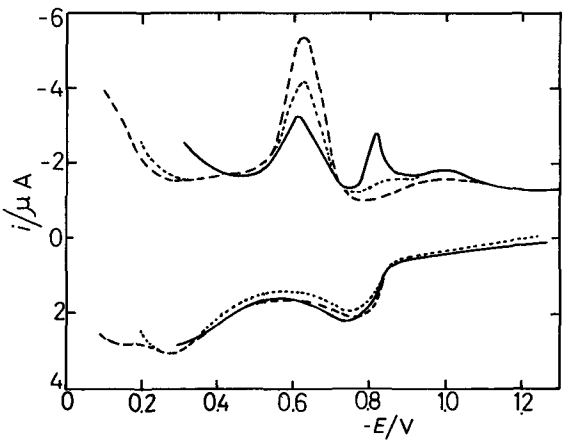


Fig. 7. Oscillopolarograms of cystine in the presence of $Cd(NO_3)_2$. 3×10^{-5} *M* Cystine, 3×10^{-5} *M* $Cd(NO_3)_2$, buffer 0.1 *M* ammonia + ammonium chloride, pH = 10.45. Starting potential: (——) 0.3, (.....) 0.2, (– – – –) 0.1 V.

The diffusion and rate controlled exchange reaction is not fast enough to maintain the equilibrium ratio during the potential sweep. The same conclusion can also be reached from the dependence of the different reduction peaks on the sweep rate. For example, the ratio of the reduction peak of mercury cysteinate to that of cadmium cysteinate for bulk concentrations 2.7×10^{-5} *M* cystine and 3×10^{-5} *M* Cd^{2+} and initial potential 0 V (relative to the silver–silver chloride electrode) is 0.33 for 1 V s^{-1}, 0.83 for 2 V s^{-1}, 2.5 for 5 V s^{-1} and 4.5 for 10 V s^{-1}. If the concentration of Cd^{2+} is raised to 10^{-4} *M* the mercury cysteinate peak disappears completely at sweep rates of 1 V s^{-1}, while at 10 V s^{-1} the size of the two peaks is about equal.

DISCUSSION

Kolthoff and his collaborators showed in their classical papers that the electro-reduction of cystine to cysteine is catalyzed by the mercury surface. They suggested that the catalytic path is through a mercury cysteinate intermediate formed on the mercury surface. This view is supported by the evidence presented in this work. Moreover, we show that cysteinates of monovalent and bivalent mercury coexist on the mercury, their ratio depending on the concentration of cystine or cysteine in the solution. The formation of mercury cysteinate is very fast when adsorbed cysteine facilitates the oxidation of mercury metal atoms on the positively polarized mercury surface. The equilibrium surface concentration of mercury cysteinate increases with the positive polarization of the surface and with the concentration of the cysteine near the surface. The diffusion controlled rate of formation of mercury cysteinate indicates

that the equilibrium concentration of cysteine required to form a monolayer of mercury cysteinate at positive polarizations is negligible with respect to the bulk concentrations used (3×10^{-5} *M*). The formation of mercury cysteinate by interaction of cystine with metallic mercury is a very slow process at low pH values but its rate increases with increasing pH. At pH 2 the accumulation of mercury cysteinate in the surface is controlled by the rate of this reaction. The equation for the rate controlled adsorption process introducing our capillary constant assumes the form:

$$N_S = k_a c_b 0.5 \times 10^{-2} t^{\frac{5}{3}}$$

from which the calculated value of k_a at pH 2 and a potential of 0 V relative to the silver–silver chloride electrode is about 10^{-5} cm s^{-1}. An electrode process or an adsorption is rate controlled[8] if $k_a t^{\frac{1}{3}}/D^{\frac{1}{2}} \ll 1$. The value of 10^{-5} cm s^{-1} is low enough to make the adsorption rate controlled at a delay time of a few (eqn. (5)) seconds, since:

$$k_a t^{\frac{1}{3}}/D^{\frac{1}{2}} = 10^{-4} \times 2.2/2.2 \times 10^{-3} = 0.01$$

Indeed, at higher pH when k_a increases, the diffusion step in series with the rate process cannot be neglected.

Heavy ions such as Cu^{2+} or Cd^{2+} compete with Hg^{2+} for the cysteinate in the mercury surface. The displacement of the mercury ions by either ionic or metallic copper and cadmium is favored by higher negative polarizations. This is to be expected since with displacement the mercury ions are immediately reduced to metallic mercury. The heavy metal cysteinates formed are then reduced at the respective potentials, which are more negative than either the reduction potentials of the free ions or that of mercury cysteinate. The competition in the surface takes place at any surface concentration of the competing components. The picture of the adsorbed monolayers of highly surface active CuRS impeding the reduction of cystine, suggested by Kůta and his coworkers, can be accepted only in the restricted sense of surface sites occupied by a cysteinate of a heavy metal ceasing to be available for interaction between cystine and mercury metal thus lowering the number of cystine molecules reduced *via* mercury cysteinate. At higher surface concentrations the adsorbed layers of surface active cysteinate may impede directly the charge transfer through the mercury electrode surface.

SUMMARY

Potential sweep oscillopolarography of solutions containing cystine and cysteine has been carried out. Mercury cysteinate is formed on the mercury electrode surface in contact with either cystine or cysteine solution at potentials more positive than the reduction potential of cystine. When a cathodic sweep is then applied, the adsorbed mercury cysteinate is reduced, producing a reversible reduction peak. The formation of mercury cysteinate from cysteine is always fast, but its formation from cystine is very slow at low pH values and its rate increases with increasing pH. The mercury in the mercury cysteinate may be in the monovalent or bivalent form but higher surface concentrations of cysteine favor the bivalent form. Heavy metal ions tend to displace mercury from mercury cysteinate shifting the reduction peaks to potentials more negative than that of mercury cysteinate or of the pure heavy metal ion.

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