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DETERMINATION OF CYSTINE AND CYSTEINE IN SEAWATER USING CATHODIC STRIPPING VOLTAMMETRY IN THE PRESENCE OF Cu(II)

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ABSTRACT

A method is described for the determination of cystine and cysteine in seawater and freshwater using cathodic stripping voltammetry in the presence of added copper(II). The optimized conditions include a copper concentration of 150 n*M*, a pH of 8.5, and a collection potential of −0.15 V; the cathodic reduction peak is located at −0.55 V. The detection limit is 0.1 n*M* after a collection period of 4 min. The sensitivity is diminished by surfactants similar to Triton X-100 in natural waters; the sensitivity therefore needs to be calibrated by internal standard additions of amino acids. It is possible to differentiate between cystine and cysteine by employing a solution pH of 6.2, where the peak due to cystine is absent. The response in seawater is different from that previously reported in buffer solutions. It is shown that the amino acid reduction peak is due to Cu(I) reduction of the adsorbed Cu(I)–cysteinate complex; this complex is formed with Cu(I) generated from dissolved copper(II) at the electrode surface at −0.15 V in the presence of cysteine; cystine is reduced to cysteine at the electrode surface during the collection process.

INTRODUCTION

The determination of trace amounts of organic compounds using voltammetric stripping techniques is a growing discipline; however, the exact mechanism by which the compound is adsorbed on the electrode is still poorly understood. Florence [1] carried out one of the first investigations of the adsorption process using a variety of different compounds. He found that compounds containing the thiol group were adsorbed and he explained this in terms of the formation of a mercury–thiol complex. However he was also able to measure several non-sulphur containing compounds (some pterins, iodides, flavones, and many flavins). He did not attempt to explain the means of their adsorption, but noted that the responses with the flavins appeared to be "true mercury compound-accumulation waves". Florence [1] further noted the existence of a third category of compounds, including

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methylene blue and haemoglobin, which adsorbed without the apparent formation of a mercury complex. In contrast, numerous other substances (including a variety of amino acids, purines and pyrimidines) did not adsorb with the conditions he employed.

Recently, a number of other workers have determined S-containing organic compounds at the mercury electrode. Thus, polarography has been used to determine cysteine [2,3], thiamine [4] and thiobarbiturate [5], and cathodic stripping voltammetry (CSV) to measure thiols [1,6–9], thiourea [10], felypressin [11], peptides [12], thioamides [13] and penicillamines [14]. Davidson and Smyth [13] postulated the mechanism for the reduction current obtained with CSV in terms of the catalytic reduction of thioamides with the concurrent formation of HgS. Banica [2] has used dc polarography to investigate the mechanism of adsorption of cysteine-containing dipeptides in the presence of nickel. He concluded that adsorption of the oxidised peptide was followed by reduction of the disulphide bond enabling complexation with Ni(II), the resulting complex being reduced to form the catalytic Ni prewave.

The current study was carried out to investigate the cystine and cysteine–copper reduction mechanism at the hanging mercury drop electrode (HMDE) in a background electrolyte of seawater. Previous determinations of cystine and cysteine using CSV, both in the presence [6] and absence [1,3,7] of added copper(II), had been carried out in buffer solutions. However, it was found that the behaviour of the two compounds in seawater was quite different from that in the synthetic solutions, and the limit of detection was found to be between one and two orders of magnitude less than that previously reported [6,7]. The data confirm Forsman's [6] finding that current enhancement in the presence of copper is due to the adsorption of Cu(I)–amino acid complexes. It will be shown that this process does not require the formation of a Cu(Hg) amalgam, but rather that the complexes are formed with Cu(I) generated at the electrode surface from dissolved Cu(II).

EXPERIMENTAL

Equipment and reagents

Voltammograms were recorded with a PAR 174A polarograph, a PAR 303 HMDE (drop surface area = 2.94 mm^2) and a PAR 305 magnetic stirrer, or with a Metrohm E506 polarograph connected to a model 663 HMDE (drop surface area = 0.38 mm^2). Reagents were supplied by BDH Chemicals Limited. Seawater ($S = 32$) was collected from the Menai Straits, and was filtered (0.45 μm) prior to use. A borate pH buffer was prepared which was 1 M with respect to boric acid and 0.4 M with respect to NaOH (Aristar). Stock copper solutions (10^{-5} M and 10^{-4} M) were prepared from a BDH atomic absorption spectrophotometry standard solution by dilution with distilled water. Stock amino acid solutions were prepared by dissolving 1 mg of cystine (or cysteine) in 20 ml of distilled water; these were found to be stable for at least a week. Distilled water was prepared using a fused silica double distillation unit. Background electrolytes were stored in fused silica

containers after irradiating for 3 h using a 1 kW mercury vapour lamp. The pH meter was calibrated against NBS pH buffers.

Procedure

A 10 ml aliquot of seawater was pipetted into a Teflon polarographic cell, and the pH was adjusted to 8.5 by addition of 100 μl borate buffer giving a final concentration of 0.01 *M* borate. The magnetic stirrer was started, and the solution was purged with oxygen-free nitrogen to remove oxygen. The potentiostat was then switched on at the required collection potential (E_{coll}), usually -0.15 V. A new mercury drop was extruded and the adsorption time (T_{ads}), usually 60 s, was measured from this point. At the end of the required collection time, the stirrer was switched off. After a quiescent period of 10 s to allow the solution to come to rest, a negative potential scan was made using the differential pulse modulation (scan rate 10 mV/s, modulation amplitude 25 mV, and a pulse time of 0.1 s). The procedure was repeated after addition of copper, usually 150 n*M*, and again after a standard addition of cysteine or cystine.

RESULTS AND DISCUSSION

Cathodic stipping voltammetry of cysteine and cystine

A narrow, clearly defined reduction peak was observed at around -0.55 V for low concentrations (n*M* level) of both cystine and cysteine in the presence of 150 n*M* copper (pH 8.5) in seawater (Fig. 1a). The exact peak potential at a given pH was dependent on the concentration ratio of the amino acid to copper, and on the amino acid concentration (Fig. 1b). The peak was preceded by the Cu(I)/Cu(0) reduction peak at -0.2 V (Cu(I) is known to stabilize in chloride media due to the formation of $CuCl^0$ and $CuCl_2$ complexes; the Cu(II)–Cu(I) reduction wave is shifted in a positive direction and masked by the mercury wave) [15–17]. Without preconcentration, the free copper (Cu(I)) reduction peak became visible only at high copper levels ($>$ 100 n*M*). It doubled in size when the CSV scan was preceded by a stirred adsorption period of 60 s ($E_{coll} = -0.15$ V), suggesting that a significant quantity of Cu(I) chloro ions were adsorbed onto the HMDE, as has also been observed previously by Nelson and Mantoura [17].

The peak resulting from the addition of either cystine or cysteine is due to the reduction of the adsorbed copper(I) cysteinate complex, cysteine having a free –SH group which can form a strong complex with copper(I). Complexation of cysteine with copper(II) was discounted as it is considered to be unstable in the presence of copper(II), being oxidised to cystine [18]. The formation constant for the copper(II) complex with cystine is low (log $K = 7.0$ [19]), so copper(II) complexation with this amino acid is negligible at the solution concentrations (10 n*M* Cu and 1 n*M* cystine) when the peak is first observed. On the other hand, the formation constant for the Cu(I) cysteine complex is thought to be very large (log $K = 20$) [18]. Further,

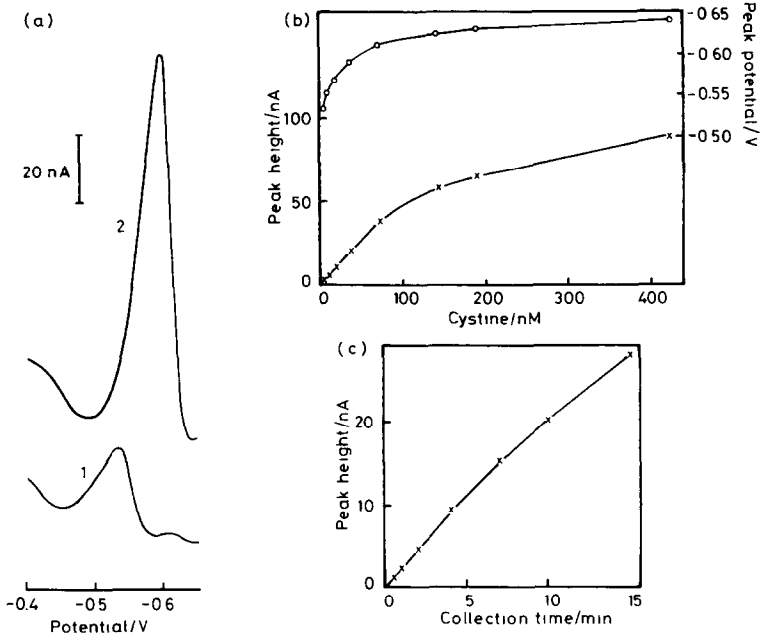

Fig. 1. DPCSV of cystine in seawater (pH 8.5) with 150 n*M* Cu added. (a) DPCSV with 1 min (scan 1) and 15 min collection (scan 2). (b) Peak height and peak potential *versus* the cystine concentration. (c) Peak height *versus* the collection time.

cystine is not stable at the mercury drop electrode, as it is easily reduced to cysteine [1,7,20]. The reaction mechanism will be discussed in more detail below.

Serial addition of cystine from 0 to 73 n*M* led to a linear increase in I_p (Fig. 1b); at higher concentrations the sensitivity (= peak height/concentration) decreased. This levelling off was not caused by saturation of the drop surface, as it was found that the peak height could be increased by extending the adsorption time or by adding more copper. A second peak appeared at −0.4 V (approximately 0.2 V more positive than peak 1) for 142 n*M* cystine, and with increased concentrations of cystine the height of both peaks increased. Addition of more Cu led to a decrease in the height of the second peak and an increase in the copper complex peak. It was therefore concluded that the second peak was due to the reduction of an adsorbed Hg(I) or Hg(II) cysteine complex [1,7].

In a background electrolyte of borate buffer (0.01 *M*) in distilled water (pH 9) the peak due to the mercury complex appeared at low cystine levels (< 10 n*M*) but this peak was strongly inhibited by addition of 30 n*M* copper, as shown in Fig. 2a and b (the small peak 0.1 V more negative than the cystine peak was due to lead traces present in the triply distilled mercury [15]). Separate experiments showed that the concentration ratio of Cu to cystine (or cysteine) determined whether the peak due to the mercury or the copper complex would appear. The mercury peak was

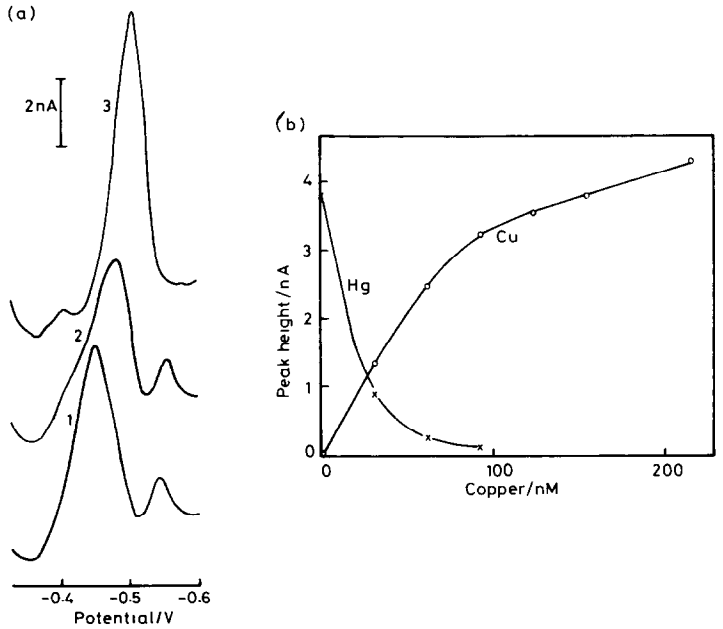

Fig. 2. Inhibition of the mercury–cysteine peak of 76 n*M* cysteine by copper in distilled water containing 0.01 *M* borate buffer (pH 9.0). DPCSV scans: (a) Without added copper (scan 1), with 16 n*M* Cu (scan 2) and with 63 n*M* Cu (scan 3). (b) The height of the copper–cysteine and mercury–cysteine peaks versus the copper concentration.

suppressed when the concentration of copper was greater than that of the amino acid. Low amino acid levels in aqueous samples also containing copper can therefore only be detected by means of the copper complex peak.

Cyclic voltammetry and tensammetry

The reversibility of the electrode reaction was studied by cyclic voltammetry in experimental conditions where the mercury as well as the copper peak were present (the amino acid > the copper concentration). It was found that the behaviours of cystine and cysteine were identical and two cathodic and their corresponding anodic peaks were resolved (Fig. 3a). The peak heights (I_{pc}) of the two cathodic peaks were found to be linearly dependent on the scan rate (v), from 0 to 100 mV/s, above which the slope levelled off (Fig. 3b). This linear relationship indicates that the peaks are produced by the reduction of a thin adsorption layer [21]. The anodic peak height was always smaller than that of the cathodic peaks, as a result of diffusion of reduced Cu^0 and uncomplexed amino acid away from the electrode surface (the anodic peak is eliminated by briefly holding the potential constant whilst stirring the solution prior to the returning scan). For both amino acids the E_p shifted in a negative direction as the scan rate was increased, as a result of non-Nernstian reactions [21].

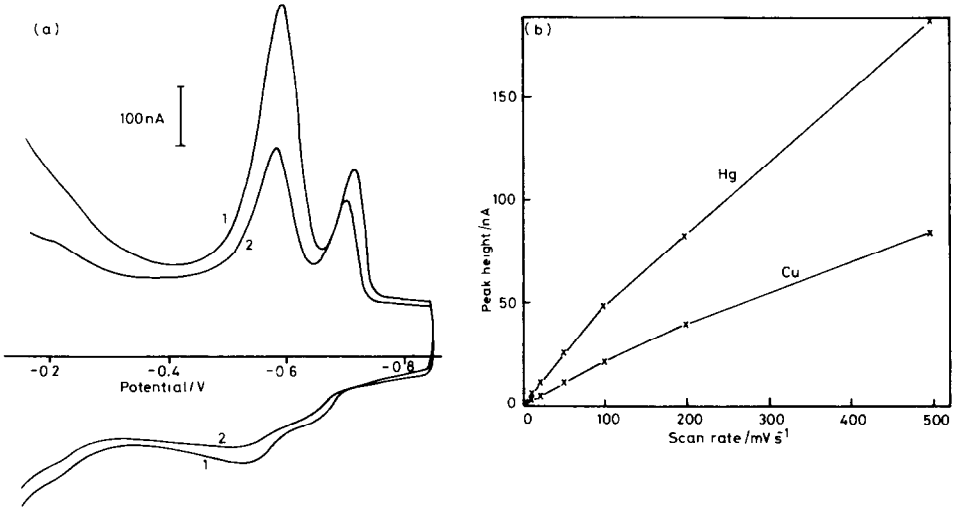

Fig. 3. Cyclic voltammetry of 400 n*M* cystine in the presence of 150 n*M* Cu in seawater (pH 8.5). (a) Scan 1 was preceded by 60 s stirred collection at −0.15 V; scan rate 100 mV; scan 2 immediately followed on scan 1. (b) Height of the mercury and copper–cysteine peaks as a function of the scan rate.

The expected capacitance-suppressing effect of the adsorption of cysteine on the HMDE was investigated using ac voltammetry, with 90° out-of-phase current sampling. Interestingly, no capacitance suppression was caused by the addition of 500 n*M* of cystine or cysteine to the solution (seawater at pH 8.5), after adsorptive collection at 0 V, at −0.2 V, and at −0.6. A small capacitance suppressing effect (approximately 2% of the current output) was produced between −0.3 and −0.6 V when 1.5 μM copper was added to the same solution (scan 3 in Fig. 4). This apparent suppression may have been caused by the disappearance of the broad mercury–cysteinate reduction wave between −0.4 and −0.55 V, cuprous replacing mercurous ions in the adsorbed complexes; the peak at −0.65 V corresponds with the reduction of cuprous–cysteinate complexes. The capacitance suppression is much less than that caused by the addition of adenine to the solution (scan 4, Fig. 4) which forms an adsorbed film of cuprous complexes [22]. This difference could be caused by the relative small size and ionic nature of cysteine, and it suggests that perhaps only the –S–Cu(I) group adsorbs on the mercury surface.

Effects of increasing the concentration of copper(II)

The peak height for cystine and cysteine increased non-linearly as the concentration of Cu was increased, as shown in Fig. 2b. At low amino acid levels (< 10 n*M*) and in seawater the peak height reached its maximum at a lower copper concentration (at 70 n*M*) than shown in Fig. 2b. This maximum of the peak height was not a result of saturation of the drop, since the peak currents increased with longer

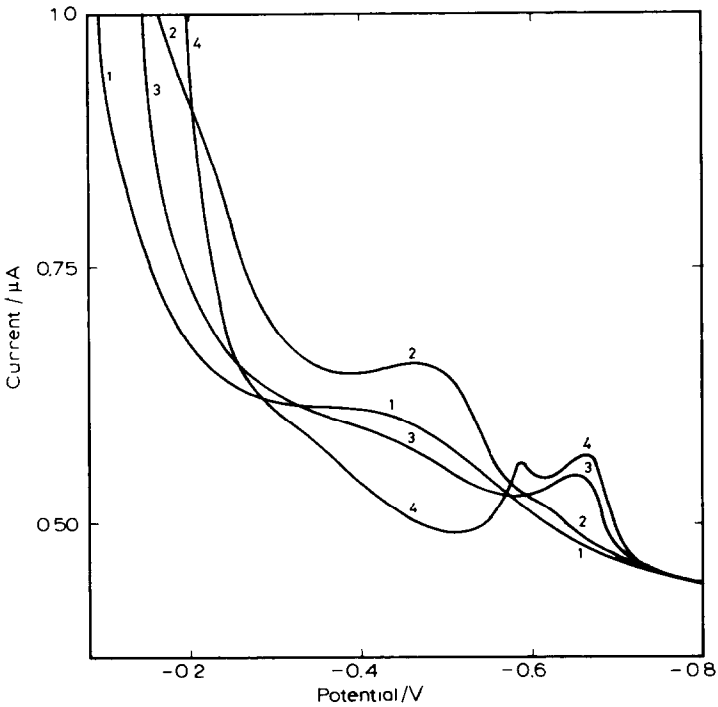

Fig. 4. Tensammetry of cystine in seawater at pH 8.6. Each scan was preceded by 60 s adsorption at −0.2 V. (1) Seawater; (2) 1 + 0.5 μM cystine; (3) 2 + 1.5 μM copper; (4) 3 + 0.5 μM adenine.

adsorption times and/or increased concentrations of cystine. In this instance, the cystine concentration was limiting.

The amino acid peak potential shifted in a negative direction with increasing copper concentration. However, this shift was only small (25 mV for Cu going from 10 nM to 150 nM) and was attributed to the enhanced adsorption of cysteine on the electrode surface. The peak potential (E_p) for 9 nM cystine was located at − 0.530 V for 70 nM Cu ($E_{coll} = -0.15$ V, $T_{ads} = 60$ s whilst stirring, in seawater with 0.01 M borate).

As a result of these experiments 150 nM Cu was chosen as a suitable concentration, as it gives a clear well-defined peak of optimal height. This is a considerably lower level of Cu than that used by Forsman [6] (10^{-5} M Cu). The free copper peak at −0.2 V at such high copper concentration interferes with the determination of low (nM) levels of cystine and cysteine in seawater.

Effect of varying the collection potential (E_{coll})

The CSV peak height was measured whilst varying the collection potential between −0.15 and −1.15 V (the stirred adsorption time was 60 s), each scan being

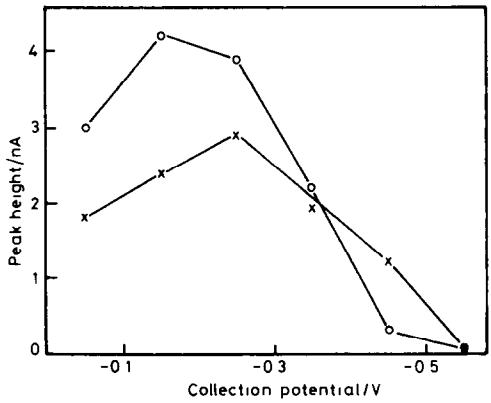

Fig. 5. DPCSV peak height obtained for 3.5 n*M* cysteine (○) and 4.7 n*M* cystine (×) in seawater (0.01 *M* borate buffer, 150 n*M* Cu) after 60 s stirred collection, as a function of the collection potential.

initiated at −0.15 V. A maximum response was obtained for cystine at a collection potential at −0.25 V, for cysteine at −0.15 V (Fig. 5).

With collection potentials more negative than the reduction potential of the copper–amino acid complex a reduction peak for the complex was no longer observed. Apparently the free amino acid (not complexed by copper) does not adsorb onto the mercury drop, as the adsorption layer is not formed upon switching the potential to −0.15 V in the presence of 150 n*M* Cu and with amalgamated copper diffusing out of the drop. A concurrent increase was seen in the free copper peak height, because amalgamated copper was released back into the solution immediately surrounding the mercury drop. The finding that free Cu(I) is required contrasts with the previous proposal that amalgamated copper is required for the formation of the copper–cysteine complex [6].

Effect of varying the collection time (T_{ads})

The peak height of the copper(I)–cysteinate peak increased linearly with the collection time up to 4 min, and the peak potential shifted in a negative direction from −0.530 to −0.595 V for 150 n*M* Cu and 9 n*M* cystine; with longer collection times the response diminished (Fig. 1c). The free Cu(I) peak, at −0.25 V, was found to decrease simultaneously. The drop surface was not saturated as the peak could be increased further by adding more copper and cystine.

The diminished sensitivity at high cystine levels (Fig. 1b) coincided with the disappearance of the free copper peak; a similar effect was observed at long collection times. These data suggest that the amount of adsorbed free Cu(I) (rather than amalgamated copper [6]) ultimately limits the subsequent formation of the amino acid–copper complex, and causes the loss of sensitivity (peak height/amino acid concentration) seen.

Effect of varying the pH and the chloride concentration

The DPCSV responses of cystine and cysteine were determined as a function of pH, at various copper concentrations, and at two salinities ($S = 3$ and $S = 32$). The behaviour of cystine and cysteine across a range of pH values was very similar; both compounds exhibited peak height responses which were highest at pH 8.0, and which diminished at lower pH values due to proton competition, and at higher pH values due to Cu(II) hydrolysis (Fig. 6). The peak potential of the Cu(I)–cysteine peak became more negative as the pH increased, as a result of complex stabilization with diminished proton competition.

The sensitivity for cysteine was greater than that for cystine at all pH values, but the difference was greatest at low pH (pH 6). Indeed, the peak for 9 nM cystine and 150 nM copper had disappeared at pH 6.2, whereas that for cysteine was still present, although at a much reduced sensitivity. This lower sensitivity of cystine is probably a reflection of the time taken for cystine to be reduced to cysteine, prior to complex formation with Cu(I). This difference in sensitivity could be used to differentiate between the two compounds when both are present in the same solution.

The sensitivity for cysteine was greater (by approximately 15%) in diluted ($S = 3$) than in undulated seawater at all pH values, probably because of less competition for Cu(I) by chloride ions, and for cysteine by calcium and magnesium ions. By varying the chloride concentration, it was shown that the effect of the chloride ions was the more important in controlling sensitivity: increasing the chloride concentration, in a stepwise manner from 0 to 0.2 M (as NaCl), in a background electrolyte of 0.01 M borate buffer, caused the cystine peak to diminish by 3% at 0.005 M, 10%

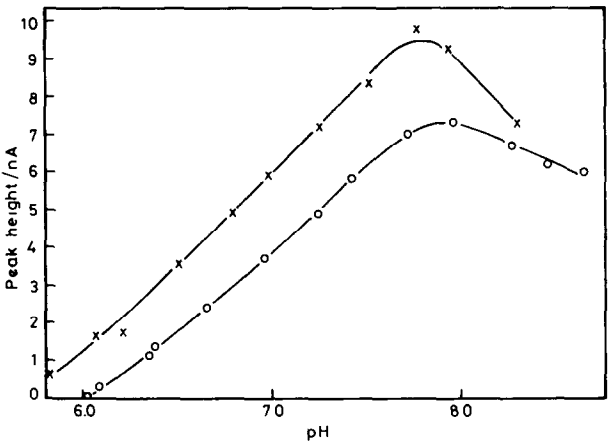

Fig. 6 Effect of pH on the DPCSV peak height obtained for 18 nM cysteine (×) and cystine (○) in seawater (pH 8.5, 150 nM Cu) after 60 s stirred collection at −0.15 V.

at 0.01 M, 23% at 0.02 M, 42% at 0.05 M, 57% at 0.10 M, and 61% at 0.2 M Cl^-. This decrease is caused by competitive complexation of Cu(I) by the chloride ions at the electrode surface.

Determination of detection limit

The relative standard deviation (SD) of 7 consecutive measurements of 4 nM cystine was 2.9%, giving a detection limit ($3 \times$ SD) of 0.4 nM ($T_{ads} = 60$ s whilst stirring, $E_{coll} = -0.15$ V in the presence of 150 nM Cu and 0.01 M borate buffer). For a collection time of 4 min the detection limit can be decreased further to 0.1 nM. This compares favourably with a detection limit calculation from Forsman's data [6] of 4.5 nM for a 60 s collection.

INTERFERENCES

A number of amino acids (ethionine, methionine, alanine, phenylalanine, hydroxyproline, glutamic acid, tyrosine) and purines (adenine, guanine, allantoin, uric acid, xanthine, hypoxanthine) were added to the experimental solution (seawater) to a concentration of 100 nM, and were found not to interfere with the formation of the cysteine–copper peak. Only some of the purines (xanthine, adenine, guanine, hypoxanthine) produced reduction peaks for the recommended conditions, but these were at E_p values which were sufficiently more positive not to interfere (these will be reported elsewhere [22]).

The presence of 1.0 mg/l of the surfactant Triton X-100 reduced the cystine peak response substantially by 50%, 2.0 mg/l causing a peak height reduction of 75%. Diminuition of the cystine/cysteine–copper complex peak was accompanied by a simultaneous increase in the "free" copper peak. The presence of surfactants present in natural waters can thus diminish the CSV sensitivity for cystine and cysteine. The sensitivity therefore has to be calibrated by standard additions to the sample.

ELECTRODE MECHANISM

Previously it has been proposed that the formation of a Cu(Hg) amalgam is required prior to the adsorption of the copper–cysteinate complex from aqueous solution [6]. However, at the adsorption potential used in the present experiments (–0.15 V), and in a background electrolyte containing chloride (seawater), copper amalgam is not formed. Instead, Cu(I) is generated continuously at the electrode surface, where it is stabilized both by the presence of chloride ions [16,17] and by cysteine. This means that the presence of amalgamated copper cannot be a pre-requisite for the adsorption of the copper(I)–cysteine complex. Futhermore, our experiments have shown that diminished sensitivity always coincided with the disappearance of the Cu(I)–Cu(0) peak, suggesting that the presence of dissolved Cu(I) at the electrode surface was required for the formation of the adsorbed cysteinate complex.

More recently the possibility has been suggested that a peak at ~ -0.4 V was caused by the reduction of a cupric dicysteinate complex; this complex was proposed to be formed on the electrode surface by reduction of a previously adsorbed mercuric–cysteinate complex [23]. However, our experiments in chloride media have shown the presence of a mercury–cysteinate reduction peak at -0.44 V in the absence of added copper (i.e. < 10 nM Cu), which is suppressed and superseded by a copper–cysteinate peak at -0.51 V when copper is added (Fig. 2). This second peak cannot be ascribed to the above-mentioned mechanism [23], as essentially Cu(II) is not present at the electrode surface at adsorption potentials < 0.0 V in chloride media, and as the formation constant for the Cu(II)–cysteinate complex is too small. Our data indicate that the copper related peak at -0.51 V is due to the reduction of a cuprous–cysteinate complex, and, analogously, that the peak at -0.44 V is due to an adsorbed mercurous–cysteinate complex.

The following electrode mechanism is therefore proposed for cysteine: during collection at -0.15 V Cu(I) is generated at the electrode surface:

$$\mathrm{Cu(II)} + e^- = \mathrm{Cu(I)}$$

This Cu(I) is stabilised by the formation and adsorption of a complex with cysteine (RS–H):

$$\mathrm{Cu(I)} + \mathrm{RS{-}H} = \mathrm{RS{-}Cu(I)} + \mathrm{H^+}$$

During the scan, copper is further reduced to Cu(0):

$$\mathrm{RS-Cu(I)} + \mathrm{Hg} + e^+ + \mathrm{H^+} = \mathrm{RS{-}H} + \mathrm{Cu(Hg)}$$

The mechanism is slightly more complicated for cystine as this compounds needs to be reduced to cysteine before copper complexation can take place: during application of the adsorption potential:

$$\mathrm{RSSR} + 2\ \mathrm{H^+} + e^- = 2\ \mathrm{RS{-}H}$$

This reaction is also thought to proceed without an applied potential as a result of a chemical reaction between cystine and mercury to form mercurous cysteinate [1,20]:

$$\mathrm{RSSR} + \mathrm{Hg} = \mathrm{Hg(RS)_2}$$

The formation of an adsorbed mercurous–cysteinate complex is inhibited when the copper concentration > the amino acid concentration, as then only the Cu(I) cysteinate reduction peak appears.

A value for the conditional stability (K'_{CuL}) for the complex of $\mathrm{Cu^+}$ with cysteine was calculated from the peak shift of the copper–cysteine peak (in seawater of pH 8.5) with respect to the free $\mathrm{Cu^+}$ peak (at pH 2.8). After correction for ion pairing with $\mathrm{Cl^-}$ (the main ion pair being $\mathrm{CuCl_2^-}$) a value of 18.5 was obtained for log K'_{CuL}, which compares well with a value of 20 estimated previously for log K_{CuL} [18] (the difference is caused by competition by $\mathrm{H^+}$, $\mathrm{Mg^{2+}}$ and $\mathrm{Ca^{2+}}$). The very great stability of these Cu(I) complexes explains the low copper concentration (< 10 nM) required to suppress the mercurous–cysteine peak in seawater.

Preliminary measurements using the given CSV procedures were carried out in filtered seawater originating in the Menai Straits and the Tamar estuary ($S = 32$ and 31 respectively). A broad peak was observed at -0.35 V, which could be removed by UV irradiation, but which was not due to cystine or cysteine as indicated by standard additions. These samples had not been obtained and stored properly to preserve amino acids, so this negative result does not indicate that these compounds were not present in these waters originally. The measurements did indicate that cystine (cysteine) could be determined in untreated seawater samples. A study is to be undertaken into the presence of Cu(I) forming organics (cystine, cysteine, and certain purines) in estuarine and coastal seawater.

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