Sensors and Actuators B: Chemical
Volume 165, Issue 1, April 2012, Pages 143-150

Use of nanohybrid materials as electrochemical transducers for mercury sensors

Daniel Martín-Yerga, María Begoña González-García, Agustín Costa-García

https://doi.org/10.1016/j.snb.2012.02.031

USE OF NANOHYBRID MATERIALS AS ELECTROCHEMICAL TRANSDUCERS FOR MERCURY SENSORS

Daniel Martín-Yerga, María Begoña González-García, Agustín Costa-García*

Departamento de Química Física y Analítica, Universidad de Oviedo, C/ Julián Clavería, 8, 33006, Oviedo, Spain

Abstract

The electrochemical behavior of mercury using different nanostructured screen-printed transducers has been studied. The first underpotential deposition (UPD) was chosen as the best electrodicprocess to detect low amounts of mercury on gold nanostructured electrodes. Several nanostructured electrochemical transducers using carbon nanotubes, graphene oxide and gold nanoparticles were generated, characterized and optimized for mercury determination in water. The transducer with a nanohybrid surface of carbon nanotubes and gold nanoparticles was the best suited to solve the analytical problem. For this sensor, a calibration plot from 0.5 to 50 µg/L was obtained in acidic solutions of Hg(II) with anintraelectrodic reproducibility of 3% (n = 5). The detection limit was 0.2 µg/L of mercury. The performance of the sensor was then evaluated using real samples of tap and river water with good accuracy.

Keywords:*Nanohybrid transducer, Underpotential deposition, Mercury sensor, Carbon nanotubes, Gold nanoparticles, Screen-printed carbon electrode.*

1. Introduction

Mercury is considered one of the most dangerous chemical pollutants, and, unfortunately, it is one of the most abundant heavy metals in the environment. It is widely distributed in air, water and soil. Although the use of mercury is being reduced in all possible areas, still 2000 t are emitted annually from anthropogenic sources[1]. The toxicity of mercury varies with its chemical form, but all mercury species are toxic. It can be accumulated in some vital organs as the liver, heart, brain and tissues as bones. Exposition to mercury can cause kidney failure, nervous system disorders, intellectual impairment and even death[2]. Therefore, mercury analysis in water is essential to prevent issues for human beings and the environment.

* Corresponding author. Tel.: + 34 98 510 34 88
E-mail address: costa@uniovi.es (Agustín Costa García)

GO: Graphene Oxide
SPCnAuEs: Screen-Printed Carbon/Gold Nanoparticles Electrodes
SPCNTnAuEs: Screen-Printed Carbon Nanotubes/Gold Nanoparticles Electrodes
SPGOnAuEs: Screen-Printed Graphene Oxide/Gold Nanoparticles Electrodes

The most used analytical methods for mercury determination are cold vapor atomic fluorescence spectroscopy(CVAFS)[3],cold vapor atomic absorption spectroscopy (CVAAS)[4] and, also, ICP-MS[5]. These methods have an expensive instrumentation, with complex sample preparation and cannot be used for in-situ analysis. The search for a fast, cheap, simple and easily portable methodology that allows performing an in-situ analysis of the environment samples is a constant concern. Hence, electrochemical analysis due to their excellent sensitivity, short analysis time, and cheap instrumentation is a good alternative to solve those challenges. Within this field, anodic stripping voltammetry (ASV) using gold electrodes has shown its applicability and has even been recommended by the US Environmental Protection Association (EPA) for the quantification of heavy metals as mercury[6].

Gold is an excellent material as a working electrode because it has high affinity for mercury enhancing thepreconcentration effect[7,8]. In addition, some metals such as mercury, arsenic, or lead present a process called underpotential deposition (UPD)[9]. The UPD is a fundamental electrochemical process andhave attracted a long-standing interest. It happens by the strong interaction between the metal and the gold electrode after the reduction of ionic metal, resulting in the formation of an adlayer. In the case of mercury, this process is of particular importance due to its special electrochemical properties and the amalgam formation.Several types of gold electrodes have been used for the electrochemical determination of mercury, such as gold disk[10], gold film[11], gold microelectrode arrays[12], and gold fiber[13]. This kind of electrodes, generally, employs instrumentation still intended for laboratory use and thus unsuitable for in-situ analysis.

The research on chemical sensors has grown exponentially in recent years, because these devices haveideal characteristics such as low cost, possibility of miniaturization and ease of use, allowing their use for in-situ analysis by non-specialist personal. Among the tools for the design of chemical sensors, the screen-printed electrodes (SPEs) stand out, since these devices fulfill many of the ideal characteristics of electrochemical sensors. Many applications have been resolved using screen-printed electrodes in sensor and biosensor technology[14]. Gold based screen-printed electrodes have been employed for mercury determination in water[15]or fish samples[16].

The excellent properties of nanomaterials can be exploited to solve analytical problems more efficiently than to date. Nanomaterials are especially interesting for its application to electrochemical sensors, producing beneficial effects such as increased mass transport and electron transfer, catalytic activity, and enhancement of the analytical signal due to its high volume-surfacerelation. Among the most widely used nanomaterials in electrochemical analysis are metal nanoparticles[17,18] and carbon nanotubes[19]. In the last years, different nanomaterials have been employed for electrochemical biosensors such as gold nanoparticles[20], carbon nanotubes[21] and graphene[22]. Forthe electrochemical determination of mercury, gold nanoparticlesare a promising electrode material, as it combines its properties as nanomaterial and the high affinity for mercury.In fact, glassy carbon electrodes have been employed for mercury analysis modified with different nanomaterials such as gold nanoparticles[23], carbon nanotubes[24], and nanohybrids such as gold nanoparticles/carbon nanotubes[25] or gold nanoparticles/graphene[26].

Screen-printed electrodes can be easily modified with different nanomaterials so that after the modification is possible the resolution of new analytical problems.Modification of SPEs with nanomaterials has been previously studied by our group. For instance,Martínez-Paredes et al.[27]studied several methodologies for the modification of SPEs with gold nanoparticles and the best results were obtained using an electrochemical method. With this methodology the nanoparticles can be optimized for a particular application, with a controllable diameter. Furthermore, the distribution of the generated nanoparticles is fairly homogeneous forming an array-type electrode.For modification with carbon nanotubes, the most significant problem is the efficient dispersion of the nanotubes in solution. Fanjul-Bolado et al. developed a method with good results for the modification of SPEs with carbon nanotubes[28].

Screen-printed carbon electrodes modified with gold nanoparticles have been employed for the determination of many analytes such as lead[29] or chromium[30], and for application togeno-[31] and immunosensors[32]. Also, carbon nanotubes are useful for modification of SPEs, resolving some problems such as p-aminophenol determination[33]. A gold film on screen-printed carbon electrodes has been used for mercury and lead determination in tap water after preconcentration with magnetic particles[34].Due to the excellent properties of those nanostructures, the modification of SPEs with nanohybrid materials is a current trend [35]. This is an innovative technology and is starting to be used to solve important clinical problems such as the detection of celiac disease[36].

In this work, we propose an original methodology using different nanohybrid materials on screen-printed carbon electrodes as electrochemical transducers for the construction of chemical sensors for mercury determination in water.

2. Experimental

2.1. Apparatus and electrodes

Voltammetric measurements were performed with an Autolab PGSTAT 12 (Eco Chemie, The Netherlands) potentiostat/galvanostat interfaced to an AMD K6 266 MHz computer system and controlled by Autolab GPES 4.9. All measurements were carried out at room temperature.

Screen-printed carbon electrodes(SPCEs) were purchased from DropSens (Spain). These electrodes incorporate a conventional three-electrode configuration, printed on ceramic substrates (3.4 x 1.0 cm). Both working (disk-shaped 4 mm diameter) and counter electrodes are made of carbon inks, whereas pseudoreference electrode and electric contacts are made of silver. An insulating layer was printed over the electrode system, leaving uncovered the electric contacts and a working area which constitutes the reservoir of the electrochemical cell, with an actual volume of 50 µL. Screen-printed gold electrodes (SPAuEs) were also purchased from DropSens and have the same design than those of carbon, but in this case, working and counter electrodes are made of gold paste cured at low

temperature. The SPEs were connected to the potentiostat through a specific connector (DropSens, ref. DSC).

A JEOL 6610LV scanning electron microscope (30 kV, Japan) was used to characterize the working electrodes. An Elmasonic P ultrasonic bath (Elma GmbG, Germany) was also employed.

An Element Finnigan MAT instrument was used for ICP-MS as the reference technique for the analysis of mercury using a standard protocol.

2.2. Reagents and solutions

Standard gold (III) tetrachloro complex was purchased from Merck (1.000 ± 0.002 g of tetracholoraurate(III) in 500 mL 1.0 M HCl). Carboxyl modified multiwalled carbon nanotubes (MWCNTs) were purchased from Nanocyl (Belgium, ref. 3151).Graphene oxide (GO) was kindly provided by Nanoinnova Technologies (Spain).Mercury acetate was purchased from Fluka (> 99.0 % purity). Lead nitrate, fuming hydrochloric acid (37.0 %), N,N-dimethylformamide (DMF) (99.8 %) and standard solutions of Cd(II), Se(IV) and Zn(II) (1000 mg/L) were purchased from Merck. Stock solutions of Hg(II) and Pb(II) (0.50 g/L) were prepared in 0.10 M hydrochloric acid. Copper sulfate was purchased from Probus. Ultrapure water obtained with a Millipore Direct Q5™ purification system from Millipore Ibérica S.A. (Madrid, Spain) was used throughout this work. All other reagents were of analytical grade.

Drinking water samples were collected from a running water tap in our lab at the Department of Physical and Analytical Chemistry, University of Oviedo. River water samples were collected from Arlos River located in Llaranes (Asturias, Spain).

MWCNTs solution was prepared by mixing 1.0 mg of MWCNT-COOH with 1.0 ml of a mixture DMF:water (1:1) by sonication using an ultrasonic bath for 2 h.A dilution of this solution was made for a final concentration of 0.10 g/L by sonication for 30 minutes. GO solutions were made in water using the same procedure. Diluted solutions of gold tetrachloroaurate and mercury acetate were prepared by suitable dilution with 0.10 M hydrochloric acid.

2.3. Procedures

2.3.1. Modification of screen-printed electrodes with nanomaterials

Gold nanostructures were generated in-situ over SPCEs (SPCnAuEs) following a method developed by Martínez-Paredes et al.[27]. It consists in dropping an aliquot of 40 µL of an acidic solution of $AuCl_4^-$ on the electrode surface and applying a constant current intensity for a specific period of time.

Modification of SPEs with carbon nanotubes was carried out following a method developed by Fanjul-Bolado et al.[28]. It consists in depositing an aliquot of 4 µL of the MWCNT-COOH dispersion (0.10 g/L in DMF:water) on the working electrode surface. The solution was left to dry at room temperature

(20 °C) until its absolute evaporation. Modified electrodes were carefully washed with water and dried at room temperature.

Modification of SPEs with graphene oxide was carried out depositing an aliquot of 10 µL of the graphene oxide dispersion (0.10 g/L in water) on the working electrode surface. The solution was left to dry at room temperature (20 °C) until its absolute evaporation. Modified electrodes were carefully washed with water and dried at room temperature.

In this work, two different nanohybrid transducers (SPGOnAuEs and SPCNTnAuEs) were prepared. Those transducers were prepared modifying the working electrode with GO or MWCNTs respectively, followed by gold nanostructuration using the explained procedures. This method has been previously used for modification of SPEs with nanohybrid materials by Neves et al.[35].

2.3.2. Characterization of the gold nanostructured sensors

Gold nanostructures were characterized by SEM and by chronoamperometry. For the optimized sensors (SPCnAuEs, SPGOnAuEs and SPCNTnAuEs), SEM images were obtained and the mean diameter of the gold nanoparticles was measured.Chronoamperometry measurements were performed to determine the amount of gold deposited on the different sensors. An aliquot of 40 µL of 0.10 M HCl was dropped onto the electrode and the oxidation of gold was carried out by holding the electrode at a potential of +0.85 V, recording the current intensity vs. time. Three electrodes were measured for each kind of sensor. The area under the curve (charge) was used to calculate the mass of gold involved in the process using the Faraday equation.

2.3.3. Voltammetric measurements of mercury

Mercury was preconcentrated over the sensor by applying a constant potential of +0.30 V for a period of time optimized for every different transducer. Then, the potential is switched between +0.30 V and +0.55 V using square-wave voltammetry (SWV), at a frequency, amplitude and step potential optimized for every transducer. All measurements were performed without removing oxygen from the solution, and using an aliquot of 40 µL of the appropriate solution.

Voltammograms obtained for mercury on gold, generally, have a broad baseline. This is consistent with data obtained by other authors as Welch et al. [37] and due to this for low concentrations is difficult to measure the peak height. For this kind of voltammograms, the GPES software has a tool to get a more defined peak and therefore to measure its height in an easier way. This tool is called “Baseline correction” and is located in the “Edit data” menu of the GPES software. This treatment was done to the voltammograms obtained for the calibration plots, and then the peak height was measured more accurately.

2.3.4. Simultaneous voltammetric measurements of mercury and lead

Mercury and lead were preconcentrated over the sensor by applying a constant potential of -0.55 V for 120 s. Then, the potential is switched between -0.55 V and +0.55 V using square-wave

voltammetry, at a frequency of 50 Hz, amplitude of 25 mV and a step potential of 4 mV. All measurements were performed without removing oxygen from the solution, and using an aliquot of 40 µL of the appropriate solution.

3. Results and discussion

3.1. Electrochemical behavior of mercury

Mercury behavior on different electrode surfaces was studied using cyclic voltamperometry (Fig. 1). For SPCEs (Fig. 1A), two cathodic processes at potentials of -0.30 (C_5) and -0.62 V (C_B) were observed. The -0.62 V process occurs in the blank solution, therefore it is not caused by mercury, while the other process corresponds to the bulk deposition of mercury on SPCEs. An anodic stripping peak appears at a potential of +0.03 V (A_5), which corresponds to the reoxidation of mercury.

For SPAuE(Fig. 1B) and SPCnAuEs (Fig. 1C) cases, it can be observed other mercury processes at potentials more positive than the bulk deposition and its corresponding stripping process on SPCEs. In both cases, 5 cathodic peaks are observed (SPAuEs: -0.20 V (C_5), -0.06 V (C_4), +0.02 V (C_3), +0.25 V (C_2), +0.42 V (C_1); SPCnAuEs: -0.23 V (C_5), -0.05 V (C_4), +0.03 V (C_3), +0.32 V (C_2), +0.47 V (C_1)). For SPAuEs, 4 anodic peaks were observed (+0.00 V (A_5), +0.07 V (A_n), +0.23 V (A_2), +0.43 V (A_1)), while for SPCnAuEs, 5 anodic peaks were observed (+0.01 V (A_5), +0.05 V (A_4), +0.15 V (A_3), +0.30 V (A_2), +0.48 V (A_1)). It is likely that in the case of SPAuEs, two of the anodic peaks perfectly observed in SPCnAuEs(A_3, A_4) are overlapped (A_n). Another visible difference is that the cathodic processes for SPCnAuEs occur to slightly more positive potentials than for SPAuEs. This behavior could be explained due to the bigger catalytic activity of gold nanoparticles, and therefore the preconcentration of mercury on the electrode occurs more easily. Using SPCNTnAuEs and SPGOnAuEs transducers, the behavior is similar to the obtained with SPCnAuEs. The observation of 5 redox processes for the mercury preconcentration on gold electrodes is consistent with those obtained in other works, such as the one realized by Herrera et al.[38].

The two peaks appearing at more positive potentials (C_1 and C_2) in the presence of gold correspond to the formation of a monolayer of atoms of mercury on the gold substrate at more positive potentials than the reversible potential of Nernst for the formation of bulk metal, referred to as underpotential deposition (UPD).

There are other processes besides the UPD ones and the bulk deposition. These are due to different redox processes of mercury, which are probably influenced by the presence of Cl^- ions[39].

UPD processes are comparable to an adsorption process where the mercury is adsorbed and reduced on gold at various stages. The presence of gold promotes the adsorption of mercury atoms on the surface, produces a mercury reduction thermodynamically more favorable, and therefore, the mercury reduction potential shifts to more positive potentials. The equivalent oxidation process also

requires a more positive potential because the stripping of the adsorbed mercury on gold will be more difficult.

Avoltammogram for SPAuEs and SPCnAuEs at the potentials where occurs the first UPD process (C_1 and A_1 processes) is shown in Fig. 2A. It can be observed as the peak intensity in the case of SPCnAuEs (continuous line) is higher than for SPAuEs (dashed line) for the same mercuryconcentration. Hence, the use of gold nanoparticles as electrode surface improves the analytical signal produced. This may be due to the bigger surface area of the nanoparticles and the array-type arrangement on the carbon surface. Then, the use of gold nanoparticles is more suitable for the determination of mercury.

Cyclic voltammograms for two different concentrations of mercury on SPCnAuEs are shown in Fig. 2B. For the case of low concentrations (dashed line), only the first UPD is observed, whereas for a higher concentration (continuous line), both UPD processes are observed. The second UPD only occurs when the first UPD is saturated, therefore for low concentrations of mercury (normally for uncontaminated and low-contaminated environmental samples) only the first UPD occurs. Thus, the first UPD process is best suited for use in the determination of very low amounts of mercury.

The variation of the intensity of the first UPD peak with the scan rate of the cyclic voltammetry was studied.Both the cathodic and corresponding anodic stripping peaks of the first mercury UPD were linear with scan rate, according to the following equations:

Cathodic peak: i_p (μA) = 0.009*v* (mV/s) + 0.510,R^2 = 0.997

Anodic stripping peak: i_p (μA) = 0.012*v*(mV/s) + 0.692,R^2 = 0.993

According to this behaviorcan be suggested thatthe UPD is a process controlled by adsorption, as is described in previous works[40].

Moreover, it was observed that the peak separation (ΔE_p) between the anodic and cathodic peaks in SPCEsand SPCnAuEs were 330 and 10 mV (first UPD), respectively. This improvement in the electrochemical performance is due to the strong interaction between mercury and gold, and to the beneficial properties of gold nanoparticles.

Due to the behavior of the first UPD process, it can be used for the determination of very low mercury concentrations when combined with anodic stripping voltammetry after a preconcentration step on the working electrode. Besides, the use of gold nanoparticles on SPCEs improves the analytical signal, showing clear advantages for its use instead of SPAuEs.

3.2. Study of different nanostructured transducers for mercury determination

Several gold nanostructures on SPCEs, SPCNTEs and SPGOEs were studied in order to obtain the most suitable nanostructured sensor for mercury analysis. Thus, different sensors were fabricated with gold nanoparticles generated under different experimental conditions (current, deposition time and $AuCl_4^-$ concentration). The analytical signal for 50 µg/L of mercury was measured using each fabricated sensor. The highest analytical signals were obtained using -100 µA of current, a deposition time of 180 s, and a concentration of $AuCl_4^-$ of 1.0mM(data not shown).

The different electrode surfaces generated by the optimized experimental conditions were characterized by scanning electronic microscopy (SEM), and the amount of gold was calculated by chronoamperometry. The data obtained is summarized in Table 1 andSEM images are shown in Fig. 3.

In the SEM images the gold nanoparticles appear as brighter spots than the carbon surface. Both the dispersion and the size of the gold nanoparticles are different for each different sensor (SPCnAuEs, SPCNTnAuEs and SPGOnAuEs), although the experimental conditions in which these nanoparticles were generated were the same. For SPCNTnAuEs, the electrode is covered by two groups of gold nanoparticles with a different mean diameter, whereas in the case of SPGOnAuEs, there are three groups of nanoparticles. One of the groups has a greaternumber of nanoparticles.The difference in diameters and groups of gold nanoparticles can be explained due to that the generation of the nanoparticles is performed on the different carbon materials. These materials (carbon, carbon nanotubes and graphene oxide)results in different electrodic surfaces where the nucleation and growth of the gold nanoparticles will be different.

This fact indicates that the carbon surface where the nanoparticles are electrochemically generated affects the size, dispersion and amount of gold nanoparticles. Therefore, depending on the application, the experimental conditions of the generation of the gold nanoparticles must be optimized for each different material of the working electrode.

3.3. Analytical response for the different transducers

The variable parameters for the analysis were optimized to find the most suitable to solve the issue of the sensible mercury determination. Different electrolytic mediums were studied by Giacomino et al.[41], and concluded that HCl was the most appropriate. Other authors have shown that a constant concentration of Cl^- ions is important for mercury analysis[42]. Therefore, HCl was chosen as the electrolytic medium and a study of several concentrations of HCl (0.01, 0.05, 0.10 and 0.50 M) was carried out. The analytical signal for 50 µg/L of mercury was measured, and the highest signal was obtained with a concentration of 0.10 M. Giacomino et al. also concluded that the square wave voltammetry technique was the most suitable for stripping of the deposited mercury. The UPD process has a good reversibility (as shown in Fig. 2B), so the characteristics of the square wave voltammetry techniquecan improve the sensitivity of the analytical signal. Thus, in this work, SWV was used as the stripping technique.

The deposition potential was set to +0.30 V because more negative potentials could produce the second UPD process or even the bulk deposition and using more positive potentials the analytical signal decreases. Thus, using +0.30 V,only wasobserved an anodic peak atapproximately +0.43 V, due to the stripping of the deposited mercury on the gold nanoparticles.

According to some authors, the major drawback of gold electrodes is the well-known phenomenon of structural changes of their surface, caused by amalgam formation[43,44]. Hence, in many of the works where mercury is electrochemically determined is necessary a pretreatment to the electrode before the analysis[37,45]. Inukai et al. described that it only happens when bulk deposition takes place[46].In our work, employing gold nanoparticles and the first UPD process, it was not necessary any pretreatment before measurements. Despite the disposable character of these electrodes, when the mercury is present in a simple matrix, the electrode can be reused rinsing it with water between each measurement.

Other parameters as the deposition time, frequency, amplitude and potential step of the square wave were optimized for each nanostructured sensor.Measurements of 20 µg/L Hg (II) standard solution in HCl 0.10 M were made and the obtained signal was evaluated. The criterium for selecting the optimized value was the evaluation of the peak height of the analytical signal. The optimized values for deposition time, frequency, amplitude and potential step for the SPCnAuEs sensor were: 240 s, 80 Hz, 30 mV and 4 mV, respectively. For the SPCNTnAuEs and SPGOnAuEs sensors were: 200 s, 40 Hz, 20 mV and 8 mV, respectively.

With the optimized methodology, calibration plots were obtained for mercury using each fabricated sensor (SPCnAuEs, SPGOnAuEs, SPCNTnAuEs).The calibration plot and the corresponding voltammograms (after the baseline correction process explained in section 2.3.3) for the SPCNTnAuE sensor are shown in Fig. 4.

Table 2 show different analytical data such as the linear range, slope of the calibration plots as well as the detection limits (calculated as the concentration corresponding to a signal that is three times the standard deviation of the intercept) and interelectrodic reproducibility for each kind of sensor. Although all sensors are able to detect low concentrations of mercury, there are significant differences in the characteristics obtained for each one.

With this data, it was observed that the use of nanohybrid materials as electrodic surface,in comparison with SPCnAuEs, improves considerably the linear range and the limit of detection. This improvement may be due to the fact that the electrodic surface in these sensors consists entirely of nanomaterials with the advantages that it entails, since both the carbon nanotubes and grapheneoxide completely covers the working electrode and the previous carbon electrode only works as an electrical contact.The obtained sensitivity (slope of the calibration plot) for the SPCNTnAuE sensors was clearly higherto the obtained for SPCnAuEs. The lowest sensitivity was obtained for the SPGOnAuE sensors. For SPCnAuEs, the saturation of the first UPD appeared to a higher concentration. This data is consistent with the amountof gold available on the different sensors (Table

1). Moreover, it was obtained the intraelectrodic reproducibilitywith similar values for each sensor: 8.5 % (SPCnAuEs), 11.4 % (SPCNTnAuEs) and 9.4 % (SPGOnAuEs) (eight repeated measurements for 50, 10 and 20 µg/L of mercury, respectively).

The best analytical characteristics were presented by the nanohybrid sensor fabricated with carbon nanotubes and gold nanoparticles (SPCNTnAuEs), since the detection limit of 0.2µg/L is lower than that obtained with the other developed sensors, and moreover the sensitivity is better. Also, the linear range for this sensor is wider, reaching two orders of magnitude. Even, the interelectrodicreproducibility is excellent, presentinga small deviation, the use of different sensors is not going to change too much the results obtained.

The analytical characteristics that the SPCNTnAuE sensor offers, allow carrying out an analysis of mercury in water because it fulfill the requirements of the guidelines set by WHO (6.0µg/L)[47], and also different regulations of the water quality for human consumption such as the U.S. EPA regulations (2.0µg/L) or the Spanish laws (1.0µg/L).

The detection limit obtained with this SPCNTnAuE sensor lower compared with other published works where portable screen-printed electrodes are used as transducers for mercury determination in water.Thus, Khaled et al.[48]obtained a 2.0µg/L detection limit using chitosan modified SPCEs.Bernalte et al. achieved a detection limit of 1.1 µg/L employing commercial gold screen-printed electrodes[15]and 0.8 µg/L using commercial gold nanostructured screen-printed carbon electrodes[49]. Other authors such as Mandil et al.[34] andLaschi et al.[42]obtained,using gold modified screen-printed carbon electrodes,1.5 and 0.9µg/L, respectively.Besides the lower detection limit, the sensor developed in the present work is able to detect a concentration as low as 0.5 µg/L while in the mentioned works the lowest concentration of the calibration plots is 2.5 µg/L. This is a clear advantage becauseif a sample is contaminated with 1.0 µg/L of mercury and therefore is above the legal limits in some countries, the SPCNTnAuE sensor is able to detect it.

The possible interferences presented by Cu (II), Se (IV), Zn (II), Cd (II) and Pb (II) were studied to evaluate the selectivity of the developed sensor (Fig. 5A). A solution of 10 µg/L of mercury was measured in presence of 100-fold concentration of the interfering metal. The results showed that the measured signal for mercury was not statistically different in presence of these metals, indicating that these metal ions do not interfere with the detection of mercury. The deposition potential applied to produce the first UPD of mercury on gold is +0.30 V, this methodology is highly selective for mercury because practically no other metal is reduced applying this potential.

To study the stability of the SPCNTnAuEs, several sensors were prepared and stored at room temperature. A solution of 10 µg/L of mercury was measured for different days of the fabrication. The results obtained are shown in Fig. 5B.While the sensor lost part of the analytical signal measuredin the day of its fabrication, it holds the 85 % of the initial signal, which remains constant during the first 30 days.

3.4. Mercury analysis in tap water with SPCNTnAuEs

In order to evaluate the performance of the SPCNTnAuE sensor in real samples, an analysis of tap and river water was carried out using the standard addition method. Mercury level was undetectable because, in normal conditions, its concentration should be below the detection limit of the method. Therefore, the samples were spiked with 1.0, 5.0, and 10.0 µg/L of mercury and were taken as the problem samples.

Every sample was analyzed by triplicate and the results obtained are shown in Table 3. The recovery yields for the river samples were, in general, lower than for the tap water samples. The more complex matrix of the river samples may be the reason of this difference. The results obtained demonstrate that the sensor developed in this work can be applied to the accurate determination of mercury in real samplesand the obtained results are in good agreement with the determination using ICP-MS as the reference technique.A student's t-test, at a 95 % confidence level, showed no statistical difference between both methods.

3.5. Analytical response for the simultaneous determination of lead and mercury

Lead is another metal that presents the underpotential deposition on gold electrodes and has been determined using gold nanoparticles on screen-printed carbon electrodes[29]. In the same way as mercury, the most interesting process is the first UPD. The first UPD of lead in HCl 0.1 M occurs at -0.25 V[27]. Therefore, the separation between the first UPD for lead and mercury allows the simultaneous electrochemical determination of both metals.

The ability of the developed sensor (SPCNTnAuE) for the simultaneous determination of lead and mercury was evaluated. Calibration plots were obtained and are described by thefollowing linear regressions: $i\ (\mu A) = 2.766 + 0.203 \cdot [Pb^{2+}]$, and $i\ (\mu A) = 0.209 + 0.228 \cdot [Hg^{2+}]$, for lead and mercury, respectively. These calibration plots showed a linear range of 2-100 µg/L and 2-60 µg/L with a limit of detection of 2.0 and 1.9 µg/L for lead and mercury, respectively.Interelectrodic reproducibility was 3.4% for lead and 4.1% for mercury.

The competition between lead and mercury for gold sites may be the main cause for obtaining a detection limit for mercury not as low as in the case of the separate determination of mercury. The results obtained demonstrate the possibility to fabricate a cheap, fast and low cost sensor for the simultaneous determination of two toxic heavy metals at µg/L range of concentration.

4. Conclusions

The UPD process is a useful analytical tool for the electrochemical analysis of some metals, such as mercury, on gold electrodes. Its behavior as an adsorption process, allows a more efficient preconcentration of mercury on the electrode, and therefore a lower concentration can be detected.

Gold nanostructures allow the electrochemical determination of mercury with a good performance, resulting in better sensitivity and better analytical characteristics than gold continuous surfaces or conventional electrodes.

Moreover, if a nanohybrid material combining carbon nanotubes and gold nanoparticles is used as electrode material, the characteristics of the method are significantly improved.

We used these excellent tools for the fabrication of an electrochemical sensor that allows a fast, cheap, and easy analysis of mercury in water detecting concentrations below the legal limits. Due to the portability of the instrumentation employed with screen-printed electrodes, the proposed method could be used for in-situ measurements of environmental samples.

This work demonstrates that the modification of SPEs with nanohybrid materials is a wide open window to explore for solving new analytical problems in a very simple way.

References

[1] E.G. Pacyna, J.M. Pacyna, F. Steenhuisen, S. Wilson, Global anthropogenic mercury emission inventory for 2000, Atmos. Environ. 40 (2006) 4048–4063.

[2] Q. Wang, D. Kim, D.D. Dionysiou, G.A. Sorial, D. Timberlake, Sources and remediation for mercury contamination in aquatic systems–a literature review, Environ. Pollut. 131 (2004) 323–336.

[3] N. Bloom, W.F. Fitzgerald, Determination of volatile mercury species at the picogram level by low-temperature gas chromatography with cold-vapour atomic fluorescence detection, Anal. Chim. Acta. 208 (1988) 151–161.

[4] J. Murphy, P. Jones, S.J. Hill, Determination of total mercury in environmental and biological samples by flow injection cold vapour atomic absorption spectrometry, Spectrochim. Acta, Part B. 51 (1996) 1867-1873.

[5] J.S. dos Santos, M. de la Guardia, A. Pastor, M.L.P. dos Santos, Determination of organic and inorganic mercury species in water and sediment samples by HPLC on-line coupled with ICP-MS, Talanta. 80 (2009) 207-11.

[6] EPA, Mercury in aqueous samples and extracts by anodic stripping voltammetry, Method 7472, n.d.

[7] J.-M. Zen, M.-J. Chung, Square-wave voltammetric stripping analysis of mercury(II) at a poly(4-vinylpyridine)/gold film electrode, Anal. Chem. 67 (1995) 3571-3577.

[8] P. Salaün, C.M.G. van den Berg, Voltammetric detection of mercury and copper in seawater using a gold microwire electrode, Anal. Chem. 78 (2006) 5052-60.

[9] E. Herrero, L.J. Buller, H.D. Abruña, Underpotential deposition at single crystal surfaces of Au, Pt, Ag and other materials, Chem. Rev. 101 (2001) 1897-930.

[10] R.D. Riso, M. Waeles, P. Monbet, C.J. Chaumery, Measurements of trace concentrations of mercury in sea water by stripping chronopotentiometry with gold disk electrode: influence of copper, Anal. Chim. Acta. 410 (2000) 97-105.

[11] E. Viltchinskaia, L. Zeigman, D. Garcia, P. Santos, Simultaneous determination of mercury and arsenic by anodic stripping voltammetry, Electroanalysis. 9 (1997) 633–640.

[12] O. Ordeig, C.E. Banks, J. del Campo, F.X. Muñoz, R.G. Compton, Trace Detection of Mercury (II) Using Gold Ultra-Microelectrode Arrays, Electroanalysis. 18 (2006) 573-578.

[13] H. Huiliang, D. Jagner, L. Renman, Simultaneous determination of mercury (II), copper (II) and bismuth (III) in urine by flow constant-current stripping analysis with a gold fibre electrode, Anal. Chim. Acta. 202 (1987) 117–122.

[14] K.C. Honeychurch, J.P. Hart, Screen-printed electrochemical sensors for monitoring metal pollutants, TrAC, Trends Anal. Chem. 22 (2003) 456-469.

[15] E. Bernalte, C. Marín Sánchez, E. Pinilla Gil, Determination of mercury in ambient water samples by anodic stripping voltammetry on screen-printed gold electrodes, Anal. Chim. Acta. 689 (2011) 60-4.

[16] V. Meucci, S. Laschi, M. Minunni, C. Pretti, L. Intorre, G. Soldani, et al., An optimized digestion method coupled to electrochemical sensor for the determination of Cd, Cu, Pb and Hg in fish by square wave anodic stripping voltammetry, Talanta. 77 (2009) 1143-8.

[17] D. Hernández-Santos, M.B. González-García, A. Costa García, Metal-nanoparticles based electroanalysis, Electroanalysis. 14 (2002) 1225–1235.

[18] F.W. Campbell, R.G. Compton, The use of nanoparticles in electroanalysis: an updated review, Anal. Bioanal. Chem. 396 (2010) 241-59.

[19] M.T. Fernández-Abedul, A. Costa-García, Carbon nanotubes (CNTs)-based electroanalysis, Anal. Bioanal. Chem. 390 (2008) 293–298.

[20] J.M. Pingarrón-Carrazón, P. Yáñez-Sedeño, A. González-Cortés, Gold nanoparticle-based electrochemical biosensors, Electrochim. Acta. 53 (2008) 5848–5866.

[21] A. Merkoc□i, M. Pumera, X. Llopis, B. Perez, M. Delvalle, S. Alegret, New materials for electrochemical sensing VI: Carbon nanotubes, TrAC, Trends Anal. Chem. 24 (2005) 826-838.

[22] T. Kuila, S. Bose, P. Khanra, A.K. Mishra, N.H. Kim, J.H. Lee, Recent advances in graphene-based biosensors, Biosens. Bioelectron. (2011).

[23] O. Abollino, A. Giacomino, M. Malandrino, G. Piscionieri, E. Mentasti, Determination of Mercury by Anodic Stripping Voltammetry with a Gold Nanoparticle-Modified Glassy Carbon Electrode, Electroanalysis. 20 (2008) 75–83.

[24] H. Yi, Anodic stripping voltammetric determination of mercury using multi-walled carbon nanotubes film coated glassy carbon electrode, Anal. Bioanal. Chem. 377 (2003) 770-4.

[25] H. Xu, L. Zeng, S. Xing, G. Shi, Y. Xian, L. Jin, Microwave-radiated synthesis of gold nanoparticles/carbon nanotubes composites and its application to voltammetric detection of trace mercury(II), Electrochem. Commun. 10 (2008) 1839-1843.

[26] J. Gong, T. Zhou, D. Song, L. Zhang, Monodispersed Au nanoparticles decorated graphene as an enhanced sensing platform for ultrasensitive stripping voltammetric detection of mercury(II), Sens. Actuators, B. 150 (2010) 491-497.

[27] G. Martínez-Paredes, M.B. González-García, A. Costa-García, In situ electrochemical generation of gold nanostructured screen-printed carbon electrodes. Application to the detection of lead underpotential deposition, Electrochim. Acta. 54 (2009) 4801–4808.

[28] P. Fanjul-Bolado, P. Queipo, P.J. Lamas-Ardisana, A. Costa-García, Manufacture and evaluation of carbon nanotube modified screen-printed electrodes as electrochemical tools, Talanta. 74 (2007) 427-33.

[29] G. Martínez-Paredes, M.B. González-García, A. Costa-García, Lead Sensor Using Gold Nanostructured Screen-Printed Carbon Electrodes as Transducers, Electroanalysis. 21 (2009) 925–930.

[30] G. Liu, Y. Lin, Voltammetric detection of Cr(VI) with disposable screen-printed electrode modified with gold nanoparticles, Environ. Sci. Technol. 42 (2008) 3117.

[31] G. Martínez-Paredes, M.B. González-García, A. Costa-García, Genosensor for SARS Virus Detection Based on Gold Nanostructured Screen-Printed Carbon Electrodes, Electroanalysis. 21 (2009) 379-385.

[32] G. Martínez-Paredes, M.B. González-García, A. Costa-García, Genosensor for detection of four pneumoniae bacteria using gold nanostructured screen-printed carbon electrodes as transducers, Sens. Actuators, B. 149 (2010) 329-335.

[33] P.J. Lamas-Ardisana, P. Queipo, P. Fanjul-Bolado, A. Costa-García, Multiwalled carbon nanotube modified screen-printed electrodes for the detection of p-aminophenol: Optimisation and application in alkaline phosphatase-based assays, Anal. Chim. Acta. 615 (2008) 30–38.

[34] A. Mandil, L. Idrissi, A. Amine, Stripping voltammetric determination of mercury(II) and lead(II) using screen-printed electrodes modified with gold films, and metal ion preconcentration with thiol-modified magnetic particles, Microchim. Acta. 170 (2010) 299-305.

[35] M.M. Pereira da Silva Neves, M.B. González-García, C. Delerue-Matos, A. Costa-García, Nanohybrid Materials as Transducer Surfaces for Electrochemical Sensing Applications, Electroanalysis. 23 (2011) 63–71.

[36] M.M.P.S. Neves, M.B. González-García, H.P.A. Nouws, A. Costa-García, Celiac disease detection using a transglutaminase electrochemical immunosensor fabricated on nanohybrid screen-printed carbon electrodes, Biosens. Bioelectron. In Press (2011).

[37] C.M. Welch, O. Nekrassova, X. Dai, M.E. Hyde, R.G. Compton, Fabrication, characterisation and voltammetric studies of gold amalgam nanoparticle modified electrodes, ChemPhysChem. 5 (2004) 1405–1410.

[38] E. Herrero, H.D. Abruña, Underpotential Deposition of Mercury on Au(111): Electrochemical Studies and Comparison with Structural Investigations, Langmuir. 13 (1997) 4446-4453.

[39] J. Li, E. Herrero, H.D. Abruña, The effects of anions on the underpotential deposition of Hg on Au (111) An electrochemical and in situ surface X-ray diffraction study, Colloids Surf., A. 134 (1998) 113–131.

[40] J. Li, H.D. Abruña, Phases of Underpotentially Deposited Hg on Au(111): An in Situ Surface X-ray Diffraction Study, J. Phys. Chem. B. 101 (1997) 2907-2916.

[41] A. Giacomino, O. Abollino, M. Malandrino, E. Mentasti, Parameters affecting the determination of mercury by anodic stripping voltammetry using a gold electrode, Talanta. 75 (2008) 266-73.

[42] S. Laschi, I. Palchetti, M. Mascini, Gold-based screen-printed sensor for detection of trace lead, Sens. Actuators, B. 114 (2006) 460-465.

[43] X. Yang, In-situ atomic force microscope observation of stripping of mercury from Hg/Au alloy films in acidic media, Surf. Sci. 324 (1995) L363-L366.

[44] L.E. Barrosse-Antle, L. Xiao, G.G. Wildgoose, R. Baron, C.J. Salter, A. Crossley, et al., The expansion/contraction of gold microparticles during voltammetrically induced amalgamation leads to mechanical instability, New J. Chem. 31 (2007) 2071.

[45] J. Wang, D. Larson, N. Foster, S. Armalis, J. Lu, X. Rongrong, et al., Remote electrochemical sensor for trace metal contaminants, Anal. Chem. 67 (1995) 1481-1485.

[46] J. Inukai, S. Sugita, K. Itaya, Underpotential deposition of mercury on Au(111) investigated by in situ scanning tunnelling microscopy, J. Electroanal. Chem. 403 (1996) 159-168.

[47] W.H. Organization, Guidelines for drinking-water quality, Fourth, Geneva, 2011.

[48] E. Khaled, H. Hassan, I. Habib, Chitosan Modified Screen-Printed Carbon Electrode for Sensitive Analysis of Heavy Metals, Int. J. Electrochem. 5 (2010) 158-167.

[49] E. Bernalte, C.M. Sánchez, E.P. Gil, Gold Nanoparticles-Modified Screen-Printed Carbon Electrodes For Anodic Stripping Voltammetric Determination Of Mercury In Ambient Water Samples, Sens. Actuators, B. In Press (2011).

Biographies

Daniel Martín-Yerga obtained her B.Sc. degree in chemistry, focus on analytical chemistry in 2010 (University of Oviedo) and the M.Sc. degree in analytical and bioanalytical chemistry in 2011 (University of Oviedo). At present, he is collaborating with the Immunoelectroanalytical Research Group of the University of Oviedo, supervised by Prof. A. Costa-García.

MaríaBegoña González-García obtained her B.Sc. degree in chemistry, focus in analytical chemistry, in 1991 (University of Oviedo) and the Ph.D. in chemistry in 1999 (University of Oviedo). Nowadays she is working as associated professor at the University of Oviedo and is a co-worker in the Immunoelectroanalytical Research Group of the same university, supervised by Prof. A. Costa-García.

Agustín Costa-García obtained his B.Sc. degree in chemistry, focus in analytical chemistry, in 1974 (University of Oviedo) and the Ph.D. in chemistry in 1977 (University of Oviedo). Since February 2000 he is professor in analytical chemistry (University of Oviedo). He leads the Immunoelectroanalytical Research Group of the University of Oviedo and has been supervisor of several research projects developed at the electrochemistry laboratories of the Department of Physical and Analytical Chemistry of the University of Oviedo. Nowadays his research is focused on the development of

nanostructured electrodic surfaces and its use as transducers for electrochemical immunosensors and genosensors employing both enzymatic and non-enzymatic labels.

Captions of Tables

Table 1: Mean diameter of the gold nanoparticles and amount of gold deposited on the different electrode surfaces. The value of mean diameter written with bold letters corresponds to the group with greater number of nanoparticles.

Table 2: Analytical characteristics obtainedfor different nanostructured sensors.

Table 3: Mercury determination in tap and river water samples using SPCNTnAuEsand ICP-MS after spiking with 1, 5 and 10 µg/L of mercury.

Captions of Figures

Figure 1: Cyclic voltammograms for 500 mg/L of mercury (continuous line) and blank solution (dashed line) for SPCEs (A), SPAuEs (B) and SPCnAuEs (C) in HCl 0.10 M. Scan rate: 100 mV/s.

Figure 2:(A) Cyclic voltammograms at potentials near the first UPD for 100 mg/L of mercury for SPCnAuEs (continuous line) and SPAuEs (dashed line). (B) Cyclic voltammograms for 5 mg/L (continuous line) and 250 µg/L (dashed line) of mercury, after deposition at 0.10 V during 60 s using SPCnAuEs. Scan rate: 100 mV/s. Supporting electrolyte: HCl 0.10 M.

Figure 3: SEM images of SPCnAuE (A),SPCNTnAuE(B) and SPGOnAuE (C). Magnification factor: $1x10^4$.

Figure 4:Calibration plot and square-wave voltammograms (after baseline correction method)obtained using SPCNTnAuEs for 0.5, 1, 2, 5, 10, 20, 40 and 50 µg/L of mercury in HCl 0.10 M. See Table 2 for experimental parameters. Deposition potential: +0.30 V.

Figure 5:(A) Effects of various metal ions on the analytical signal of Hg(II) using SPCNTnAuEs. Mercury concentration: 10 µg/L. Metal concentration: 10 mg/L. (B) Stability of the mercury SPCNTnAuE sensor for a month. Mercury concentration: 10 µg/L. Supporting electrolyte: HCl 0.10 M.

576
577
578 **Table 1**
579

	Diameter (nm)	Mass Au (ng)
SPCnAuEs	135 ± 16	(1.28 ± 0.08) x 10^2
SPGOnAuEs	298 ± 35 **154 ± 33** 90 ± 10	(8.4 ± 0.9) x 10^2
SPCNTnAuEs	**148 ± 12** 87 ± 10	(9.0 ± 0.8) x 10^2

580
581
582

582

583 **Table 2**

584

	Linear range (µg/L)	Slope (µA·L/µg)	R^2	DL (µg/L)	RSD (%)[1]
SPCnAuEs	5-100	0.120	0.996	3.3	7.3
SPGOnAuEs	2-50	0.082	0.996	1.9	16.4
SPCNTnAuEs	0.5-50	0.237	0.9998	0.2	3.0

585 [1] Reproducibility obtained for five electrodes

586

587

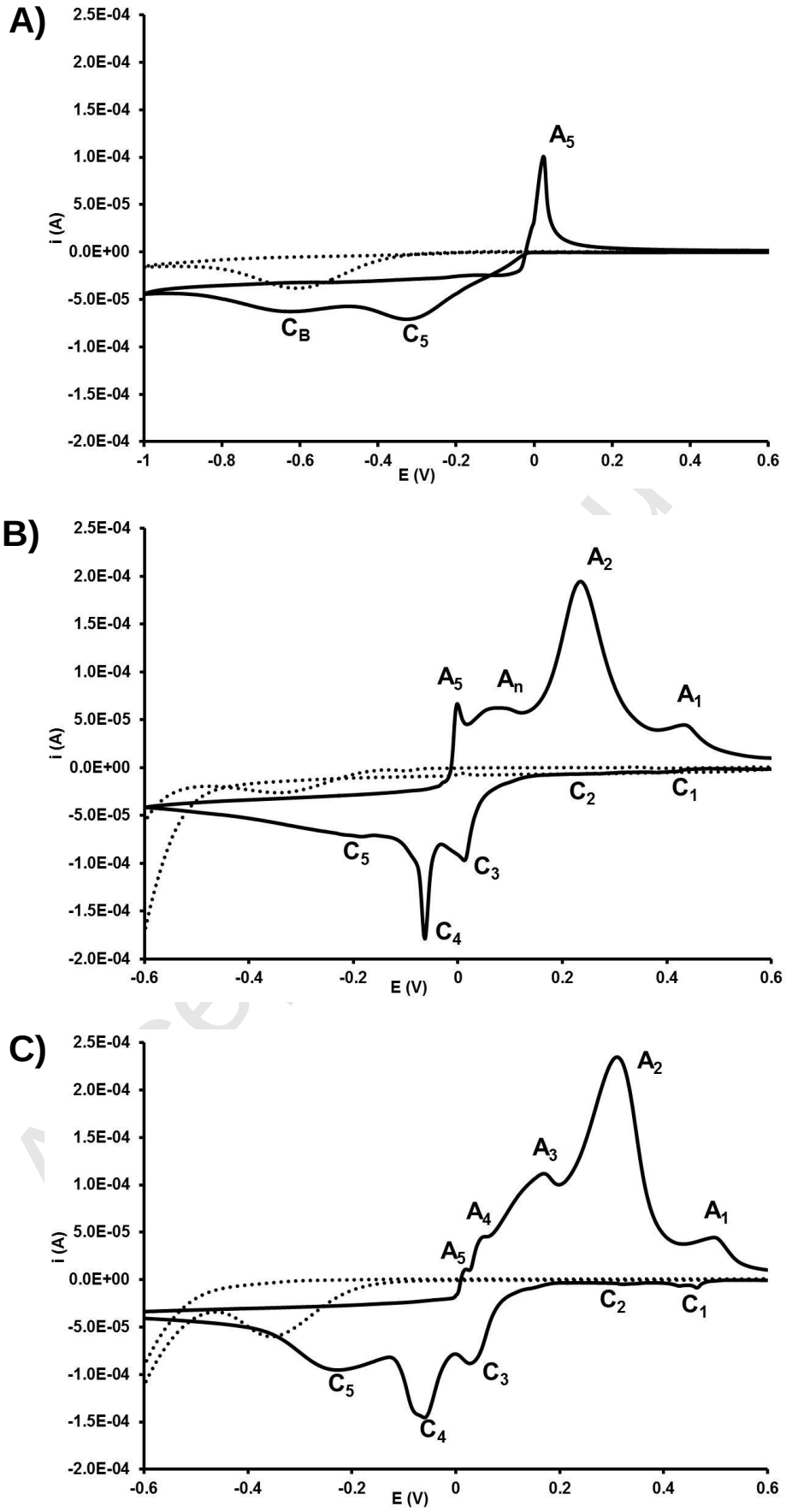
588

588
589
590
591

Table 3

	Added (µg/L)	Found (µg/L)	Recovery (%)	ICP-MS (µg/L)
Tap water	1	1.03 ± 0.07	95.3-108.3	0.98 ± 0.02
	5	4.9 ± 0.4	91.0-106.2	5.4 ± 0.2
	10	10.3 ± 0.8	95.7-111.5	10.6 ± 0.1
River water	1	0.8 ± 0.1	68.0-92.4	0.61 ± 0.04
	5	3.8 ± 0.4	70.6-83.8	3.80 ± 0.05
	10	7.7 ± 0.8	71.1-82.1	7.1 ± 0.2

592
593



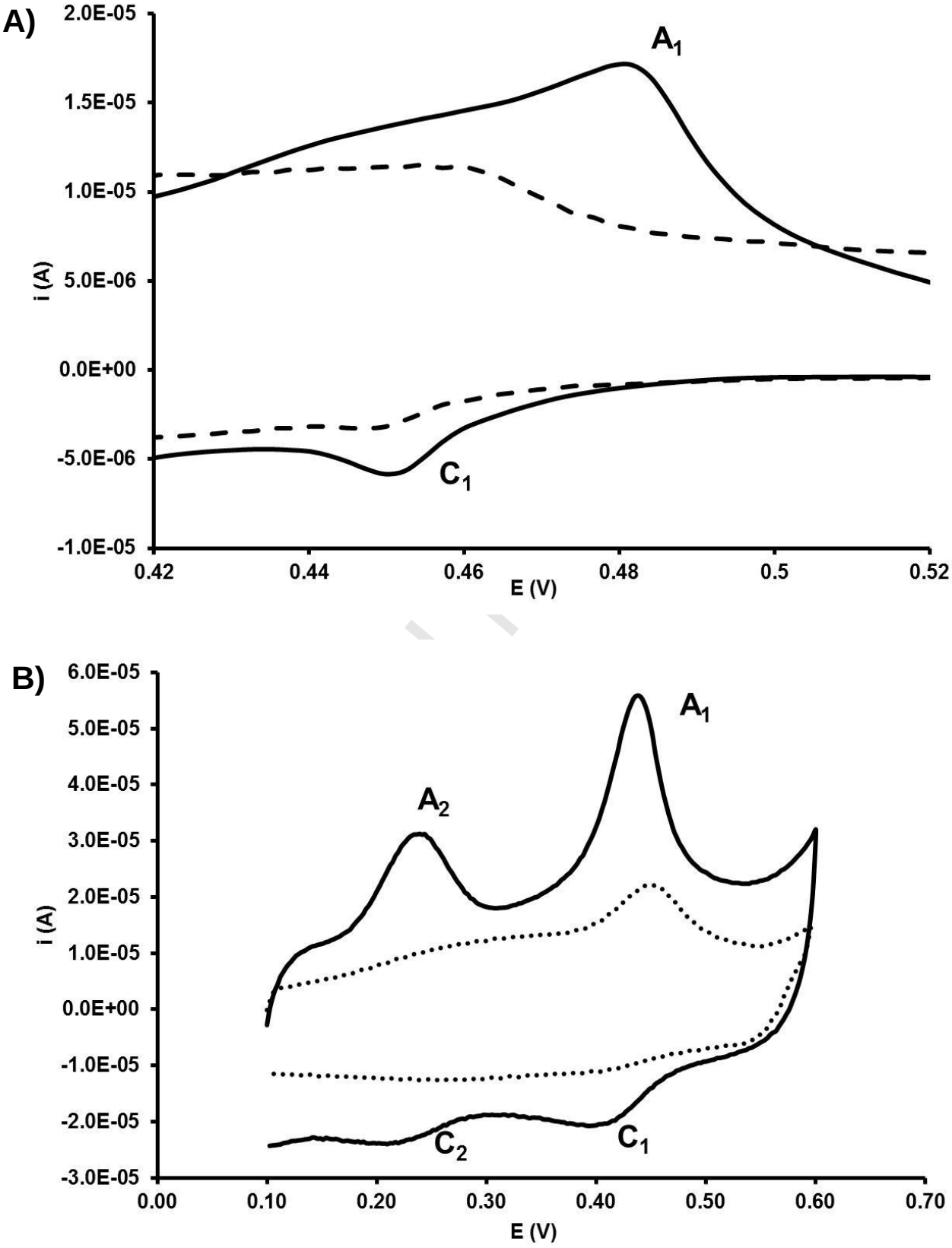


Figure 3