Analytical Applications of a Nanoparticle-Based Sensor for the Determination of Mercury

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The Determination of Mercury by Anodic Stripping Voltammetry with a Gold Nanoparticle-modified Glassy Carbon Electrode

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Abstract

The aim of this work is the development of a procedure for the determination of aqueous Hg (II) by anodic stripping voltammetry at a gold nanoparticle-modified glassy carbon electrode (AuNPs-GCE). The signal of aqueous Hg (II) was measured in the square wave mode; the effect of potential scan parameters, deposition potential and deposition time on the analytical signal was examined. The supporting electrolyte was 0.06 M HCl. The repeatability, the linearity, the accuracy, the detection limit of the procedure and the interferences of other cations and of anions were evaluated. The performance of the AuNPs-GCE was compared with those of a solid (SGE) and a film (FGE) gold electrode:the AuNPs-GCE showed to provide lower detection limits and higher repeatability. The renewable surface permits to eliminate memory effects, to maintain a stable baseline and response, and to avoid frequent mechanical cleaning steps. The applicability of the AuNPs-GCE for Hg(II) determination in drinking waters, sediments and pharmaceuticals was demonstrated.

Keywords

Mercury, Nanoparticles, Gold electrode, Anodic stripping voltammetry, Real samples.

1. Introduction

In the last few years, nanoparticle research has witnessed tremendous growth. One of the reasons for the considerable current interest in nanoparticles is because such materials frequently display unusual physical (structural, electronic, magnetic and optical) and chemical (catalytic) properties [1]. Especially noble metal nanoparticles are of fundamental interest and technological importance owing to their sensoric and catalytic applications [2].

Metal nanoparticles can be exploited in electroanalysis for their ability to catalyze the redox processes of some molecules of analytical interest, since they facilitate the electron transfer, and can be modified with a wide range of biomolecules and ligands [3]. Moreover, the large surface area of the deposited nanoparticles could permit an improvement of the analytical performance (lower detection limit and shorter deposition time) of voltammetric techniques in comparison to conventional electrodes.

Mercury has many unique properties that make it useful in many industrial applications, e.g. in lamps, batteries, thermometers, and in the electrolytic manufacture of chlorine and sodium hydroxide [4]. Mercury compounds are used as catalysts, fungicides, herbicides, disinfectants, pigments and are present in several drugs, e.g. diuretics, antiseptics, remedies for skin and eyes. At the same time mercury and its compounds are highly toxic, even at low concentrations: they accumulate in vital organs and tissues, such as liver, heart muscle and brain, and cause kidney injury, central nervous system disorders, intellectual deterioration and even death [5].

For these reasons, there is an increasing necessity for quantification of mercury in different samples, such as in environmental compartments, food, humans (e.g. hair and blood) and pharmaceuticals. Therefore it is important to develop sensitive analytical methods for its determination.

The methods commonly used for the measurement of total mercury are: cold vapour atomic absorption spectrometry (CVAAS) [6], cold vapour atomic fluorescence spectrometry (CVAFS) [7], inductively coupled plasma mass spectrometry (ICP-MS) [8].

These methods, though highly sensitive, have some drawbacks: CVAAS and CVAFS can be applied only to the determination of mercury, while ICP-MS has high purchase and running costs. Voltammetric methods represent an interesting alternative. These methods are sensitive, relatively inexpensive and they also enable the determination of a number of metals and organics at trace or ultra-trace concentrations [9].

The most common electroanalytical technique for mercury determination is anodic stripping voltammetry (ASV), [10, 11, 12] but also other techniques, such as potentiometric stripping analysis (PSA) [13], chronopotentiometry [9, 14], pulsed amperometry coupled with HPLC [15] were adopted.

Different types of electrode materials have been utilized, mainly gold [10, 14], glassy carbon [16], carbon paste [17] and chemically modified graphite [18]. Gold was found to be the superior substrate for working electrodes owing to its high affinity for mercury, which enhances the preconcentration effect [19]. However, the major drawback of gold-based electrodes is the well-known phenomenon of structural changes on the surface, caused by amalgam formation [20], that requires complex electrochemical and mechanical pretreatments to achieve reproducibility [21, 22].

Relatively few papers concerning the use of gold nanoparticles in electrochemical analysis were published; these articles describe the electrochemical reduction of oxygen on gold nanoparticles [23], or the determination of histamine [3], aromatic compounds [24], antimony [25] and in particular arsenic (III) [26, 27, 28].

Several kinds of gold electrodes were used for the voltammetric determination of mercury: solid [e.g. 10, 29], film [e.g. 14, 21], microwire [e.g. 30, 31, 32], fiber [e.g. 33], ultramicroband array [34] gold electrodes; nevertheless, to our knowledge, published studies concerning the use of gold

nanoparticles are limited to the work of Gao et al. [35] who electrodeposited gold nanoparticles onto a gold electrode, followed by a modification with a mercaptoethanesulfonate monolayer.

The aim of this work is the development of a procedure for the determination of aqueous Hg(II) with ASV using a gold nanoparticle-modified glassy carbon electrode (AuNPs-GCE) without further surface modification. The electrode response was investigated, in order to identify the best operating conditions; working in the square wave (SW) mode, the effects of potential scan parameters (amplitude, frequency, step potential), deposition time and deposition potential on the mercury peak shape and intensity were examined.

Moreover the repeatability, the linearity, the accuracy, the detection limit and the possible interferences of other cations and of anions were evaluated. The performance of the AuNPs-GCE was compared with those of a solid (SGE) and a film (FGE) gold electrode. The procedure developed was applied for the determination of Hg in real samples, namely drinking waters, sediments and pharmaceuticals.

2. Experimental

2.1. Apparatus and reagent

Voltammetric analyses were performed with a PGSTAT 10 potentiostat (Eco Chemie, Utrecht, The Netherlands) coupled to a 663 VA Metrohm (Herisau, Switzerland) stand. The analyzer was interfaced to a personal computer. The working electrode was an AuNPs-GCE, prepared from a commercial Metrohm glassy carbon electrode; a glassy carbon counter electrode and an Ag/AgCl/KCl (3M) reference electrode were employed.

Analytical grade reagents were used. A 1000 mg/l standard solution of mercury was prepared from $HgCl_2$ in 0.012 M HCl. More diluted Hg(II) standard solutions were prepared from the concentrated standards in the supporting electrolyte. Hydrochloric acid was purified by sub-boiling distillation. High purity water (HPW) obtained from a Milli-Q apparatus (Millipore, Bedford, USA) was used throughout.

100 mg/l stock solutions of $HAuCl_4 \cdot 3H_2O$ (Sigma, > 49% as Au) in HPW were prepared and used for the deposition of gold nanoparticles onto the electrode. The characterisation of the electrode surface was performed by scanning electron microscopy (SEM) using a LEICA-Stereo scan 410 SEM.

Dissolutions of sediment and ocular lubricant gel samples were performed in tetrafluormethoxyl (TFM) bombs, with a Milestone MLS-1200 Mega microwave laboratory unit (Milestone, Sorisole, Italy).

2.2. Procedures

2.2.1. Deposition of gold nanoparticles on the electrode

A 100 mg/l $HAuCl_4 \cdot 3H_2O$ solution (corresponding to 50 mg/l of Au) was prepared in Milli-Q water previously filtered through a 0.45 μm cellulose acetate filter and deaerated by passing a N_2 stream. The GCE was polished with a suspension of 0.3 μm alumina in HPW for 1 min, then it was rinsed three times with ethanol and water, alternatively, and dried using a nitrogen stream. Modification with gold nanocrystals was performed by dipping the electrode into the $HAuCl_4$ solution and applying a potential of -0.80 V for 6 min. The modified electrode was washed with Milli–Q water and kept in 0.1 M NaOH until use [3].

The presence of gold nanoparticles, visible through a colour change of the glassy carbon surface from black to red-orange, was confirmed by SEM analyses. The Au nanoparticles in the SEM image (see Figure 1) appear as circular bright spots and their average diameter is 125 ± 25 nm.

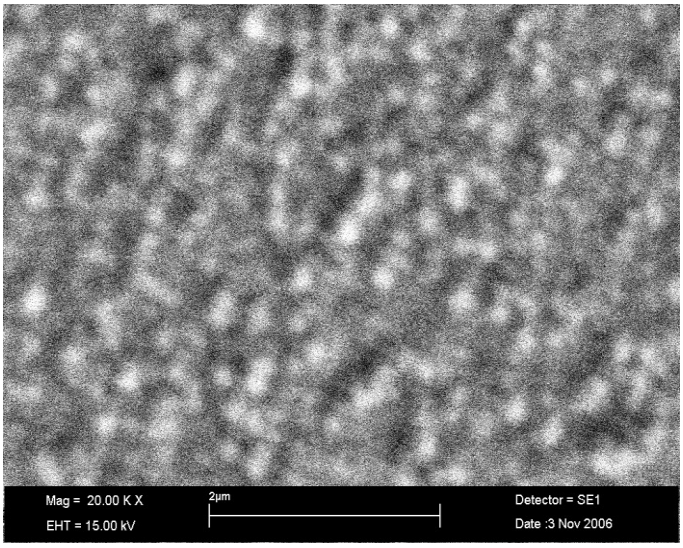

Fig.1. SEM image of gold nanoparticles electrochemically deposited on a glassy carbon electrode.

Before proceeding with the voltammetric determinations, it was necessary to effectuate an activation step by applying a potential of 0.6 V for 60 s while the working electrode was stirred in 0.06 M HCl. Activation may strive to remove any native oxides on Au [36]. This treatment is also called "poisoning of the electrode" [9].

When required (see section 3.2), the dissolution of the gold layer was performed by varying the potential from 0 V to 1.6 V in 6 M HCl whilst stirring the electrode.

2.2.2. ASV determination of mercury

10 ml test solutions of supporting electrolyte were delivered into the voltammetric cell. After 120 s of deposition a voltammetric scan was performed. Initially, the scan parameters were: frequency (the number of square waves applied per second) 100 Hz, step potential (the potential increment between two successive current measurements) 0.002 V, wave amplitude (the half of the peak-to-peak value in the square wave perturbation) 0.02 V. Initial and final potentials were 0 V and 0.80 V respectively. For the values of the scan parameters after optimization, please refer to section 3.1.

In all the determinations the working electrode was stirred (2000 rpm); after recording the voltammogram of the blank, aliquots of Hg were added and the corresponding signals were recorded.

The removal of dissolved oxygen prior to analysis was found to be unnecessary, in agreement with the findings of Jayaratna and Wu [37, 38].

After each determination the working electrode was maintained in a mixture of 0.2 M $HClO_4$, 3 mM NaCl and 1 mM EDTA·for 30 s at 0.80 V [39]. This procedure was necessary to remove residues of mercury from the active surface of the electrode; the presence of EDTA, probably thanks to its complexing properties, favoured this removal.

All experiments were performed in duplicate, unless otherwise stated.

2.2.3. Cyclic voltammetry (CV)

Linear potential sweep CV experiments were performed on 1 mg/l Hg in 0.06 M HCl in the following conditions: start potential, -0.60 V; vertex potential, +0.80 V; end potential, -0.60 V; step potential, 2 mV; scan rate, 10 mV; three cycles. Other experiments were carried out after reversing the values of the start and end potentials.

2.2.4. Samples and sample pretreatment

Drinking water was collected from the laboratory tap and filtered through a 0.45 µm cellulose acetate filter. 9 ml of water were transferred into the voltammetric cell, added with 1 ml of 0.6 M HCl and analyzed as such of after spiking with 0.45 µg/l of mercury.

Three aliquots of a standard reference sediment, ("Estuarine Sediment" BCR CRM 277) were digested in a microwave oven: 3 ml of HNO_3 and 3 ml of H_2O_2 were added to 100 mg of sample and this heating program was followed: 250 W for 5 min; 400 W for 5 min; 600 W for 5 min; 250 W for 5 min; ventilation for 25 min. The resulting solutions were diluted to 15 ml with HPW. 5 ml of each of these sample solutions were transferred into the voltammetric cell and added with 15 ml of 0.06 M NaCl. The determinations were performed using a deposition potential of +0.3 V with the medium exchange procedure (see section 2.2.4).

An ocular lubricant gel containing $2 \cdot 10^{-3}$ % (w/w) of Thimerosal (sodium ethylmercurithiosalicylate, $C_9H_9HgNaO_2S$), equivalent to 0.98 mg of Hg for 100 g of gel, was purchased from a local chemist. Two procedures were followed. In the first one, the product was digested in microwave oven using the same procedure adopted for the sediments and analyzed with the medium exchange procedure. In the second procedure, the sample was analyzed directly, i.e. without digestion and medium exchange, after diluting 0.05 g of gel in 100 ml of the supporting electrolyte (0.06 M HCl).

The standard addition method was adopted for the evaluation of the concentration of mercury in all investigated samples. Each sample was analyzed in triplicate.

2.2.5. Medium exchange

Medium exchange was done after the electrodeposition step: the potential was maintained at 0 V with the "Hold" function of the voltammetric analyzer and the sample solution cell was replaced by a solution of 0.06 M NaCl; then the stripping step was performed. The exchange took less than 20 s.

3. Results and discussion

3.1. Optimization of experimental conditions

The effect of the experimental conditions on the signal of mercury was investigated by spiking the supporting electrolyte solutions with two 20 μg/l-aliquots of Hg standard solution and evaluating the shapes and the heights of the peaks obtained.

The findings of a previous study performed in our laboratory on a SGE [40] were used as a starting point to optimize the performance of the AuNPs-GCE. In fact, the signal of mercury was recorded using SW scan mode in the electrolyte which provided the best results with the SGE, namely 0.06 M HCl.

The effect of SW parameters on the mercury peak height and intensity was investigated. Different values of frequency (25-50-100-150-200 Hz), wave amplitude (0.005-0.01-0.02-0.03-0.04 V) and step potential (0.0005-0.001-0.002-0.003-0.004 V) were examined. The results obtained are reported in Table 1.

When frequency and step potential were increased, the signal increased and shifted to more positive potentials, in agreement with the increase in scan rate; at frequency higher than 150 Hz a distortion of the signal shape was observed with a consequent decrease of the peak height. An increase of wave amplitude was responsible of an increase of the peak height and no shift of the peak potential was observed. The optimal values were 150 Hz, 0.004 V and 0.03 V for frequency, step potential and wave amplitude respectively.

Using the optimized parameter values, the effect of different deposition potentials (-0.40, -0.30, -0.20, -0.10, 0, +0.10, +0.20, + 0.3, 0, + 0.40 V) and of deposition times (30, 60, 90, 120, 150 s) on the signal of 20 μg/l of Hg was evaluated. Table 2 reports the heights and the potentials of the peaks observed in the different experimental conditions.

Table 1. Peak intensities (i_p) and potentials (E_p) obtained for a 20 μg/l Hg solution with different values of the scan parameters. Supporting electrolyte: 0.06 M HCl. Scan mode: SW.

Parameter	Value	i_p (μA)	E_p (V)
Step potential (V)	0.0005	11.4	0.54
	0.001	10.7	0.55
	0.002	14.8	0.55
	0.003	18.0	0.57
	0.004	23.8	0.57
Frequency (Hz)	25	3.52	0.55
	50	5.98	0.55
	100	15.3	0.57
	150	26.3	0.57
	200	20.7	0.58
Wave amplitude (V)	0.005	7.48	0.57
	0.01	12.9	0.57
	0.02	19.5	0.57
	0.03	24.5	0.57
	0.04	24.5	0.57

Table 2. Peak intensities (i_p) and potentials (E_p) obtained for a 20 μg/l Hg solution with different deposition potentials and deposition times. Supporting electrolyte: 0.06 M HCl. Scan mode: SW.

Parameter	Value	i_p (μA)	E_p (V)
Deposition potential (V)	-0.40	17.2	0.56
	-0.30	20.7	0.56
	-0.20	21.7	0.56
	-0.10	23.8	0.56
	0	24.2	0.56
	+0.10	22.1	0.56
	+0.20	21.8	0.56
	+0.30	19.4	0.56
	+0.40	18.7	0.56
Deposition time (s)	30	17.9	0.59
	60	29.7	0.57
	90	27.0	0.57
	120	40.6	0.57
	150	40.1	0.57

As to the deposition potential, different values were adopted by different researchers with gold electrodes. For example, 0.30 [37], and -0.20 V [11, 38] were used with SGEs, while 0.20 [21] and 0.30 V [14] were adopted with GFEs. Bonfil, working with 10 mM NaCl and 10 mM HNO_3 with a SGE, observed that the repeatability and the magnitude of the analytical signal were independent of

the deposition potential in the range +0.55 ÷ -0.40 V [10]. In our study, the highest peak for mercury was obtained with 0 V as deposition potential, in agreement with Ermakov [29]. The optimum value for this parameter also depends on the composition of the matrix: in fact a deposition potential of 0.30 V was found to provide the best results for samples digested with HNO_3 and H_2O_2 (see above).

As expected, the height of the mercury peak increased with increasing deposition time, up to 120 s; longer deposition times did not give rise to an increase of the signal, probably because the amount of mercury deposited on the electrode is not completely removed during the stripping step [41].

The analysis does not require the purging of the sample solutions with nitrogen, since no interference due to dissolved oxygen takes place [37, 38].

The effect of the optimisation of all parameters is well shown in Figure 2.

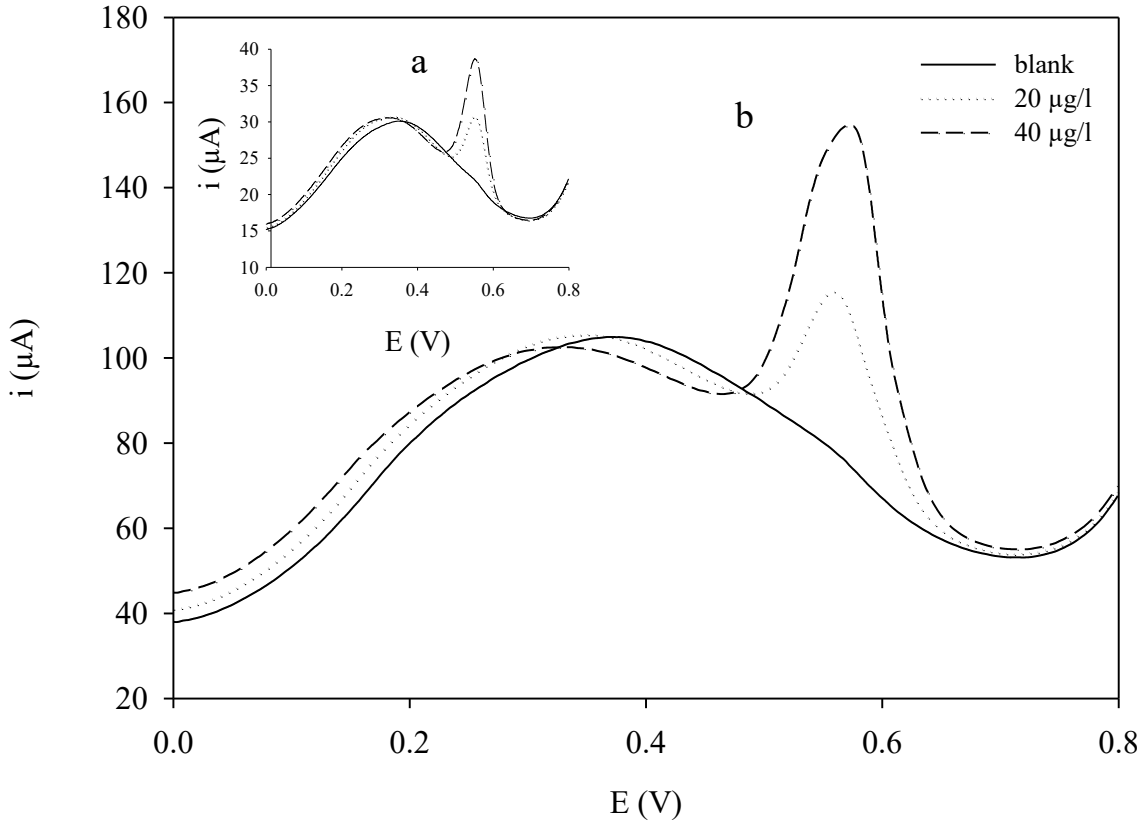

Fig. 2. Comparison between the mercury peaks before (a) and after (b) optimization of experimental parameters.

3.2. Repeatability, linearity, accuracy, detection limit, blank subtraction

The performance of the analytical method was evaluated in the following conditions: frequency: 150 Hz, step potential: 0.004 V, amplitude: 0.03 V, deposition potential: 0 V and deposition time: 120 s.

In these experiments we measured the peak heights after subtracting the blank from the voltammograms of the sample solutions, in order to decrease the uncertainty in the choice of the baseline. In fact, in agreement with literature data [20], the voltammograms of mercury on gold electrodes are characterized by a broad baseline, which makes difficult to measure the peak height directly, especially at low (≤ 3 µg/l) analyte concentrations. According to previous studies, the precipitation of calomel on the electrode as a result of the rapid mercury oxidation during the voltammetric scan might be the cause of the high background [30]. The presence of chloride results in the formation of Hg_2Cl_2 which is scarcely soluble in water (pKs = 17.9) and which precipitates onto the electrode surface [19]. After blank subtraction, a well defined peak was obtained (Figure 3).

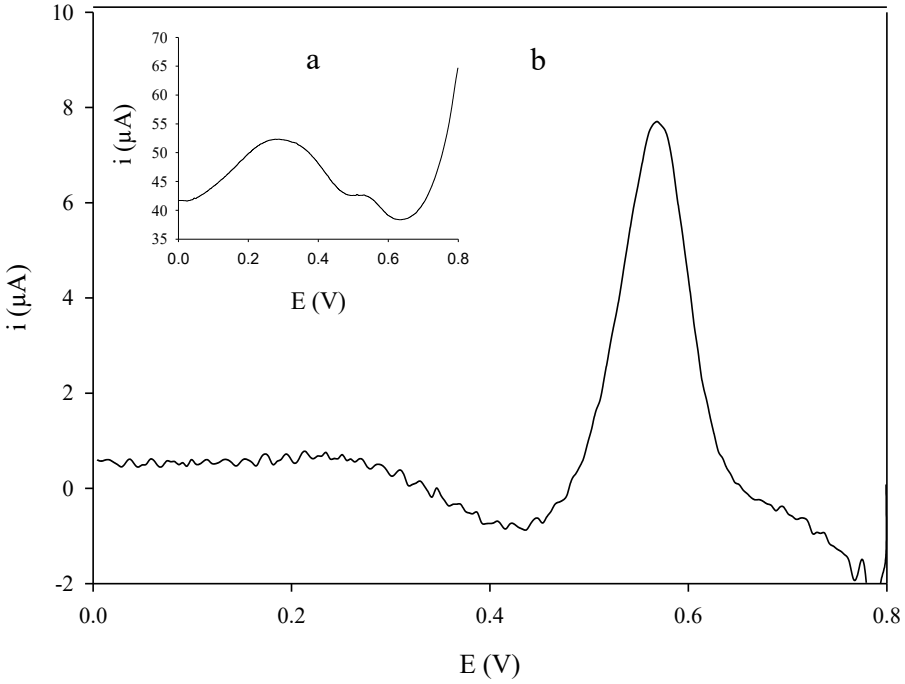

Fig. 3. Voltammogram of a 1 µg/l Hg solution before (a) and after (b) blank subtraction.

The repeatability of the response of 10 µg/l of mercury was evaluated with ten replicates on ten different cells. The relative standard deviation was 2.8 %. This value can be considered satisfactory, taking into account the relatively low concentration level involved.

In order to evaluate the linearity of the method, the signals of different concentrations of mercury (0.01, 0.05, 0.5, 1, 2, 3, 4, 5, 50 µg/l) were measured in different experiments. Table 3 shows the equations of the calibration curves, R^2 values, and average sensitivities obtained.

Table 3. The equations of the calibration curves, R^2 values and the corresponding sensitivities obtained in different concentration ranges.

Concentration range (µg/l)	Equation of the calibration curve	R^2	Average sensitivity ($\mu A/\mu gl^{-1}$)
0.01 - 0.5	$y = 6 \cdot 10^{-6} \times + 6 \cdot 10^{-7}$	0.9999	6.5
1 – 5	$y = 3 \cdot 10^{-6} \times + 2 \cdot 10^{-6}$	0.9998	2.6
5 – 50	$y = 2 \cdot 10^{-6} \times + 1 \cdot 10^{-5}$	0.9827	1.4

The value of R^2 and the sensitivity increase as the concentrations decrease. This change in sensitivity must be taken into account when analyzing real samples and indicates that it is convenient to perform the calibration with standard solutions having concentrations close to the ones present in the samples. According to previous studies [41], mercury is adsorbed onto gold electrodes and does not diffuse deeply inside them: therefore, the higher sensitivity for low concentrations is probably due to the lower competition for the electrode surface, which ensures a more efficient deposition of the analyte. At concentrations higher than 50 µg/l a broad and ill-shaped mercury peak appeared and a loss of linearity was observed.

The accuracy of the response was tested by analysing three sample solutions containing 10 ng/l of mercury with the standard addition method, adopting a deposition time of 120 s. The concentration found was 9.92 ± 0.05 ng/l, with a relative error of –0.8 %. This value can be considered quite satisfactory, taking into account the low concentration level involved. The detection limit, evaluated as three times the standard deviation of these data, was found to be 0.15

ng/l, which compares well with the value of 0.13 µg/l (deposition time 180 s) found by Gao et al [35]. This difference might be due to the use of different supporting electrolytes: in fact in our previous studies we found a lower sensitivitity with the mixture HNO_3/NaCl, used in their work, than with HCl [40].

Typically after about 100 measurements, the electrode performance in terms of sensitivity and reproducibility started to worsen; the gold layer was dissolved and a new one was deposited. The same accuracy level was obtained with different Au-nanoparticle layer depositions.

3.3. CV experiments

CV experiments show that mercury gives rise to an oxidation peak at 0.591 V and a reduction peak at 0.537 V (Figure 4). The other reduction peak at –0.15 V is present also in the blank, therefore it is not related to mercury; its source is unexplained. The same voltammograms were obtained when the start and end potentials were reversed.

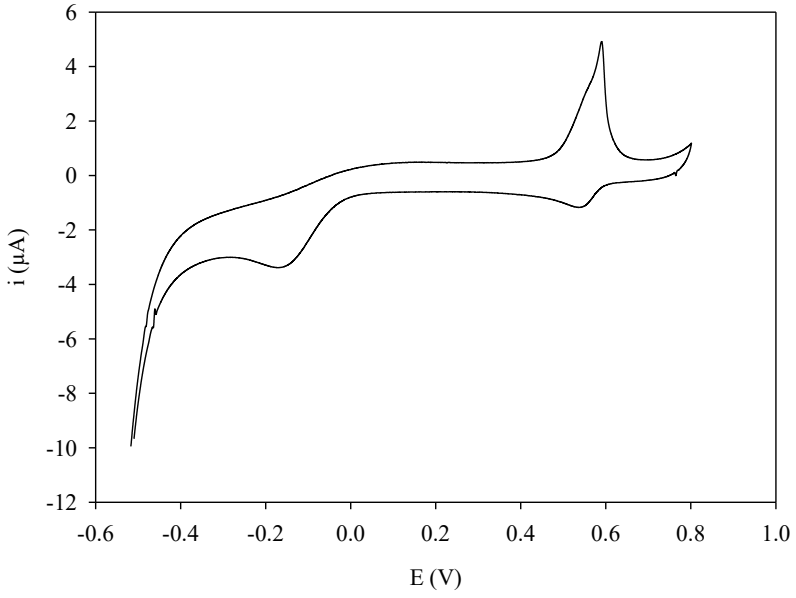

Fig. 4. Cyclic voltammogram of a 1 mg/l Hg solution.

A preliminary hypothesis on the processes occurring at the electrode maybe that Hg(0) is oxidized to Hg(I) with the subsequent formation of calomel, while during the cathodic scan the

reduction of Hg(I) to Hg(0) takes place. The difference between peak potentials is 54 mV, close to the value expected for a reversible one-electron process. The hypothesis is in agreement with the conclusions of Nolan et al. [42], who studied the behaviour of Hg^{2+} at carbon electrodes, and of Ribeiro et al., who investigated the redox mechanism of methylmercury at carbon microelectrodes [43].

The proposed mechanism is presumably valid in solutions containing chloride ions, since it was observed that for $[Cl^-] < 0.001$ M, Hg(0) is oxidized to Hg(II) [42].

3.3. Interferences

The possible interference of Al(III), As(V), Bi(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(II), Mn(II), Ni(II), Pb(II), Sb(III), Sc(II), Se(IV) and Zn(II) on the mercury stripping signal was evaluated.

The voltammogram of a solution with 5 μg/l of mercury was recorded in the presence of each element in 1:1 and 1:100 concentration ratios with respect to Hg. The only peak which appeared in the considered potential range was that of Cu at 0.38 V, which did not interfere with the mercury signal. However, a small increase of the sensitivity of response for mercury was noticed in the presence of the considered elements, probably because of a modification of the background; this effect took place both for the mercury initially present in the test solutions and after the addition of further aliquots of the analyte. When standard solutions were analyzed in the presence of each cation, the expected concentration was measured, even with an analyte:element ratio of 1:100: therefore it is sufficient to use the standard addition method in order to take the variation of sensitivity into account.

The effect of different anions, namely PO_4^{3-}, ClO_4^-, $HCOO^-$, BO_3^{3-}, NO_3^-, F^-, SO_4^{2-}, CH_3COO^-, Br^-, CO_3^{2-}, S^{2-} and I^-, on the mercury peak was also investigated. Each anion was added in 1:1, 1:10, 1:100, 1:1000 concentration ratios with respect to Hg (1 μg/l). PO_4^{3-}, ClO_4^-, $HCOO^-$, BO_3^{3-}, NO_3^-, F^-, SO_4^{2-}, CH_3COO^- and CO_3^{2-} were found not to interfere.

The presence of I^- and S^{2-} caused i) a decrease of the background current, probably because of their interaction with the gold surface, and ii) a sharp decrease of the mercury signal and a loss of linearity, which hindered the quantification of the analyte, due to the low solubility of HgS (K_{ps} = $2 \cdot 10^{-53}$) and Hg_2I_2 (K_{ps}= $1.1 \cdot 10^{-28}$).

Also in the presence of bromides the background shifted to low currents, but the accuracy of the determination was maintained up to a 1:100 $Hg:Br^-$ ratio. For higher content of bromides, the analyte concentration was overestimated. It is possible to determine mercury in presence of bromides (up to 100 µg/l) only for [Hg] > 3 µg/l, i.e. when background subtraction is not necessary. The determination of lower mercury contents is possible only if the concentration of Br^- in the sample solution is known and it is added to the blank.

3.4. Comparison between nanoparticle-based electrodes and other types of gold electrodes

The performance of the AuNPs-GCE was compared with that of a SGE using the same supporting electrolyte and operating conditions.

The large surface area of the deposited nanoparticles permitted an improvement of the sensibility in comparison to conventional electrodes: 3.5 $\mu A/\mu gl^{-1}$ for AuNPs-GCE versus 1.71 $\mu A/\mu gl^{-1}$ for SGE in the concentration range 0 - 50 µg/l. The sensitivity for AuNPs-GCE is even better below 0.5 µg/l (see Table 3).

The detection limit measured in our laboratory for the SGE was 0.40 µg/l after 120 s of deposition [40]. For comparison, other values of the detection limit reported in the literature for this electrode are: 0.022 µg/l with deposition times longer than 30 minutes [38]; 0.02 µg/l (480 s) [16]; 0.002 µg/l (300 s) [29]; 1 µg/l (90 s) [39]; 0.005 µg/l (10 min) using stripping chronopotentiometry [9]. The AuNPs-GCE allows to obtain a lower detection limit, that is 0.0015 µg/l.

A slightly better repeatability for the AuNPs-GCE (relative standard deviation 2.78 % for 10 µg/l) than for the SGE (relative standard deviation 4.40% for 50 µg/l) [40] was found.

Another advantage of the nanostructured electrode over the SGE is in the cleaning procedure. The importance of electrochemical electrode cleaning after a single mercury determination at a gold surface, in order to maintain the reproducibility of the response, is well known [37]. However, there is also the evidence in the literature that the cleaning step may not result in a fresh, analyte-free, gold surface. In the early 1980s, Schadewald and co-workers reported that mercury accumulated on the electrode in subsequent CV experiments and was not completely removed when the CV experiment was continued in fresh (mercury-free) electrolyte solution [44]. These observations were confirmed by Watson et al. [41] with CV and X-ray photoelectron spectroscopy studies. In addition, other components of the sample solutions may adsorb on the electrode surface and eventually modify its properties and affect electron transfer between electrode and solution.

Therefore a more drastic treatment than the electrochemical cleaning is required, once in a while, when a decrease of performance is observed. Usually SGEs are mechanically polished with alumina powder, but this treatment progressively damages their surface.

A great advantage of the AuNPs-GCE is the possibility to work with a renewable surface, dissolving the gold nanoparticle layer and depositing a new one when a worsening of instrumental response is noticed. Furthermore, the AuNPs-GCE permits to attempt to work in drastic conditions, e.g. with very positive potentials or with aggressive or complex matrices, since in the worst of the hypothesis only the surface layer of gold would be damaged, and a new deposition would be possible.

The response of a GFE was tested, following Okcu's procedure [21] (three successive electrolysis steps at –0.5 V for 300 s using a solution containing 1 x 10^{-5} M Au in 0.1 M HCl). The presence of the film was evidenced by the formation of a green-yellow layer, whose colour was clearly different from that of the nanoparticle coating. Also this kind of electrode allows to work with a renewable surface. The performance of the GFE was investigated at different concentration levels between 0.2 and 10 μg/l). Its average sensitivity in this concentration range was found to be

1.06 μA/μgl^{-1}, which is lower than that of the AuNPs-GCE, probably because the latter has an higher surface area.

For concentrations greater than or equal to 1 μg/l the response of the GFE was satisfactory. Instead, the repeatability obtained for concentrations below 1 μg/l was low: for example the relative standard deviation for 0.4 μg/l Hg was 18 %. Our findings are in agreement with the indications of EPA method 7472 [45], which reports a detection limit of 0.1 μg/L using a 10-minute plating time and of 3 μg/L after 1-minute plating. Therefore it can be stated that the AuNPs-GCE is more suitable than the GFE for analyses at low concentrations.

3.5. Analysis of real samples

The applicability of the AuNPs-GCE to the analysis of real samples (drinking water, sediments and ocular lubricant gel) was tested. Table 4 summarizes the results obtained.

No mercury peak was observed in drinking water, therefore the sample was spiked with 0.45 μg/l of the analyte. The results obtained were satisfactory in terms of accuracy and they show that mercury can be determined at concentrations lower than the maximum admissible level according to the Italian Legislation [46] and to the International World Health Organization (WHO) [47], that is 1 μg/l.

Aqua regia was not used for the digestion of the estuarine sediment samples in order to avoid the formation of nitrosyl chloride which attacks gold electrodes [15]. In any case, the high sensitivity of the technique permits to made significant dilutions of the samples (depending on analyte content): therefore, the high acid concentration decayed with solution dilutions and this increased the lifetime of the gold deposition. Before the voltammetric analysis, chloride ions were added to the sample solution (final concentration 0.06 M) in order to enhance the sensitivity of the mercury stripping signal [11]. The peak of mercury in this solution was well defined but it could not be quantified accurately (see Table 4). The difficulty in determining the analyte in this matrix was not due to the reagents used for the digestion, because the accuracy of the determination of the mercury in the

blank spiked with 2 µg/l was good (relative error 3.3 %); probably some residual component of the sediment matrix interfered with the analysis. This difficulty was overcome by performing the stripping step in a solution of 0.06 M NaCl, using the medium exchange procedure: with this method the result obtained was in good agreement with the certified value (see Table 4). Medium exchange was used also by other researchers for the analysis of sediments [16] and natural (saline and river) waters [38] with gold electrodes.

Thimerosal is commonly used as both an antiseptic and an antimicrobial preservative in pharmaceutical formulations. The concentration of mercury measured in the ocular lubricant gel was in very good agreement with the value reported by the manufacturer, with both adopted procedures. In fact, due to the high concentration of Hg and a relatively simple matrix, also the direct determination of the analyte in the sample was possible, avoiding both digestion and medium exchange.

Table 4. Determination of mercury in real samples.

Sample	[Hg] expected	[Hg] found	Recovery (%)
Drinking water	0.45 µg/l[a]	0.47 ± 0.015 µg/l	104
Sediment (BCR CRM 277)	1.77 ± 0.06 mg/kg	1.71 ± 0.28 mg/kg[b]	97
		2.72 ± 0.11 mg/kg[c]	154
Ocular lubricant gel	0.98 mg/100g	1.03 ± 0.06 mg/100g[b]	105
		1.00 ± 0.06 mg/100g[d]	102

[a]Concentration of mercury added to clean drinking water; [b]after digestion and medium exchange; [c]after digestion without medium exchange; [d]direct analysis

4. Conclusions

In this work the suitability of the AuNPs-GCE for the determination of mercury by ASV was demonstrated.

The main advantages of the procedure developed are:

- the improvement of the analytical performance with respect to solid and film gold electrodes. In particular, a very low detection limit, with short deposition times, was attained, thanks to the increase of specific surface of the gold nanoparticles;

- mercury concentrations in the low ng/l range are easily quantified with high accuracy and precision. Consequently, it is possible to work with small sample amounts or with high factors of dilution in the supporting electrolyte; this is useful i) when only small quantities of sample are available, ii) in the presence of complex matrices or iii) when aggressive reagents are needed for dissolution;

- the renewable active surface of the AuNPs-GCE permits to eliminate the problem of irreversible contamination of the gold layer and to minimize memory effects; this is convenient in routine work, but it also allows researchers to test new electrolyte compositions without spoiling the electrode. The response of the AuNPs-GCE is repeatable even with different nanoparticle layers, i.e. when a layer is dissolved and a new one is deposited. This is a great advantage with respect to SGEs, whose performance tend do worsen with time and which require frequent time-consuming and dangerous mechanical cleaning;

- many kinds of real samples, both aqueous and (after dissolution) solid ones, can be analyzed. The use of medium exchange allows to overcome interferences which cannot be eliminated simply by dilution in the supporting electrolyte.

The versatility and sensitivity of the procedure developed confirm that ASV is an effective alternative to other techniques used for the determination of mercury. The main advantages of using voltammetry are the low purchase and running costs of the instrumentation and the applicability to the determination of many inorganic and organic analytes in different fields, e.g. for environmental, industrial, pharmaceutical, clinical and food analysis.

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