

# Electrochemical study of a metallothionein modified gold disk electrode and its action on $\text{Hg}^{2+}$ cations

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## Abstract

A novel protein monolayer modified electrode has been prepared by the self-assembly of metallothionein (MT) at a gold disk electrode. The properties of MT in Tris–HCl buffer and in the monolayer are studied by using cyclic voltammetry and differential pulse voltammetry with a gold disk electrode. In the negative sweep, the voltammogram of MT in buffer shows two small peaks and different electrochemical behaviour from that at a mercury electrode.  $\text{Cd}^{2+}$  complexed to the thionein can easily be replaced by  $\text{Hg}^{2+}$  ions, and  $\text{Hg}^{2+}$  ions can firmly adsorb in the MT monolayer with a saturation coverage of  $(2.78 \pm 0.29) \times 10^{-10}$  mol  $\text{cm}^{-2}$ . This behaviour has been used to preconcentrate trace  $\text{Hg}^{2+}$  for its determination by cathodic stripping differential pulse voltammetry. The cathodic stripping peak current is proportional to  $\text{Hg}^{2+}$  concentration in the range of 0.15–3  $\mu\text{M}$  and the detection limit is ca. 0.08  $\mu\text{M}$  (16 ppb) with a 2 min open circuit accumulation step. The relative standard deviation is 7.2% at 0.4  $\mu\text{M}$   $\text{Hg}^{2+}$  concentration ( $n = 4$ ). At higher concentration the adsorption of  $\text{Hg}^{2+}$  exhibits a response similar to that expected for a Langmuir adsorption isotherm with the stability constant of  $(4.0 \pm 0.2) \times 10^5 \text{ M}^{-1}$ . © 2000 Elsevier Science S.A. All rights reserved.

**Keywords:** Chemically modified electrodes; Self-assembled monolayer; Metallothionein; Gold disk electrode; Determination of Hg(II); Cathodic stripping voltammetry

## 1. Introduction

Metallothioneins (MT) are a group of low molar mass and cysteine rich proteins. Their cysteine content is high, up to 30 mol% of the amino acids, thus they possess a selective capacity to bind metal ions such as zinc, cadmium, mercury and copper through metal–thiolate bonds [1]. The binding capabilities play an important biological role in the storage and transport of essential metal ions, and the detoxification or sequestration of toxic metal ions [2]. In the metal-saturated thionein, the cysteine residues generally bind seven bivalent ions. Furey et al. [3] calculated the 2.3 Å resolution electron density map of MT and proposed that the seven metal sites are distributed in two do-

mains ( $\alpha$  and  $\beta$ ) of MT and that each domain is globular with a diameter of 15–20 Å. Stillman et al. [4] studied the mercury-binding properties of rabbit liver MT using absorption and magnetic circular dichroism spectroscopies. They then reported the structures of the Cd, Hg and Zn thiolate clusters based on an EXAFS study [5]. In order to obtain a better understanding of the biological functions of MT, many different analytical methods such as X-ray diffraction, NMR, ELISA and RIA have been used to study the structure and properties of MT, particularly the complexation of metal ions [5–10]. These contributions have been gathered in monographs [11,12].

In recent years, electroanalytical methods such as direct current polarography, cyclic voltammetry, square wave voltammetry and differential pulse (DP) polarography have also been used by a few groups to study MTs [8,13–21]. In particular a series of electrochemical studies have been reported by the group of Rodríguez

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[22–32]. Many studies on the electrochemical behaviour of several Cd, Zn thioneins have been published [15–17,22–31]. Most of these reports used mercury as a working electrode, thus the polarograms of MT exhibited one peak (at  $-0.390$  V vs. Ag | AgCl | KCl (sat.) at pH 7.0 [22,23]) which was attributed to the oxidation of the mercury electrode in the presence of the peptide acting as a chelating agent previously complexed with cadmium. This peak has been used for the quantitative determination of Cd and Zn-thioneins [32].

Several solid electrodes have been used as working electrodes to study the voltammetric characteristics of MT [18,33]. Sestakova et al. [18] compared the difference of electrochemical behaviour of MT at mercury and carbon electrodes and reported that metals such as Zn and Cd in the MT structure were substituted by Hg from the mercury electrode surface. Limson et al. [33] used cobalt(II) tetrasulfonated phthalocyanine modified carbon paste and glassy carbon electrodes to study the electrochemical behaviour of MT and obtained an oxidation peak for cysteine at  $+0.82$  V (vs. Ag | AgCl) in acidic media.

The fact that MT contains a large number of cysteine groups leads us to believe that it may firmly adsorb on gold surfaces through Au–thiolate bonds. Therefore, we report here, for the first time, the electrochemistry of self-assembled MT protein at a gold disk electrode. The selective binding of self-assembled MT on gold surfaces to metal ions provides an effective determination method of trace  $\text{Hg}^{2+}$ . This work studies the binding capacity of MT monolayers to  $\text{Hg}^{2+}$  cations and the adsorption stability constant of  $\text{Hg}^{2+}$  on MT monolayer.

## 2. Experimental

### 2.1. Reagents and materials

Metallothionein, with a cadmium content of 6.7% and zinc content of 0.5%, from rabbit liver was obtained from Sigma (St. Louis, USA) Tris(hydroxymethyl)-aminomethane hydrochloride and tris(hydroxymethyl)aminomethane (reagent grade) were purchased from Sigma for preparing 0.025 M pH 7.4 Tris–HCl buffer solutions. Electrolyte solutions were prepared with deionised water from a Milli-Q purification system. Other reagents were of analytical reagent grade. All experimental solutions were prepared with 0.025 M pH 7.4 Tris–HCl buffer solution.

The bulk gold electrodes were prepared by sealing polycrystalline gold wires ( $>99.99\%$ , Goodfellow, Cambridge, UK) in glass tubes using a gas + air flame, followed by attaching copper wires with tin solder for electrical connection to the back of the electrode. The gold electrodes were abraded with successively finer

grades of SiC paper to expose gold disks of macroscopic area about  $7 \times 10^{-3}$  cm<sup>2</sup>. The roughness factor of bare polished gold electrodes was  $2.1 \pm 0.3$ , which was determined from the ratio of the real to the geometric area. The real area was obtained by measuring the charge that evolved during oxidation of the superficial Au in 1.0 M  $\text{H}_2\text{SO}_4$  [34].

### 2.2. Preparation of MT self-assembled monolayer

The gold disk electrodes were first polished to a mirror-like finish with 0.3  $\mu\text{m}$ , followed by 0.05  $\mu\text{m}$  alumina slurry on microcloth pads (Buehler). After removal of the trace alumina from the surface, by rinsing with water and brief cleansing in an ultrasonic bath, they were immersed in Tris–HCl buffer containing  $1.0 \times 10^{-5}$  M MT for over 48 h for self-assembly of MT on the gold surface. The modified MT electrodes were kept in Tris–HCl buffer following formation.

### 2.3. Electrochemical measurements

All electrochemical measurements were performed in a Faraday cage with a BAS-100B electrochemical analyser (Bioanalytical Systems Inc., USA) and a conventional three-electrode system comprising a bare or modified working electrode, a platinum wire as the auxiliary and an Ag | AgCl | 3.0 M NaCl electrode (from BAS) as the reference. All potentials are reported versus this reference at room temperature (r.t.) ( $\sim 20^\circ\text{C}$ ). All experimental solutions were deaerated with nitrogen for at least 10 min, and maintained under a nitrogen atmosphere during the course of the experiment. The differential pulse voltammetric experiments were performed under the conditions of 20 mV s<sup>-1</sup> scan rate, 20 mV pulse amplitude as well as 50 ms pulse and 17 ms sample width.

## 3. Results and discussion

### 3.1. Voltammetric behaviour of MT at a bare gold electrode

According to results reported previously, the DP polarograms of Cd- and Zn-MT at dropping mercury working electrodes at pH 7.5 exhibited three peaks at  $-0.40$ ,  $-0.83$  and  $-1.10$  V (vs. Ag | AgCl) [22,23]. These peaks have been attributed to the oxidation of the mercury electrode in the presence of free thiol groups of MT [22,23] and to the reduction of cadmium ( $E_{p,\text{CdMT}}$ ) and zinc ( $E_{p,\text{ZnMT}}$ ) complexes of MT, respectively. The reduction of Cd- and Zn-MT appeared at more negative values than those corresponding to the free cations,  $-0.58$  and  $-1.01$  V for  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$ , respectively.

In our studies, the cyclic voltammogram of a bare gold electrode in pH 7.4 Tris–HCl buffer exhibits a large hydrogen wave at potentials more negative than  $-0.85$  V, thus the reduction peaks of both zinc complexes and zinc cations cannot be observed. The cyclic voltammogram of a  $10\ \mu\text{M}$  MT solution at pH 7.4 exhibits two small peaks at ca.  $-0.37$  and  $-0.70$  V for the cathodic processes. The peak at  $-0.37$  V is very poorly defined and its potential is slightly more positive than that of the mercury electrode oxidation [22,23], thus it may be attributed to the oxidation of Au in the presence of thiol groups. The peak at  $-0.70$  V is proposed to be due to reduction of CdMT at the Au electrode. It appears at a more positive potential than those at a mercury electrode, but is yet  $0.31$  V more negative than the reduction potential of  $-0.39$  V for free  $\text{Cd}^{2+}$  cations at the Au electrode.

Upon addition of cadmium cations to  $10\ \mu\text{M}$  MT solutions, a new shoulder at ca.  $-0.69$  V, between the peaks corresponding to  $\text{Cd}^{2+}$  reduction and to CdMT

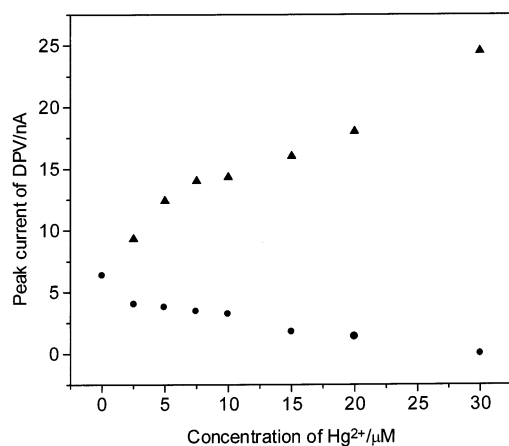


Fig. 1. Plots of reduction peak currents of CdMT (●) and HgMT (▲) versus  $\text{Hg}^{2+}$  concentration in 0.025 M pH 7.4 Tris–HCl buffer including  $10\ \mu\text{M}$  MT.

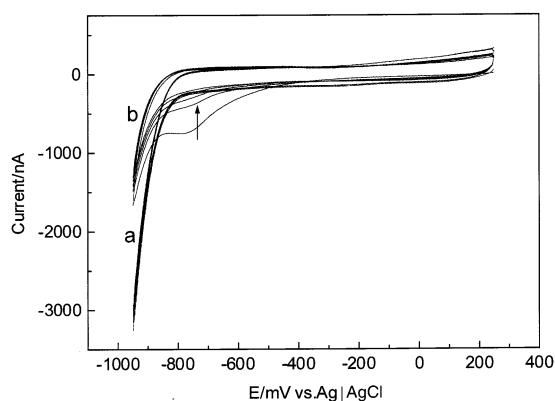


Fig. 2. Continuous cyclic voltammograms of bare (a) and MT modified gold electrode (b) in 0.025 M pH 7.4 Tris–HCl buffer at  $100\ \text{mV s}^{-1}$ .

reduction, appeared at mercury electrodes, proposed to be due to reduction of a different form of CdMT [26]. At a Au electrode, the DP voltammograms of  $10\ \mu\text{M}$  CdMT solution show only a small peak for CdMT at ca.  $-0.70$  V and a reduction peak for free  $\text{Cd}^{2+}$  at a potential of  $-0.39$  V when low concentrations ( $< 10\ \mu\text{M}$ ) of  $\text{Cd}^{2+}$  are added. These differences possibly result from strong adsorption of the uncomplexed thiols of MT at the Au electrode surface, which prevents further binding of metal cation to the protein. The reduction peak current and width of the peak for free  $\text{Cd}^{2+}$  ion reduction increase upon addition of further  $\text{Cd}^{2+}$ . At high  $\text{Cd}^{2+}$  concentrations, this peak is very wide so that it covers the small reduction peak of CdMT.

When  $2.5\ \mu\text{M}$   $\text{Hg}^{2+}$  is added to the  $10\ \mu\text{M}$  MT solution, the DP voltammogram exhibits a new reduction peak at a potential of  $-0.32$  V, and the reduction peak for CdMT is decreased greatly. This new peak differs from that for the reduction of free  $\text{Hg}^{2+}$  at a bare gold electrode: the latter shows a DPV peak at  $-0.153$  V. The new peak is attributed to the reduction of HgMT produced from the cation exchange between  $\text{Cd}^{2+}$  in MT and  $\text{Hg}^{2+}$  in solution. Upon addition of  $\text{Hg}^{2+}$ , the reduction peak current of HgMT increases and the reduction peak current of CdMT decreases (shown in Fig. 1). These phenomena are consistent with the stability constants for the complexes ( $K'_{\text{HgMT}} > K'_{\text{CdMT}}$ ) [22,23]. When the concentration of  $\text{Hg}^{2+}$  is higher than  $20\ \mu\text{M}$ , the reduction peak current for CdMT tends towards zero; almost all  $\text{Cd}^{2+}$  ions in MT are substituted by  $\text{Hg}^{2+}$  ions. At the same time the reduction peak current for free  $\text{Hg}^{2+}$  increases so that the reduction peak of HgMT is obscured.

### 3.2. Cyclic voltammetry of MT modified electrode

When a Au electrode was immersed in  $10\ \mu\text{M}$  MT solution for some time (e.g. 48 h for Fig. 2), its cyclic voltammogram upon transfer to a solution containing Tris–HCl buffer alone showed a reduction peak at  $-0.77$  V. This peak is proposed to result from the reduction of adsorbed CdMT. The peak current is about 25 times that of  $10\ \mu\text{M}$  MT in buffer, indicating adsorption/pre-concentration of MT on the electrode surface. The reduction potential of this adsorbed CdMT is more negative than that observed for CdMT in solution. With increasing immersion time in MT solution, the peak current of the first sweep increases and then tends towards a constant value.

The reduction peak current of CdMT adsorbed on the surface decreases rapidly and tends to the base line of the cyclic voltammogram (see curve b in Fig. 2) upon continuous cyclic sweeps. Comparing the cyclic voltammogram of the CdMT adsorbed electrode with that of a bare gold electrode (curve a in Fig. 2) in Tris–HCl

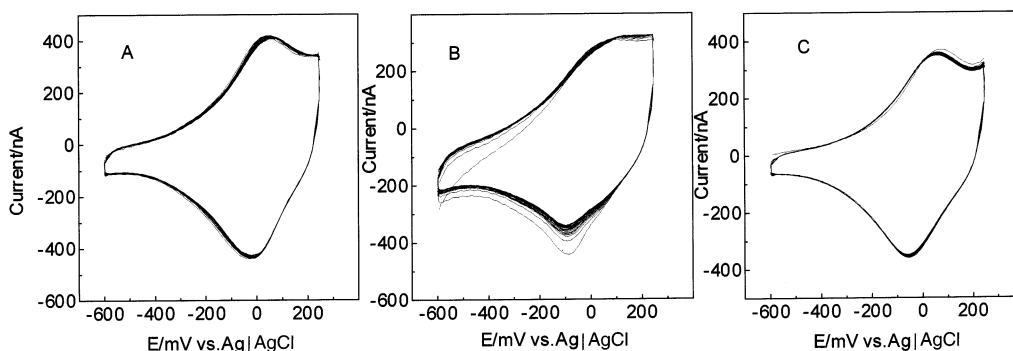


Fig. 3. Continuous cyclic voltammograms of an electrodeposited Hg–Au layer (A), adsorbed  $\text{Hg}^{2+}$  at a Au electrode (B) and a  $\text{Hg}^{2+}$  saturated MT-modified Au electrode (C) in 0.025 M pH 7.4 Tris–HCl buffer at  $100 \text{ mV s}^{-1}$ .

buffer, the capacitive current is lower and the overpotential of the hydrogen wave becomes more negative in the former. These phenomena seem to indicate that MT has adsorbed firmly at the Au electrode. The oxidation peak of cadmium is not observed in the positive direction of the cyclic sweep. Thus, when  $\text{Cd}^{2+}$  ions in CdMT adsorbed on the surface are reduced, the reduction product, cadmium, may dissociate from the MT complex and diffuse into the bulk solution.

### 3.3. Electrochemistry of $\text{Hg}^{2+}$ at a bare gold electrode

It has been noted that bare Au electrodes show considerable memory effects and poor reproducibility for both positive and negative sweeps in solutions containing  $\text{Hg}^{2+}$  [35]. After  $\text{Hg}^{2+}$  is reduced at a bare Au electrode, a layer of Au–Hg is formed, which makes the reduction current of  $\text{Hg}^{2+}$  increase non-linearly with increasing  $\text{Hg}^{2+}$  concentration. In pH 7.4, Tris–HCl buffer without the presence of  $\text{Hg}^{2+}$ , the cyclic voltammogram of the Au–Hg layer, which is formed previously by cyclic sweeps between  $-0.35$  and  $+0.3$  V in  $\text{Hg}^{2+}$  solution, shows redox peaks at ca.  $-0.02$  and  $+0.045$  V corresponding to the reduction of  $\text{Hg}^{2+}$  and the oxidation of Hg in the Au–Hg layer (Fig. 3A). Furthermore, these peaks are very stable to continuous cyclic sweeps. After a fresh bare Au electrode is immersed in the buffer containing  $2.0 \mu\text{M}$   $\text{Hg}^{2+}$  for some time (e.g. 10 min shown in Fig. 3B) redox peaks of  $\text{Hg}^{2+}/\text{Hg}$  can also be seen in buffer without  $\text{Hg}^{2+}$ , indicating  $\text{Hg}^{2+}$  can adsorb on the Au surface. These peaks are unstable, they decrease with continuous cyclic sweeps, and the peak separation is larger than that of the Au–Hg layer. When the accumulation time at the open circuit of a bare electrode in buffer solution containing less than  $1.5 \mu\text{M}$   $\text{Hg}^{2+}$  is increased, the peak currents of DP voltammograms of  $\text{Hg}^{2+}$  solution increase. However, there is no simple relationship between the peak current and the open circuit time and the reduction wave becomes more complex. After a longer open circuit time, the DP voltammogram shows

two reduction peaks. With increasing  $\text{Hg}^{2+}$  concentration from  $1.5 \mu\text{M}$ , two reduction peaks also occur, their peak currents showing poor reproducibility.

### 3.4. Action of MT modified electrode on $\text{Hg}^{2+}$

When a MT modified electrode is immersed in buffer solution containing  $\text{Hg}^{2+}$  for some time the cyclic voltammogram of this electrode after rinsing and immersion in buffer alone shows redox peaks of  $\text{Hg}^{2+}/\text{Hg}$  at potentials slightly more negative than those at the Au–Hg layer. This indicates substitution of  $\text{Hg}^{2+}$  for  $\text{Cd}^{2+}$  in the MT monolayer. The peak-to-peak separation is slightly larger than that of  $\text{Hg}^{2+}/\text{Hg}$  at the Au–Hg layer. The redox peaks are very stable upon continuous cyclic sweeps (Fig. 3C for 2 min immersion time in  $2.0 \mu\text{M}$   $\text{Hg}^{2+}$  solution). The reduction peak potential of HgMT in the monolayer is ca.  $0.30$  V more positive than that in solution, thus HgMT in the monolayer is easier to reduce than that in solution and slightly more difficult to reduce than the oxidation product of the Au–Hg layer.

With increasing scan rate, the peak-to-peak separation of cyclic voltammograms of  $\text{Hg}^{2+}$  saturated MT modified electrodes in Tris–HCl buffer increase and both reduction and oxidation peak currents increase linearly with the same slope (Fig. 4), indicating a surface-controlled electrode process. Thus, the  $\text{Hg}^{2+}$  ions adsorb firmly on the surface of the MT modified electrode by the complexation by cysteine groups of MT. From the peak area of cyclic voltammograms, the surface coverage of  $\text{Hg}^{2+}$  can be estimated. Considering the roughness of the electrode surface, the average surface coverage of  $\text{Hg}^{2+}$  at a saturated adsorption electrode is  $(2.78 \pm 0.29) \times 10^{-10} \text{ mol cm}^{-2}$  for 12 determinations.

The MT molecule is composed of two globular domains ( $\alpha$  and  $\beta$ ). The diameter of each globular domain is  $15\text{--}20 \text{ \AA}$ . The two domains are linked at residues 30 and 31 to form a molecule with the maximum linear dimension of  $30\text{--}35 \text{ \AA}$  [3]. If the MT monolayer is

adsorbed onto gold surfaces through the cysteine groups in a single domain (case 1) or in both domains (case 2), then, the theoretical coverage for a complete MT monolayer is between  $5.2 \times 10^{-11}$  and  $9.4 \times 10^{-11}$  mol  $\text{cm}^{-2}$  for case 1 or between  $1.7 \times 10^{-11}$  and  $2.4 \times 10^{-11}$  mol  $\text{cm}^{-2}$  for case 2. The coverage of  $\text{Hg}^{2+}$  at the MT modified electrode is about 2.95–5.3 (average value 4.1) times and 11.8–16.2 (average value 14.0) times the theoretical coverage for these two cases, respectively. Usually, one molecule of MT can complex seven metal ions, and four ions are in the  $\alpha$  domain and the others in the  $\beta$  domain [3]. Thus, case 2 is impossible. The average value of 4.1 times indicates that one MT molecule in the monolayer can complex ca. four  $\text{Hg}^{2+}$  ions. As is well known, the adsorption of thiol molecules on the Au surface forms a self-assembled layer by the formation of Au–S bonds. Thus, the MT

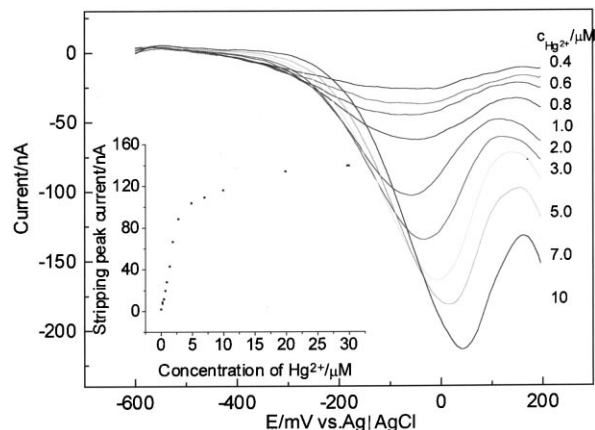


Fig. 6. Cathodic stripping differential pulse voltammograms of  $\text{Hg}^{2+}$  using 120 s open circuit preconcentration at a MT-modified electrode in 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 5.0, 7.0 and 10  $\mu\text{M}$   $\text{Hg}^{2+}$ . Inset: plot of stripping peak current versus  $\text{Hg}^{2+}$  concentration.

molecule binds to the Au surface to form Au–S bonds by cysteines in a domain, possibly the  $\beta$  domain, and another domain is exposed to the solution and can complex four  $\text{Hg}^{2+}$  ions by Hg–S bonds. The complexation may be used to preconcentrate  $\text{Hg}^{2+}$  ions for the determination of trace  $\text{Hg}^{2+}$ .

### 3.5. Determination of trace $\text{Hg}^{2+}$ by cathodic stripping differential pulse voltammetry

In solutions containing trace  $\text{Hg}^{2+}$ , the reduction peak current of cathodic stripping differential pulse voltammograms (CSDPV) increases linearly with increasing immersion time for the open circuit accumulation of  $\text{Hg}^{2+}$  ions at the MT modified electrode. After a longer immersion time, the stripping peak current tends to a constant value, indicating that the adsorption of  $\text{Hg}^{2+}$  or the formation of HgMT in the MT layer reaches saturation. The time for reaching the constant value is different for various concentrations of  $\text{Hg}^{2+}$ . The lower the  $\text{Hg}^{2+}$  concentration, the longer is this time. Fig. 5 shows the curves of CSDPV in a solution containing 2.0  $\mu\text{M}$   $\text{Hg}^{2+}$ . From the inset in Fig. 5 an estimate of about 140 s is obtained for saturated complexation of  $\text{Hg}^{2+}$ . With increasing preconcentration time, the peak shifts slightly to more positive potentials. All reduction peak potentials of HgMT on the surface are ca. 0.30 V more positive than those in solution, indicating that the reduction of  $\text{Hg}^{2+}$  in the MT layer is easier than that in solution or that their electrode processes are different.

When the open circuit time is set at 2 min, for CSDPV of MT modified electrodes, a reduction peak for HgMT is observed for  $\text{Hg}^{2+}$  concentrations of more than 0.08  $\mu\text{M}$ . The stripping peak current increases linearly with increasing  $\text{Hg}^{2+}$  concentration from 0.15 to 3  $\mu\text{M}$  (Fig. 6). The coefficient of variation

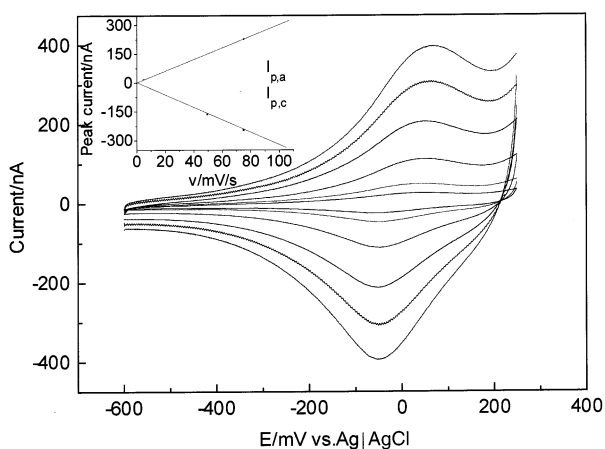


Fig. 4. Cyclic voltammograms of a  $\text{Hg}^{2+}$  saturated MT-modified Au electrode in 0.025 M pH 7.4 Tris–HCl buffer at 5, 10, 25, 50, 75 and 100  $\text{mV s}^{-1}$ . Inset: plots of peak current versus scan rate.

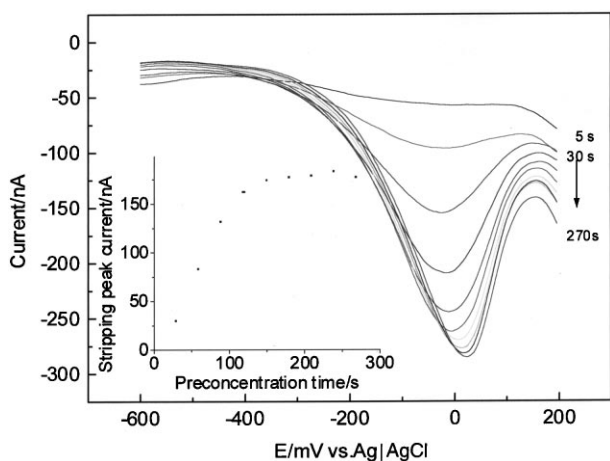


Fig. 5. Cathodic stripping differential pulse voltammograms of  $\text{Hg}^{2+}$  at a MT-modified electrode using open circuit preconcentration in 2.0  $\mu\text{M}$   $\text{Hg}^{2+}$  for 5, 30, 60, 90, 120, 150, 180, 210, 240 and 270 s. Inset: plot of stripping peak current versus open circuit accumulation time.

for this calibration curve is 0.994. When the concentration of  $\text{Hg}^{2+}$  is more than 3  $\mu\text{M}$ , the peak current tends to a constant value. With an increase in open circuit time, a narrower linear range is observed because of saturation of the protein binding sites. The relative standard deviation of stripping peak currents in the Tris–HCl solution containing 0.4  $\mu\text{M}$   $\text{Hg}(\text{OAc})_2$  is 7.2% for four determinations.

### 3.6. Adsorption equilibrium constant of $\text{Hg}^{2+}$ on MT monolayer

At higher  $\text{Hg}^{2+}$  concentrations the adsorption of  $\text{Hg}^{2+}$  with an open circuit time of 2 min tends to saturation. The plot of the stripping peak current versus  $\text{Hg}^{2+}$  concentration shows a response similar to that expected for a Langmuir adsorption isotherm. Therefore, the process of complexation can be considered as an adsorption equilibrium at the modified electrode|solution interface. The adsorption equilibrium constant,  $b$ , can be determined according to the Langmuir equation (Eq. (1)):

$$\theta = bc/(1 + bc) \quad (1)$$

Here  $\theta$  is the ratio of the surface concentration  $\Gamma$  of  $\text{Hg}^{2+}$  at concentration  $c$  to its maximum surface concentration  $\Gamma_{\text{max}}$ . Thus,

$$c/\Gamma = 1/(b\Gamma_{\text{max}}) + c/\Gamma_{\text{max}} \quad (2)$$

Assuming that the stripping peak current is proportional to the surface concentration of  $\text{Hg}^{2+}$ , Eq. (2) may be written as

$$c/I_p = 1/(bI_{p,\text{max}}) + c/I_{p,\text{max}} \quad (3)$$

A plot of  $c/I_p$  versus  $c$  is shown in Fig. 7, which yields good linearity, with a correlation coefficient of 0.9996. From the slope and intercept of the line, an adsorption equilibrium constant of  $(4.0 \pm 0.2) \times 10^5 \text{ M}^{-1}$  can be

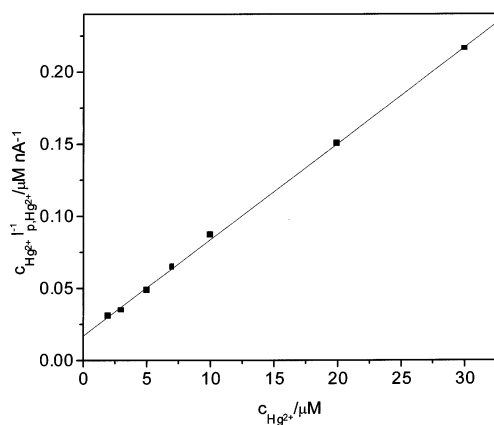


Fig. 7. Analysis of the cathodic stripping peak current and  $\text{Hg}^{2+}$  concentration at a MT-modified electrode using the Langmuir equation and the data of Fig. 6.

obtained. Thus, the Gibbs energy change due to the adsorption is  $-32.0 \text{ kJ mol}^{-1}$  calculated from  $\Delta G^0 = -RT \ln b$ .

## 4. Conclusions

The electrochemical behaviour of MT at a Au electrode differs slightly from that observed at a mercury electrode. Upon addition of  $\text{Hg}^{2+}$  to MT solutions, a reduction peak for HgMT appears and the reduction peak of CdMT decreases. It is proposed that  $\text{Cd}^{2+}$  cations complexed to the MT are substituted by  $\text{Hg}^{2+}$ . In addition, MT can adsorb firmly on Au electrode surfaces via the formation of Au–thiolate bonds with the cysteine in MT domains to form a self-assembled layer.  $\text{Cd}^{2+}$  cations in the MT layer can also be replaced by  $\text{Hg}^{2+}$  cations. Each MT molecule in the monolayer can complex approximately four  $\text{Hg}^{2+}$  cations. This exchange process has been used in an accumulation step at open circuit potentials, to preconcentrate  $\text{Hg}^{2+}$  from solution for the determination of trace  $\text{Hg}^{2+}$ . The formation of HgMT complexes at the MT modified electrode follows a Langmuir-type adsorption isotherm.

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## References

- [1] D.A. Fowle, M.J. Stillman, *J. Biomol. Struct. Dyn.* 14 (1997) 393.
- [2] M.J. Stillman, *Coord. Chem. Rev.* 114 (1995) 461.
- [3] W.F. Furey, A.H. Robbins, L.L. Clancy, D.R. Winge, B.C. Wang, C.D. Stout, *Science* 231 (1986) 704.
- [4] W.H. Lu, M.J. Stillman, *J. Am. Chem. Soc.* 115 (1993) 3291.
- [5] D.T. Jiang, S.M. Heald, T.K. Sham, M.J. Stillman, *J. Am. Chem. Soc.* 116 (1994) 11004.
- [6] A. Yoshida, B.E. Kaplan, M. Kimura, *Proc. Natl. Acad. Sci. USA* 70 (1979) 486.
- [7] C. Hogstrand, C. Haux, *Toxicol. Appl. Pharmacol.* 103 (1990) 56.
- [8] F.A. Armstrong, *Probing Metalloproteins by Voltammetry*, in: M.J. Clarke, et al. (Eds.), *Structure and Bonding*, vol. 72, Springer–Verlag, Berlin, 1990.
- [9] *Talanta, Special Issue on Metallothioneins* 46 (1998).
- [10] S. Duquesne, M.A. Janquin, C. Hogstrand, *Fresenius J. Anal. Chem.* 352 (1995) 389.

- [11] J.H.R. Kagi, M. Nordberg (Eds), *Metallothionein*, Birkhäuser-Verlag, Basel, 1979.
- [12] M.J. Stillman, C.F. Shaw III, K.T. Suzuki (Eds.), *Metallothioneins, Syntheses, Structure and Properties of Metallothioneins, Phytochelatins and Metal-thiolate Complexes*, VCH, New York, 1992.
- [13] M.T. Stankovich, A.J. Bard, *J. Electroanal. Chem.* 75 (1977) 497.
- [14] C. Hogstrand, C. Haux, *Toxicol. Appl. Pharmacol.* 103 (1990) 56.
- [15] S. Duquesne, M.A. Janquin, Hogstrand, *Fresenius J. Anal. Chem.* 354 (1995) 589.
- [16] B. Raspor, J. Pavicic, *Fresenius J. Anal. Chem.* 354 (1996) 529.
- [17] R. Wagemann, J.G. Dick, J.F. Klaverkamp, *Intern. J. Environ. Anal. Chem.* 54 (1994) 147.
- [18] I. Sestakova, D. Míhlova, H. Vodickova, P. Mader, *Electroanalysis* 7 (1995) 237.
- [19] M. Fedurco, I. Sestáková, *Bioelectrochem. Bioenerg.* 40 (1996) 223.
- [20] M. Studnicková, J. Turánek, H. Zabrsová, M. Krejci, M. Kysel, *J. Electroanal. Chem.* 421 (1997) 25.
- [21] M. Erk, B. Raspor, *Anal. Chim. Acta* 360 (1998) 189.
- [22] J. Mendieta, J. Chivot, A. Munoz, A.R. Rodríguez, *Electroanalysis* 7 (1995) 663.
- [23] A. Munoz, A.R. Rodríguez, *Electroanalysis* 7 (1995) 670.
- [24] C. Ruiz, J. Mendieta, A.R. Rodríguez, *Anal. Chim. Acta* 305 (1995) 285.
- [25] O. Nieto, A.R. Rodríguez, *Bioelectrochem. Bioenerg.* 40 (1996) 215.
- [26] C. Ruiz, A.R. Rodríguez, *Anal. Chim. Acta* 325 (1996) 43.
- [27] C. Harlyk, G. Bordin, O. Nieto, A.R. Rodríguez, *Electroanalysis* 8 (1995) 473.
- [28] O. Nieto, G. Hellemans, G. Bordin, M. De ley, A.R. Rodríguez, *Talanta* 46 (1998) 315.
- [29] C. Harlyk, G. Bordin, O. Nieto, A.R. Rodríguez, *J. Electroanal. Chem.* 446 (1998) 139.
- [30] C. Harlyk, G. Bordin, O. Nieto, A.R. Rodríguez, *J. Electroanal. Chem.* 451 (1998) 267.
- [31] C. Harlyk, G. Bordin, O. Nieto, A.R. Rodríguez, *J. Electroanal. Chem.* 458 (1998) 199.
- [32] A. Munoz, A.R. Rodríguez, *Analyst* 120 (1995) 529.
- [33] J. Limson, T. Nyokong, *Electroanalysis* 9 (1997) 255.
- [34] H.X. Ju, D. Leech, *Phys. Chem. Chem. Phys.* 1 (1999) 1549.
- [35] N.Y. Stojko, K.Z. Brainina, C. Faller, G. Henze, *Anal. Chim. Acta* 371 (1998) 145.