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The Use of Bimetallic Au(Cu)-Coated Microelectrodes for Improved Detection of Cystine

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Abstract: Au shell – (Au-Cu) core coatings on carbon microdisc electrodes (30 μm diameter) have been prepared by a two-step technique whereby Cu particles electrodeposited onto carbon supports (first step) had their surface layers replaced by Au (second step). The latter has been achieved by means of spontaneous partial replacement of the non-precious metal deposits of Cu by Au upon their immersion in the chlorolaurate-based solution: $3\ Cu/C + 2\ AuCl_4^- \longrightarrow 2\ Au\ (Cu)/C + 3\ Cu^{2+} + 8\ Cl^-$. The Au-Cu coated microelectrodes, μAu(Cu)/C, were subsequently used for voltammetric determination of cystine and their performance compared to a bulk Au RDE. The voltammetric picture was similar at both electrodes. A peak corresponding to adsorbed cystine oxidation at ca. +1.2 V vs. Ag/AgCl and a plateau at ca. +1.6 V corresponding to cystine oxidation from the bulk solution under mass transfer control, were recorded. The latter permitted an estimate of the apparent number of electrons associated with cystine oxidation which were calculated to be around 5 in both cases. However, the peak current was found to be much higher at the Au-Cu microelectrodes, even after correction for increased roughness was taken into account. This increase is attributed to an electronic effect of Cu on Au which decreases the affinity of the latter for excessive oxide formation and/or poisonous carbonaceous intermediates of cystine oxidation. Both the peak and plateau currents varied linearly with the concentration range of $1 \times 10^{-5} – 1 \times 10^{-4}$ M cystine and, in the former case, with a detection limit (3σ) estimated to be about 2×10^{-6} M.

Keywords: Microelectrodes; Bimetallic particles; Gold; Cystine determination.

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Introduction

Cystine is one of the few aminoacids that is electroactive in its uderivatised form on C, Pt and Au electrodes. Its oxidation proceeds via either a 5, 6 or 10e^- loss resulting in sulfone, sylfinyl sulfone or cysteic acid, respectively [1-3].

For direct voltammetric or amperometric detection of organics using bare electrodes, high signal magnitude and stability are sought. This is particularly important in the case of microelectrodes where their advantages (enhanced mass transfer rates, low ohmic losses, high S/N ratios) are often offset by low signals and contamination problems [4].

Au has been the electrode of choice for direct amperometric and voltammetric determination of cystine since the oxidation of the latter occurs at relatively low potential values and Au has a wide potential window. The voltammetric picture is however complicated [1] and is proven to depend on electrode/surface history [5] that affects the adsorption of the reactant and its intermediates. Furthermore, early attempts to use bare Au microelectrodes for the determination of cystine were not very successful since they were characterized by unusually small signal-to-background ratios [6]. There is therefore the need for improving the catalytic activity of Au-based microelectrodes towards cystine oxidation and coupling Au with another metal that affects its morphology and electronic properties could be a route worth investigating.

During the last decade an alternative method for the introduction of a noble metal into a bimetallic system has been developed. The method is based on the spontaneous electroless-galvanic replacement of surface layers of a non-noble metal, Me (where Me: Pb, Cu, Fe, Co, and Ni) by a noble metal, such as Pt, Au, Pd, or Ir, upon immersion of the former in a solution of metal ions of the latter. For example, in the case of Cu and Pt or Au:

$$2\ Cu + PtCl_6^{2-} \longrightarrow Pt + 6\ Cl^- + 2\ Cu^{2+} \quad (1)$$

$$3\ Cu + 2\ AuCl_4^- \longrightarrow Au + 8\ Cl^- + 3\ Cu^{2+} \quad (2).$$

The method was first applied by Adzic and co-workers to underpotentially deposited (UPD) Cu monolayers [7-9] and Cu and Pb bulk deposits by Kokkinidis and co-workers [10-11]. It has since been extended to other transition metals (Fe, Co, Ni) and substrates (glassy carbon) by Sotiropoulos and co-workers [12-17]. Initial experiments with such Pt(M) catalysts formed on flat glassy carbon substrates where M had been electrodeposited, showed encanced activity towards methanol electrooxidation (and for some of them towards oxygen

reduction too) [15-17]. Tri-metallic PtAu(Cu) coatings were also prepared and tested in borohydride oxidation [18]. However, such multi-metallic systems have not yet been tried as modified electrodes in electroanalysis.

The aim of the present work has been the development of modified microelectrodes that show enhanced currents and improved signal stability for the voltammetric/amperometric detection of cystine. Its objectives have been: (a) to prepare bimetallic Au(Cu) coatings on carbon microdisc electrodes, consisting of a Au shell and a bimetallic Au-Cu core [12-18] (Au(Cu)/μC electrodes); (b) to study the voltammetry of cystine oxidation at modified Au(Cu)/μC microelectrodes and, for comparison, Au(Cu)/Glassy Carbon (GC) RDEs; (c) to identify conditions for cystine determination at these electrodes**.**

Experimental

Electrodes

Carbon Microdisc Electrodes (μC). The C microdisc electrodes were prepared by sealing the corresponding C microfibres (30 μm diameter; WPI Inc., US) into borosilicate capillary tubes (WPI Inc., USA) with a two-component epoxy resin glue (RS Components, UK). Thorough polishing of the assembly on emery polishing paper in a direction vertical to its axis removed the excess glue from the tip and revealed a microelectrode disc. The microdisc radius was confirmed to be within 5% of its nominal value by the reversible reduction of ferricyanide (Aldrich) from its 5mM solution in 0.1 M NaOH.

Glassy Carbon (GC) and Gold (Au) Rotating Disc Electrodes (RDEs). GC (3 mm diameter) and Au (2 mm diameter) RDEs, used for comparative macro-electrode experiments, were controlled by a Taccusel EDI101T motor.

Au-Cu Modified Carbon Microelectrodes, Au(Cu)/μC, and Glassy Carbon RDEs, Au(Cu)/GC. Electrodeposition of Cu on the μC and GC RDE electrode substrates was carried out from 0.1 M $HClO_4$ + 0.01 M $CuSO_4$ deaerated solutions at –0.40 V vs. Ag/AgCl (3 M NaCl). The total charge density passed in the case of the GC RDE macroelectrode was 340 mC cm^{-2} range, corresponding to an estimated Cu film thickness of 125 nm (based on the bulk density of Cu and assuming a uniform deposit, limited on the GC substrate area).

The total charge used in the case of the μC microelectrode was 15 μC (corresponding to 2122 mC cm^{-2} of original μC substrate), to ensure a significant final Au(Cu) content. The Cu-coated macro- and micro-electrodes were then immersed in a 0.1 M HCl + 10^{-3} M $AuCl_3$ solution for 30 min so that spontaneous Cu replacement by Au occurred.

Chemicals, Reagents, Stock and Standard Solutions. $HClO_4$ from Riedel, (puriss p.a., ACS reagent, ≥70%) and $CuSO_4.6H_2O$ from Sigma-Aldrich (ACS reagent) were used in the preparation of Cu deposition solutions. Au exchange solutions were prepared with $H(AuCl_4).3H_2O$ from Merck. L-Cystine >99% was from Fluka, Biochemica.

Electrochemical Apparatus and Other Instrumentation. A modular electrochemical system AUTOLAB equipped with PGSTAT-12 and ECD modules (Eco Chemie; Utrecht, Holland) was used in combination with GPES software (the same manufacturer). An Ag/AgCl electrode (containing 3M KCl as the inner electrolyte, BAS Inc., USA) as the reference electrode and a Pt coil (wire with 1 mm in diameter; Goodfellow, UK) used as the auxiliary electrode completed the cell. Scanning Electron Microscopy (SEM) was carried out using a JEOL JSM-5510 microscope and elemental analysis of the coatings was performed by the accompanying EDS (EDAX) system.

Results and Discussion

Microelectrode Characterization

Preparation and Microscopic Characterisation. Au shell – (Au-Cu) core coatings on carbon microdisc electrodes (30 μm diameter) or GC RDEs have been prepared by a two-step technique whereby Cu particles electrodeposited onto carbon supports (first step) had their surface layers replaced by Au (second step). The latter has been achieved by means of spontaneous partial replacement of the non-precious metal deposits of Cu by Au upon their immersion in chlorolaurate solutions (see chemical equation (2) in the Introduction above).
Figure 1A shows the SEM of the coated microdisc (where the deposit is seen to expand beyond the carbon disc periphery) and 1B shows the particulate nature of the Au(Cu) coating.
EDS X-ray analysis showed that the relative Au÷Cu % atomic concentration ratio is 76÷24.

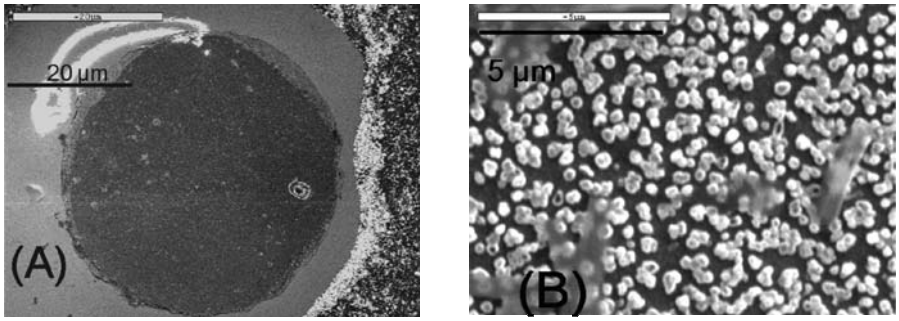

Fig. 1: *SEM micrographs of a Au(Cu)/µC prepared by initial electrodeposition of Cu (15 µC passed) at a 30 µm diameter carbon microdisc, followed by partial electroless replacement of Cu by Au during immersion in 1 mM $AuCl_3$+0.1 M $HClO_4$ solution.* (**A**) Overall microelectrode view; (**B**) Details of the deposit.

Electrochemical Characterisation of Au(Cu)/µC.

Electrochemical characterisation was first carried out by continuous cyclic voltammetry in perchloric acid (serving also as a cleaning-activation procedure for the bare Au electrodes and ensuring the anodic dissolution of uncovered Cu of Au(Cu) coatings). These experiments allowed calculation of microelectrode roughness, r (true to nominal electrode area), based on Au oxide surface electrochemistry and the charge associated with the oxide stripping peak (see Fig. 2, as a cathodic peak). After continuous cycling up to a stable voltammetric picture, r reached values in the 7-10 range.

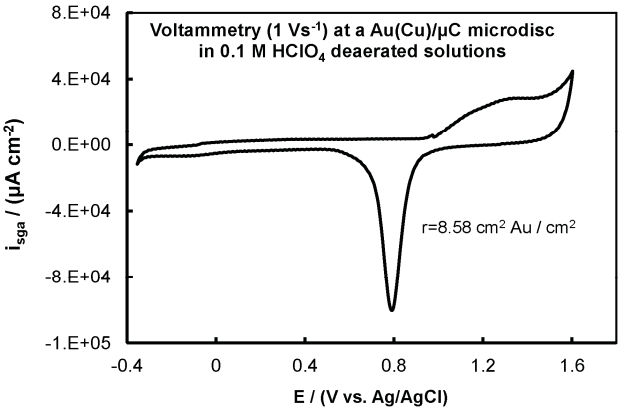

Fig. 2:
Cyclic voltammogram at 1V/s of a Au(Cu)/µC electrode in deaerated 0.1 M $HClO_4$.

Note: I_{sga} is the current density per substrate geometric area (sga).

Microelectrode behavior, as well as the estimate of electrode geometric area, were asserted through the reduction of the reversible electroactive probe of ferricyanide (see Fig. 3 and also Eqn. 7 below). The resulting electrode radius was 2-3× higher than the nominal C-microdisc substrate value, due to extension of the original Cu deposit beyond the microdisc periphery.

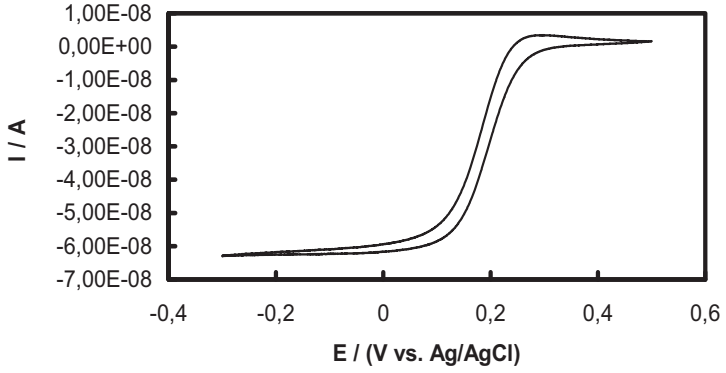

Fig. 3: *Nearly steady-state voltammogram for reduction of ferricyanide at a Au(Cu)/μC microelectrode in deaerated solution of 5 mM $K_3Fe(CN)_6$ + 0.1 M KCl* (recorded at a 5 mVs^{-1} potential scan rate).

Electrochemical Oxidation of Cystine and Its Determination

Oxidation at the Au- and Au(Cu)/GC RDE Macro-Electrodes. The voltammetry of cystine oxidation was first investigated at bulk Au RDE and Au(Cu)/GC RDE macro-electrodes, at high cystine levels (1 mM; Figures 4(A) and 4(B)). The voltammetric picture was in general similar for bulk and modified electrodes. A peak corresponding to adsorbed cystine oxidation (and hence little influenced by rotation rate) at ca. +1.2 V vs. Ag/AgCl has been observed. The ill-defined wave recorded in the +1.3 - +1.6 V range (transformed into an S-shaped wave after background subtraction) is attributed to cystine oxidation from the bulk solution under mass transfer control.

The variation of the ill-defined plateau current with rotation rate for RDEs permitted an estimate of the apparent number of electrons associated with cystine oxidation, through the use of the Koutecky-Levich equation:

$$\frac{1}{I}=\frac{1}{I_K}+\frac{1}{I_L}=\frac{1}{I_K}+\frac{\nu^{1/6}\mathrm{A}^{-1}}{0.62nFC(2\pi f)^{1/2}D^{2/3}} \qquad (3)$$

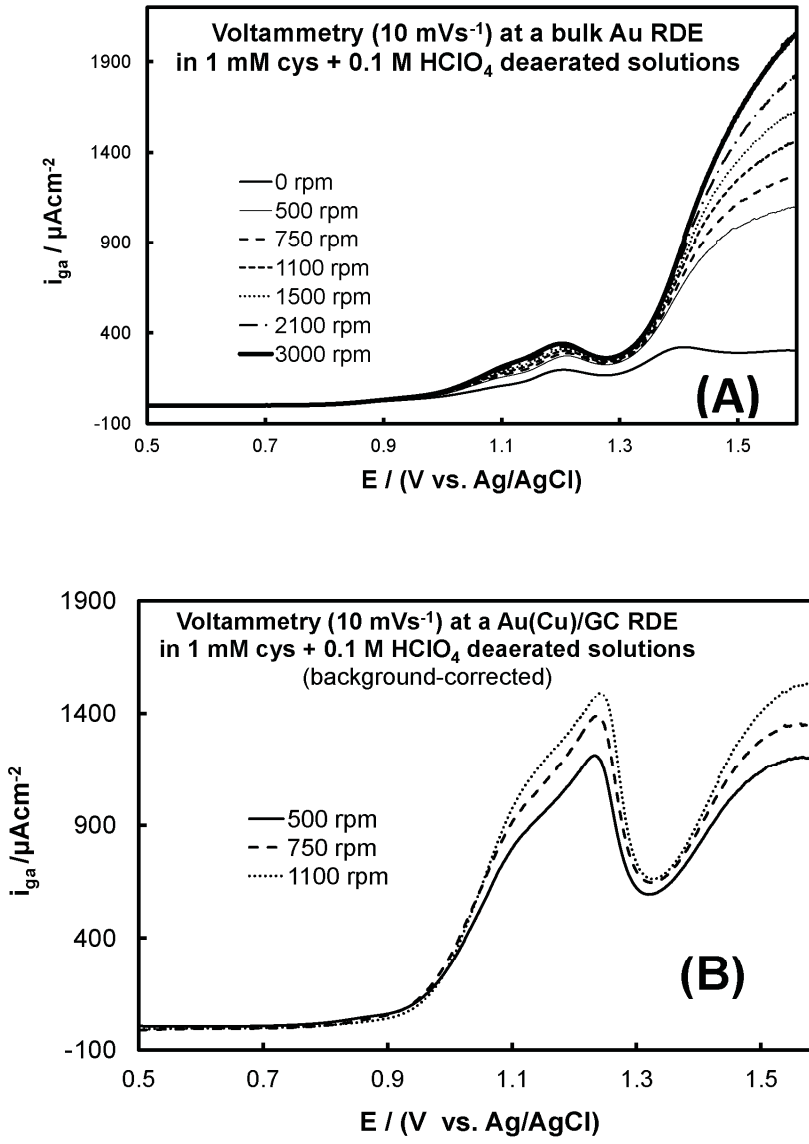

Fig. 4: *Voltammograms for oxidation of cystine at the Au-macroelectrodes recorded at a scan rate of at 10mV/s*. Legend: **A**) Au RDE; **B**) Au(Cu)/GC RDE. Note: I_{ga} is the current density per nominal C microdisc substrate geometric area.

where I is the total, experimentally recorded, current,

$$I_K = nFAkC \qquad (4)$$

is the kinetic current (due to electron transfer kinetics and/or any chemical reaction kinetics present, k being the corresponding rate constant) and

$$I_L = 0.62nFAD^{2/3}\nu^{-1/6}(2\pi f)^{1/2}C \qquad (5)$$

is the mass transfer limiting current given by the Levich equation (ν being the solution kinematic viscosity and f the electrode rotation rate in Hz). Using values of D = 4.8 x 10^{-6} cm^2s^{-1} and ν = 0.011 cm^2s^{-1} [19], the number of electrodes involved in oxidation of cystine in that potential range is estimated as n ≈ 5, indicating an oxidation to the cationic sulfone, both for plain Au and modified Au(Cu) electrodes:

$$R-S-S-R + 2H_2O \rightarrow \left(R-SO_2-S-R\right)^+_{ads} + 4H^+ + 5e^- \qquad (6)$$

However, the peak current at ca. +1.2 V was found to be much higher at the Au(Cu) modified electrodes, even after correction for increased roughness. This increase is attributed to an electronic effect of elemental Cu at the Au material, decreasing the affinity of the latter for excessive oxide formation and/or poisonous carbonaceous intermediates from the oxidation of cystine. The enhancement of peak current in the case of modified Au(Cu) electrodes (when compared to their plain Au analogues) could be of electroanalytical value since this peak is recorded at relatively low potentials where the background current is low (when compared with the onset of oxygen evolution at higher overpotentials). This potentiality is explored below in the case of microelectrodes.

Oxidation and Determination of cystine at Au(Cu)/μC microelectrodes. Cystine oxidation at modified Au(Cu)-coated carbon microdiscs (*Au(Cu)/μC)* was studied both from high concentration solutions (10^{-3} M; for oxidation mechanism verification) and from dilute solutions (10^{-4} – 10^{-5} M; relevant to biological fluid analysis). Fig. 5(A) presents voltammograms for 1 mM cystine and a similar picture with that of Fig. 4(B) obtained at a Au(Cu)/GC macroelectrode can be seen. The main difference between voltammetry at a Au(Cu)/GC RDE macroelectrode and a Au(Cu)/μC microelectrode is the fact that the oxidation wave at more positive potentials is less well-defined and a clear plateau cannot be obtained. A possible explanation is due to the different carbon substrates (a glassy carbon and a graphitic one) which gives rise to a higher oxygen evolution background current at graphitic uncovered areas (see Fig.1).

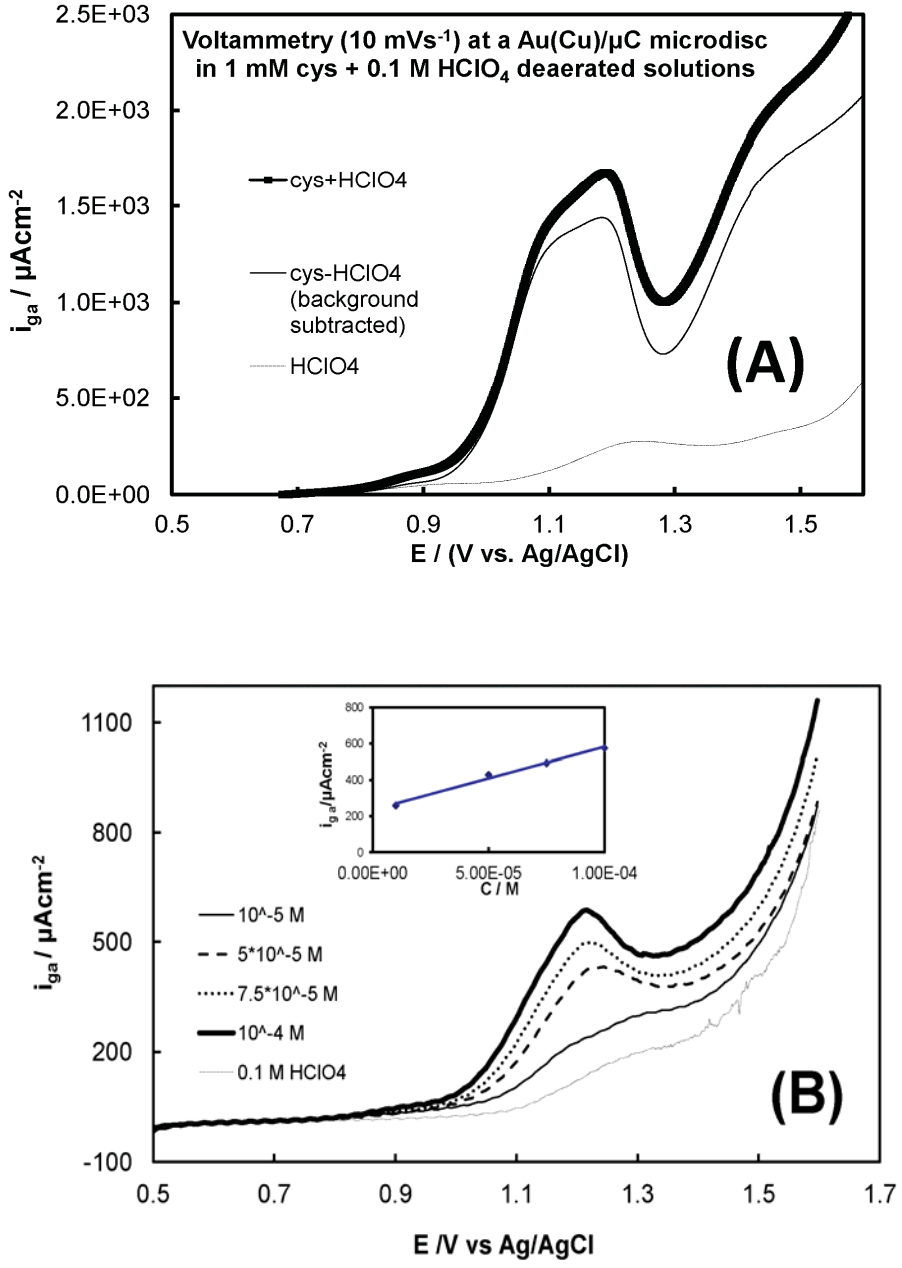

Fig. 5: *Voltammograms for the oxidation of cystine at Au(Cu)/µC microelectrodes recorded with a scan rate of at 10mV/s*. Legend: **A**) 1mM cystine + 0.1 M $HClO_4$ solutions; **B**) 10^{-5} - 10^{-4} M cystine + 0.1 M $HClO_4$ solutions. Notes: the arrow indicates potential scan direction; i_{ga} is the current density per nominal C microdisc substrate geometric electrode area.

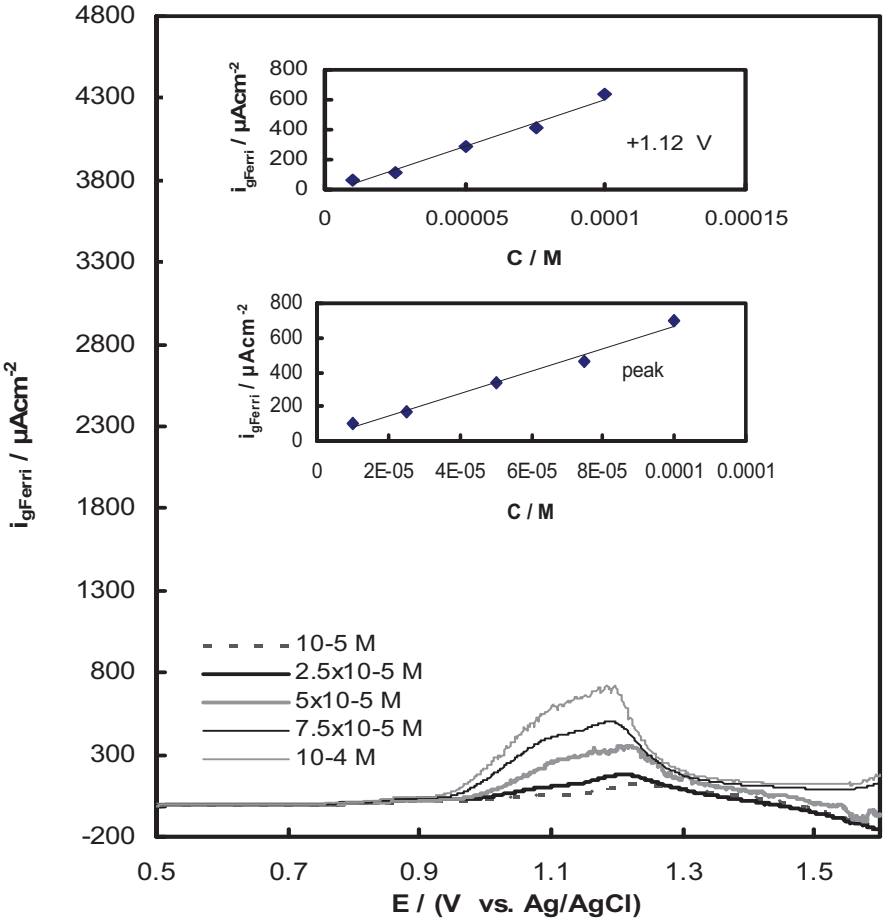

Fig. 6: *Background-corrected voltammograms at 10mV/s for cystine oxidation at Au(Cu)/µC microelectrodes*. Background electrolyte: 0.1 M $HClO_4$. Insets: variation of voltammetric peak currents with cystine concentration. Note: i_{gFerri} is the current density per actual geometric electrode area, as estimated from ferricyanide reduction data via Eqn. (7).

An attempt to estimate the number of electrons associated with this wave can be made at a potential where signs of a mass transfer control appear (namely, at ca. +1.50 V vs. ref.), via the equation of the diffusion limiting current, I_d, at microdisc electrodes or radius r_d:

$$I_d = 4nFDCr_d \qquad (7)$$

An estimate of n = 5.5 and n = 4.7 from the un-corrected and corrected curves can be made, indicating a similar mechanism of cystine oxidation (to cationic sulfone) both at macro- and micro- Au(Cu)-coated electrodes.

Fig. 5(B) presents a number of voltammograms recorded for various cystine concentrations in the 10^{-4} – 10^{-5} M range. At these low concentrations the second oxidation wave seems to be so small that is lost into the onset of oxygen evolution. Nevertheless, the peak current has varied linearly with the concentration of cystine in the same range (see the inset).

Finally, Fig. 6 presents the background-corrected voltammograms at a Au(Cu)/μC microelectrode for various cystine levels and its Insets the calibration plots at two different potentials in the peak region. Very good linearity was observed and a detection limit of $2x10^{-6}$ M was estimated for the 10^{-5} M concentration.

Conclusions

Based on the above-discussed experimental material and the corresponding interpretation and discussion, it can be concluded that:

(i) Au(Cu) modified bi-metallic microelectrodes have been prepared on carbon microdisc substrates via a two step electrodeposition-galvanic replacement method.

(ii) For cystine, the Au(Cu) electrodes showed higher oxidation currents at moderately applied potentials than those obtained with the plain Au-analogues.

(iii) Determination of cystine at Au(Cu)-coated carbon microdiscs via its electrooxidation peak showed good linearity in a range of $1x10^{-4}$ – $1x10^{-5}$ M with a detection limit of $2x10^{-6}$ M.

Similarly as the recent study [20], the method is another contribution to the electrochemistry of sulfur-containing biologically important compounds when using non-mercury electrodes.

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European Union – European Social Fund

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