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Development and evaluation of a glassy carbon electrode modified with silver and mercury nanoparticles for quantification of cysteine rich peptides

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**ABSTRACT**

A glassy carbon electrode, covered with a Nafion film, on which nano-structured bimetallic particles of Ag-Hg were deposited; from now on “the AgHgNpNf/GC electrode”, was used for the indirect determination of cysteine, glutathione and metallothioneins by measuring the catalytic hydrogen evolution signal according to the Brdička reaction using cobalt as a catalyst. Upon successful formation and deposition of the Ag-Hg bimetallic nanoparticles cyclic voltammetry (CV), scanning electron microscopy (SEM) and atomic force microscopy (AFM) were used to characterize the electrode for that specific task. The results showed the electrode to be mechanically resistant even under sonication, stable in its performance, porous, sensitive in its response and of low cost to be built. Differential pulse voltammetry signals for cysteine and glutathione obtained using the AgHgNpNf/GC electrode were qualitatively similar to those reported using a hanging mercury drop electrode, (HMDE), dropping mercury electrode (DME) and static mercury drop electrode (SMDE). Detection limits based on the variability of a blank solution, (3 s criterion) calculated for those tests were 0.463 μg L\(^{-1}\) and 0.064 μg L\(^{-1}\), for cysteine and glutathione, respectively. Based on the Brdička procedure and considering the particular characteristics of the new electrode, some analytical tests were carried out for the indirect determination of the metallothioneins content of samples of blood serum of rats exposed to lead by measuring the evolution of the catalytic hydrogen signal. An average accuracy value around 102%, n = 6, and a precision of 0.87% ± 0.09 were obtained for these tests.

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1. Introduction

In the presence of cobalt salts, cysteine rich peptides and proteins act as catalysts for hydrogen evolution on mercury electrodes. This catalytic process was discovered and studied by Rudolf Brdička in 1933 [1]. Brdička showed that when the sulfur atom from the –SH group is bound to cobalt (or even to nickel) forming a complex, its hydrogen atom gets activated for easier involvement in the electrode process of hydrogen evolution as well as to act as a proton donor to the solution [2]. In the case of cobalt, the catalytic part of the organo-metallic complex could probably have the structure:

\[
\text{Co} - \text{S} - \text{R} \\
\downarrow \text{H}
\]

The Brdička reaction was a subject of great interest and consequently it was widely used [3–8]. To date, the procedure based on this reaction is used for determination of peptides rich in cysteine such as glutathione, glutathione oxide, metallothionein and phytochelatin [9–16]. The interest in these peptides lies in the following general facts: they have been shown to be part of a defense mechanism by plants and animals in response to exposure to high levels of essential and non-essential metals (Pb as an example); the presence of these peptides in concentrations above certain limits in living organisms could be associated to resistance of such organisms to certain therapies against cancer [12,17–24].
Most studies reported involving Brdička's reaction have been performed using a dropping mercury electrode (DME), a static mercury drop electrode, (SMDE) [24–26] or a hanging drop mercury electrode (HDME) [19]. However, these electrodes are not at all convenient when the determinations have to be carried out directly in the field because they are not amenable to miniaturization, do not provide results in real time and because their use brings about the possibility of ambient contamination with relatively high amounts of mercury either in liquid or gaseous form. Given these facts, development of solid electrodes which keep the best features of the traditional mercury electrode and excludes or minimizes the negative ones, has been the focus of special attention for several researchers [27–38]. Although mercury has not been eliminated in some of them, an important benefit of most of these electrodes is the reduction of the amount of mercury they contain as compared to the traditional ones. Mercury present in these electrodes has been added as a meniscus [39] or, in the most used ones, as an electrolytically deposited thin layer [40–43]. Both types of electrodes are alternatives which contain much lesser amounts of mercury than the conventional type.

Mercury thin layers could be electrolytically deposited on glassy carbon or in metals such as platinum, iridium, copper or silver thus rendering very useful electrodes [44–48]. However, there are some negative aspects that hinder the usefulness of these electrodes: insufficient mechanical stability due to the weakness of the attachment of the mercury layer on the bulk of the electrode [49–51], the possibility of dissolution of the sample substrate in the mercury phase [52–54] and deactivation of some parts of the electrode surface due to insertion of organic substances from samples with complex matrices [54–58]. A better way of overcoming the deficiencies just mentioned is by improving the mechanical stability of the electrode. This has been achieved covering the electrode surface with organic membranes or templates made out of cellulose acetate or Nafion [59–65]. Thin films of Nafion deposited on a glassy carbon structure and subsequently doped with metallic nanoparticles results in a mechanically stable electrode and resistant to deactivation. This last characteristic, is due to the fact that Nafion is a hydrophobic polymer constituted by short, laterally placed and regularly spaced perfluorovinyl branches [66]. The net-type of the Nafion film is of the upmost importance to our goals because it could allow for regular distribution, size control and uniform density of nanoparticles electrodeposited on the glassy carbon electrode that it covers [67,68]. Based on the above, we constructed electrodes by depositing Nafion films on top of glassy carbon substrates and subsequent electrodeposition of alloyed Ag-Hg bimetallic nanoparticles on them. The electrodes were optimized to measure the catalytic hydrogen evolution signals, according to the Brdička reaction, using cobalt as a catalyst. These hydrogen evolution signals were used for the indirect determination of cysteine and glutathione in standard aqueous solutions, as well as for determination of metallothioneins in blood serum of laboratory rats exposed to lead contaminated ambiances. During electrode construction, special attention was placed on the following parameters: electrode mechanical stability, chemical inertness, sensitivity, reproducibility, high hydrogen over reaction potential, ample working potential window and minimum possibility of mercury contamination during its use.

2. Experimental

2.1. Reagents

Cysteine (Cys), glutathione (GSH), K₃Fe(CN)₆·anhydrous methanol 99.8% purity, dimethylformamide (DMF), Nafion 5% (w/w) and Sodium acetate (>99%) were purchased from Sigma-Aldrich. Acetic acid (99.80%), ethanol (99.80%), and nitric acid (65%) were purchased from Riedel-de Haën. A 1.000 mg L⁻¹ Hg(II) standard aqueous solution was supplied by Merck. The supporting electrolyte, 1 mol L⁻¹ NH₄Cl + 1 mol L⁻¹ NH₄OH (ammonia buffer, pH 9.5) + 0.6 mmol L⁻¹ Co(NO₃)₂ 6H₂O, was prepared from ACS Chemicals purchased from Sigma-Aldrich. Working standard solutions were prepared daily by dilution of the stock solutions with distilled/deionized water, 18 MΩ cm⁻¹ resistivity. Metallothioneins (MTs) standard solutions for working curves for the analysis of blood samples were prepared from a purified rabbit liver certified reference material containing 5.9% Cd, 0.5% Zn and 1.0 mg L⁻¹ MTs in distilled/deionized water. The rabbit liver certified reference material was purchased from Sigma Chemical Co, as the product No. M 7641 (MW 7143).

2.2. Instrumentation

Voltammograms were obtained using an EG&G Princeton Applied Research (PAR) model 273A potentiostat interfaced to a computer system with PAR M270 software, using a standard cell able to support three electrodes: a working electrode, a Ag/AgCl (3.5 mol L⁻¹ KCl) and a graphite as auxiliary and reference electrodes, respectively. Working electrodes were: glassy carbon (GC), glassy carbon modified with silver nanoparticles on a Nafion film (AgNP/Nf/GC) and glassy carbon modified with silver and mercury nanoparticles on a Nafion film (AgHgNP/Nf/GC) electrodes. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analyses were performed using a PHENOM PROX table top scanning electron microscope coupled to an X-ray microanalysis system. A Bruker dimension Incon equipment was used for the ex-situ atomic force microscopy (AFM) studies, operated in Peakforce Tapping mode, and employing silicon nitride probes (5–40 nm radius).

2.3. Electrodeposition of Ag-Hg alloyed nanoparticles on a glassy carbon electrode using a nafion film as a template

Nanostructured material was obtained following a modification of the procedure reported by Xing et al., [68]. A GC disk electrode with a diameter of 3.0 mm was first mechanically polished with alumina powder until a mirror-like surface was obtained. The disk was then sequentially sonicated for three minutes in each of the following solutions: acetic; nitric acid: H₂O₂ (1:1); sodium hydroxide (1.0 mol L⁻¹) and doubly distilled water and dried under an air stream at ambient temperature (−25 °C). A 5 wt% Nafion solution in lower aliphatic alcohols/H₂O was diluted to 1 wt% with ethanol. A 5 μL volume of the diluted Nafion solution was casted on the cleaned GC surface, dried at room temperature for 10 min, followed by immediate addition of 3 μL of DMF casting solvent to the electrode surface by means of a micropipette, in agreement with the work reported by G. Kefala et al., [69]. The solvents were then gently evaporated from the electrode in a warm air stream (30 °C) from an air gun while the electrode was rotated at 50 rpm. The electrode was then exposed to a hotter air stream (65 °C) for 1 min, holding the air gun at a short distance above the electrode surface to finish its drying, to cure the Nafion film and to increase its adherence to the glassy carbon substrate. The electrode covered with the Nafion film was immersed for three hours in an aqueous solution containing 0.85 mg L⁻¹ Ag and 0.15 mg L⁻¹ Hg to promote insertion of metals in the Nafion template. The electrode was then removed and washed with double distilled/deionized water to remove non-absorbed material. Finally, the electrode was submitted to coulombimetry at controlled potential (CPC) at −1.2 V for 300 s, in a 1 mol L⁻¹ in KNO₃ + 0.1 mol L⁻¹ HNO₃ solution to reduce the ions Ag(I) and Hg(II) trapped inside the Nafion to bimetallic Ag-
Hg alloyed particles. A 300 s time was selected in order to achieve the smallest nanoparticles size.

2.4. Analytical procedure

Appropriate standard test solutions of the cysteine-rich peptides (cysteine, glutathione or metallothioneins) were added to the cell and differential pulse voltammetry (DPV) measurements were carried out according to the Brdička procedure from −0.2 to −1.4 V under the following settings: pulse width 80 ms, pulse amplitude −50 mV, initial potential $E_{\text{fin}}$ −0.2 V, final potential $E_{\text{fin}}$ −1.4 V, $E_{\text{quiescent}}$ −0.1 V. $t_{\text{quiescent}}$ 2 s, scan rate 20 mV s$^{-1}$. A degassing step for at least 10 min with an argon stream was carried out prior to each voltammetry measurement.

Samples of blood serum of rats were prepared by heat treatment and solvent precipitation. They were kept at 99 °C in a thermomixer stirring for 10 min and then cooled to 4 °C. The denatured homogenates, maintained at that temperature, were centrifuged at 4000 rpm and 5 mL of the supernatant serum were taken for analysis by differential pulse voltammetry. Quantification was done by measuring the Brdička reaction current against standard calibration curves.

3. Results and discussion

3.1. Electrode characterization

3.1.1. Electrochemical characterization

Cyclic voltammetry, CV, was used to examine the electrochemical behavior of the nanostructured Ag-Hg deposits on the GC electrodes covered with the Nafion film. Fig. 1 (red line) shows a cyclic voltammetry scan of a AgNpNf/GC modified electrode prepared by immersing for three hours a GC electrode covered with Nafion in an aqueous solution containing 0.85 mg L$^{-1}$ Ag. This voltammogram is consistent in the occurrence of six anodic peaks with those previously published for a conventional Ag electrode in a NaOH solution [70–73]. Distinctive of the anodic sweep, as seen in Fig. 1 are the peaks “$a_1$” at 256 mV due to electroformation of Ag$_2$O; the peak “$a_2$” at 346 mV due to nucleation and three-dimensional growth of the Ag$_2$O layer and the peak “$a_3$” at 703 mV indicating formation of AgO. The cathodic part of the cyclic voltamgrams is characterized by the presence of an activated anodic peak “$b_1$” at 541 mV due to electrooxidation of Ag to Ag$_2$O and two cathodic peaks, “$b_2$” and “$b_3$”, at 353 mV and at −58 mV corresponding to electroreduction of AgO to Ag$_2$O and then Ag$_2$O to Ag, respectively. The CV response of the nanostructured AgHgNpNf/GC electrode (Fig. 1, black line), is similar to the response of the conventional Ag-Hg amalgam electrode [74]. This indicates that mercury is effectively forming an amalgam with silver in the electrode being characterized. The waves obtained in the sweep using the modified AgNpNf/GC electrode, are still present in the sweep using the AgHgNpNf/GC (peaks “$b_1$”, “$b_2$” and “$b_3$”, respectively in the figure) but with certain changes in potential values and an additional current plateau which appears after the potential value labeled “$a_2$” (about 433–610 mV). The plateau could be assigned to stripping of mercury as [Hg(OH)$_2$] and [HgOH], in addition to the formation of Hg$_2$OH and HgO.

Fig. 1. Cyclic voltammetry scan of a AgNpNf/GC (red line) and a AgHgNpNf/GC (black line) electrode, in a solution containing 0.1 mol L$^{-1}$ NaOH, at a scan rate of 20 mV s$^{-1}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Cyclic voltammetry scan of the bare GC (a), Ni/GC (c) and AgHgNpNf/GC (b) electrodes, in a 0.1 M NaClO$_4$ + 1 mmol L$^{-1}$ K$_3$Fe(CN)$_6$ solution at a scan rate of 100 mV s$^{-1}$. 

The electron transfer capacity at the AgHgNpNf/GC was also studied using the [Fe(CN)₆]³⁻⁻⁴⁺⁺⁺⁻⁺⁺⁺⁺ couple as an electrochemical probe. Fig. 2 shows the cyclic voltammograms (CVs) of the bare and modified GC electrodes in 0.1 M NaClO₄ + 1 mmol L⁻¹ K₃Fe(CN)₆ solution, at a scan rate of 100 mV s⁻¹. A reversible electrochemical response for the [Fe(CN)₆]³⁻⁻⁴⁺⁺⁺⁺ couple was observed using the bare glassy carbon electrode (Fig. 2a). Using the glassy carbon electrode modified by addition of a Nafion film, Nf/GC electrode, but with no Ag or Hg added (Fig. 2c), very low peak currents with a large peak separation (ΔEp) were observed for the redox couple. We have associated this to the electrostatic repulsion between the negatively charged [Fe(CN)₆]³⁻⁻ and –SO₃ groups in the Nafion film which results in slow kinetics for the electron-transfer process at the Nf/GC electrode. When the Ag–Hg alloyed bimetallic nanoparticles were introduced into the Nf/GC electrode (Fig. 2b), the electrochemical behavior of the redox couple was considerably improved. In agreement with Xing et al., [68], it can be said that the Ag–Hg alloyed nanoparticles introduced in the Nafion template provide a conduction pathway thus accelerating the kinetics for the electron transfer process. The ΔEp values observed were 69, 257 and 120 mV for the GC, the Nf/GC and the AgHgNpNf/GC electrodes, respectively.

3.1.2. Scanning electron microscopy (SEM)

The morphologies and the structures of the pure Nf film and the AgHgNpNf composite film deposited on the GC electrodes were characterized using SEM. Fig. 3a presents a micrographic view of the Nafion layer deposited on the GC electrode, which shows a flat, porous and continuous film, with holes in diameter around 300–500 nm for the AgHgNpNf film. Fig. 3b shows the glazed surface of bare GC with no Nafion. When the GC was modified with the AgHgNpNf film (Fig. 3c), many Ag–Hg alloyed nanoparticles were deposited across the whole surface, the alloyed nanoparticles were immobilized on the electrode surface giving origin to a
3.2. DPV of cysteine and Co(II) ions at AgHgNpNf/GC modified electrode

Fig. 5 shows the DPV responses of the supporting electrolyte (1 mol L\textsuperscript{-1} NH\textsubscript{4}Cl + 1 mol L\textsuperscript{-1} NH\textsubscript{4}OH + 0.6 mmol L\textsuperscript{-1} Co(NO\textsubscript{3})\textsubscript{2} 6H\textsubscript{2}O) at pH 9.5, in the absence (Fig. 5a) and the presence (Fig. 5b) of cysteine, using the AgHgNpNf/GC electrode. In the absence of cysteine a single peak corresponding to Co(II) reduction was observed at –1.0 V. In the presence of cysteine the Co(II) peak was shifted by about 0.15 V to the less negative potential region and a new peak appears at –1.25 V. The shift of the Co(II) peak to the less negative potential, could be associated to presence of transition metals and some other hydrated ions undergoing a relatively slow electrode reaction, while they get reduced more easily in presence of organic complexes.

The new peak, at –1.25 V, the so-called Brdička signal, shown in the DPV in Fig. 5b, corresponds to the catalytic hydrogen evolution according to the Brdička reaction.

The Brdička signal is most frequently used for determination of the concentration of low-molecular mass peptides and/or proteins rich in cysteine such as cysteine itself, glutathione, phytochelatins and metallothioneins, which are frequently used as biomarkers. Due to the linear dependence of the signal of the catalytic hydrogen evolution on the amount of low molecular mass peptides or proteins rich in cysteine, it is possible to correlate the Brdička signal with the concentration of those analytes in a given sample. This approach does not require laborious and tedious deconvolution procedures in order to calculate the analyte content of the samples. This in turn facilitates faster and simpler completion of the analysis [75]. Evaluation of the AgHgNpNf/GC electrode shows that the Brdička peak height at –1.25 V followed a linear dependence on the cysteine concentration in the range of 2–35 μg L\textsuperscript{-1} (Inset Fig. 5). Calculation of the detection limit of these measurements based on the variability of a blank solution (3σ criterion), for 10 measurements at the AgHgNpNf/GC electrode gave a detection limit (DL) of 0.463 μg L\textsuperscript{-1}. This value is lower than those reported by other research groups using electrodes with more complicated structural features [76,77]. Additional evaluation of the AgHgNpNf/GC electrode showed that the slope of the straight line does not have a very marked dependence on the concentration of the Co(NO\textsubscript{3})\textsubscript{2} 6H\textsubscript{2}O depolarizer. The effect of temperature on the intensity of the signal was also assessed in the interval from 7 to 25 °C. Maximum signal intensity, that is, highest sensitivity of the measurement, was obtained for a 0.6 mmol L\textsuperscript{-1} Co(NO\textsubscript{3})\textsubscript{2} 6H\textsubscript{2}O at 18 °C (Table 1).

3.3. DPV of glutathione and Co(II) ions at AgHgNpNf/GC modified electrode

DPV voltammograms obtained in the absence (Fig. 6A-a), and the presence (Fig. 6A-b) of glutathione using the AgHgNpNf/GC electrode, showed different electrochemical responses. In the
absence of glutathione a single peak corresponding to Co(II) reduction was observed at −1.0 V. In the presence of glutathione the reduction peak of Co(II) is observed at a lower current, −0.94 V, and a new peak appeared at less negative potentials (about −1.1 V), which could be associated to reduction of a glutathione–cobalt complex, as has been described earlier [78–80]. Finally, a Brdička signal in a region around −1.38 V corresponding to the glutathione catalysed hydrogen evolution is observed.

Analytical curves were obtained using the AgHgNpNf/GC modified electrode plotting Brdička signals as a function of glutathione concentration (Fig. 6B). Two linear responses, two different slopes, were found in the ranges from 0.5 to 4 and 20–70 µg L⁻¹ glutathione (Fig. 6B a and b). This could be reasoned in the following way: the electrode works more efficiently at low analyte’s concentrations i.e. below approximately 4 µg L⁻¹ glutathione. Its response becomes somehow restricted at higher glutathione concentrations probably by partial saturation of its active surface by the presence of an
excess of glutathione. The best representation of the linear dependence of the Brdička signal on the concentration of glutathione is shown in the analytical curve covering the range, from 0.5 to 4 μg L⁻¹. This curve presented the highest R² and its extrapolation towards lower concentration values would get closer to zero than would be the case for the curve covering the higher range of glutathione concentration. This curve is most suited to analysis of samples containing glutathione at very low concentrations. Using this curve a calculated detection limit of 0.064 μg L⁻¹ was obtained which is one order of magnitude lower than the one for cysteine, section 3.2. Maximum sensitivity as a function of temperature and amount of polarizer used was achieved with 1.0 x 10⁻¹ mol L⁻¹ Co(NO₃)₂·6H₂O at 7 °C (Table 2). On the other hand, the curve at higher concentrations could be useful in those cases where glutathione is found in concentrations relatively high which, according to the literature, are quite frequent [81]. It is interesting to notice that the analytical curve at these higher concentration levels does not cross the zero point in the calibration graph. This could be due to the effect of the hydrogen evolution residual current. Similar tests performed using the unmodified GC electrode showed just a well-developed peak at −0.98 V due to Co(II) reduction but no signals at all from glutathione (data not shown).

3.4. AgHgNpNg/GC modified electrode performance in the determination of metallothioneins (MTs)

3.4.1. Voltammograms of metallothioneins using the AgHgNpNg/GC modified electrode following the brdička procedure

Fig. 7A, shows the voltammograms of the MTs plus the Brdička supporting electrolyte (1 mol L⁻¹ NH₄Cl + 1 mol L⁻¹ NH₄OH + 0.6 mmol L⁻¹ Co(NO₃)₂·6H₂O, pH 9.5). Fig. 7A line (a), corresponds to the signal without metallothionein. It shows a peak at −1.0 V which could be ascribed to reduction of Co(II) ions. Remaining lines (b, c and d) are voltammogram signals obtained in the presence of MTs. Fig. 7A, line (b), illustrates the signal due to the MTs complex with cobalt ions, so-called RS₂Co signal, appearing at −1.22 V. This signal is shifted to more positive potentials with increasing MT concentration (Fig. 7A c-d). The signal at −1.43 V is the signal belonging to the cobalt-catalysed reduction of thiolic hydrogen according to the Brdička reaction [82–85]. The dependence of the height of that signal on the MTs concentration is represented in Fig. 7B. It can be seen that said dependence is linear in a range from 0.1 to 1.8 μg L⁻¹ (Y = 1.09x + 0.111, R² = 0.9985).

3.4.2. Application of the AgHgNpNg/GC electrode to the analysis of real samples

Metallothioneins (MTs) belong to a group of proteins and peptides of the glutathione type which play an important role in the metabolism of metals in animals, plants and microorganisms [83]. MTs are a class of intracellular proteins rich in cysteine which have metal binding properties. Such characteristics make them useful as biomarkers for the detection and assessment of heavy metal concentrations in studies of environmental pollution [84]. If animals are exposed to environments contaminated with heavy metals they liberate metallothioneins as part of their defense mechanism. In agreement with findings described in section 3.4.1, the new AgHgNpNg/GC electrode, in combination with the Brdička procedure, should be a valid choice for determination of MTs in order to indirectly assess metal contamination in animals. To check this
assumption, samples of blood serum of laboratory rats exposed to an environment contaminated with Pb, were chosen in the present study to test the new electrode in the analysis of a real sample.

3.4.3. Analytical procedure

The supernatant serum was grouped in three series of six samples each: MT addition 1; MT addition 2 and MT addition 3. To each of the 18 samples an appropriate volume of a Pb solution was added as to reach 1.0 μg mL⁻¹ of Pb. The three series of samples were analysed for lead during each analysis session. Once each series of measurements was completed, the AgHgNpNf/GC modified electrode was withdrawn from the last solution of the series, rinsed thoroughly with deionized/demineralized water and then immersed in the first solution of the next series to continue the measurements. Results shown in Table 3, attest that the method provides recoveries within 101 and 104% expressed as the percentage of relative error between the found mean concentration and the added concentration for each MTs solution, accuracy is 0.87% and precision of the measurement for intra-day measurements for the same solution, is not higher than ± 0.09.

4. Conclusion

In general, the AgHgNpNf/GC modified electrode in connection with the Brdička procedure can be applied to determination of thiols (cysteine, glutathione and/or metallothionein) containing peptides and proteins. In particular, the indirect determination of cysteine and/or glutathione based on evaluation of the catalytic hydrogen evolution signal according to Brdička is a viable approach which simplifies calculations of the analytical results. The determination of Metallothionein, as described in this paper, is a convenient way for indirectly assessing Pb concentration in biological samples such as blood serum. The glasseye carbon electrodes modified with nano-structured bimetallic deposits of Ag–Hg, having just nano amounts of mercury, have, besides their analytical advantages, the advantage of being more environmentally friendly and of lower cost than the normally used mercury macro-electrodes.

Availability of an electrochemical system with temperature control allowed us to check the dependence of the signal intensity on temperature in our first essays as shown in Tables 1 and 2. However, this system was not available for the rest of the measurements which therefore were done at ambient temperature (approx. 24 °C). Further studies, using a cell with temperature control, will be conducted at a maximized temperature value in order to increase the sensitivity of the modified electrode to check it for determination of the presence of other heavy metals, different from Pb, in biological samples. Development and evaluation of such a methodology would be very useful for assessing the extent of such contamination in some particular environments.

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References


Table 3
Results of electrochemical determination of MTs in samples blood serum of rat exposed to Pb using the AgHgNpNf/GC electrode (n = 18).

<table>
<thead>
<tr>
<th>Sample series</th>
<th>Pb(II) added (μg L⁻¹)</th>
<th>Concentration found (μg L⁻¹) by this method</th>
<th>Reclaimed (μg L⁻¹)</th>
<th>% Recovery (R) (a)</th>
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<tr>
<td>MT addition 1</td>
<td>1.0</td>
<td>1.55</td>
<td>2.56</td>
<td>101</td>
</tr>
<tr>
<td>MT addition 2</td>
<td>1.0</td>
<td>1.58</td>
<td>2.71</td>
<td>104</td>
</tr>
<tr>
<td>MT addition 3</td>
<td>1.0</td>
<td>1.63</td>
<td>2.63</td>
<td>102</td>
</tr>
</tbody>
</table>

(a) = [% R = (Reclaimed value – Found value)/Added value x 100]


P. Stenberg, F.P. Portante, Potentiodynamic extraction of surface processes and kinetics for the Ag/OAg/AgOI-system, Electrochim. Acta 13 (1968) 1805–1814.


Biographies

Danny Valera received a BS degree in chemistry from the Universidad Simón Bolívar in 2007. Shortly after that she joined the Laboratory of Electroanalysis at the Universidad Simón Bolívar, to work as young research (M.Sc. student), in Caracas Venezuela. His research interest lies on development of composite films for electroanalytical detection in environmentally friendly processes.

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