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ORIGINAL PAPER

Voltammetric determination of methylmercury and inorganic mercury with an home made gold nanoparticle electrode

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Abstract This study outlines the development of a procedure for the determination of methylmercury by anodic stripping voltammetry at a gold nanoparticle-modified glassy carbon electrode (AuNPs-GCE) and for the differentiation between methylmercury and inorganic mercury. The signal of methylmercury was measured in the square wave mode using HCl as the supporting electrolyte. The procedure had good accuracy, repeatability and linearity. The determination of total mercury in solutions containing both methylmercury and inorganic mercury was performed after converting the former into the inorganic form. Different sample solution pre-treatments were tested for this purpose, and an acid digestion in a microwave oven with HNO_3 and H_2O_2 was found to be the most effective. The selective determination of methylmercury in the presence of inorganic mercury was possible after masking the latter through reduction to the elemental state with $SnCl_2$. The amount of inorganic mercury was determined by difference.

Keywords Methylmercury · Nanostructured gold electrode · Anodic stripping voltammetry · Mercury

1 Introduction

Mercury is among the trace metals most highly bioconcentrated in the human food chain. The behaviour of mercury is very complex: its toxicity, like that of many other heavy metals, varies with its chemical form, which in turn influences its degree of absorption, transport, biotransformation, retention and mode of excretion in living organisms; in addition, its toxic effects also depend on the route of entry, on dosage, frequency, and age at exposure [1].

Although all forms of mercury are poisonous, its ecological and human health effects are generally related to the environmental transformations of inorganic Hg to methylmercury (MM) [2]. Methylmercury is the most commonly occurring methylated forms of mercury and is one of the most toxic mercury species due to its chemical nature, which causes higher solubility in lipids, higher membrane permeability and greater tissue fixation in comparison to inorganic mercury [3], and consequently gives rise to high levels of bioaccumulation and biomagnification.

Inorganic mercury compounds can undergo methylation by microorganisms present in the environment, and thus be bioaccumulated through the food chain, and can potentially result in severe effects to humans if consumed in sufficient quantities [4, 5]. Inorganic mercury is a potent neurotoxin that impairs the central nervous system and, in severe cases, causes irreversible brain damages. The rate of CH_3Hg^+ production depends on a complex interaction of a variety of environmental variables [6] such as biological activity, nutrient availability, pH, temperature, redox potential, and inorganic and organic complexing agents [7].

Given the different toxicities of inorganic and organic mercury compounds, the determination of total mercury is not sufficient for understanding its toxicological impact on biota and on human health. For this reason the availability of a simple method for the determination of methylmercury at trace levels and for the speciation between inorganic mercury and methylmercury is useful.

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Various analytical techniques have been developed to differentiate between these two species. Such techniques include selective extraction in an organic solvent, extraction via complex formation followed by separation by gas chromatography [8, 9] or high-performance liquid chromatography [10, 11] combined with a detection technique such as atomic emission [12, 13], absorption [14, 15] or fluorescence spectrometry [16, 17], inductively coupled plasma mass spectrometry [18, 19] or electrochemical methods, like amperometry [20] or coulometry [21].

Cold vapor atomic absorption spectrometry (CV-AAS) is the most common technique for trace level determination of mercury: one possibility of using CV-AAS for mercury speciation is based on the different reactivity of mercury species with some reducing agents [22, 23].

A microwave oven assisted mineralization of the methylmercury previously extracted by liquid-liquid extraction is another possibility of using CV-AAS for Hg speciation [2, 24].

Typical non-electrochemical methods for the determination and speciation of organomercury compounds at trace levels are quite well established, leading to high sensitivity and selectivity, but they require rather complicated and expensive instrumentation and time-consuming procedures. Voltammetric techniques are very attractive for the determination of trace and ultra-trace elements because they require relatively unexpensive instrumentation, offer low detection limits and in some cases allow for the direct determination of the species of interest.

Electrochemical methods for the quantification of trace levels of mercury are usually based on anodic stripping voltammetry using electrodes made of glassy carbon [25], carbon paste [26], chemically modified graphite [27] or gold [28]. In the last years, gold electrodes have received great attention for trace mercury determination [29] because of the high solubility of mercury in gold. However, all these types of electrodes are generally used for the determination of total mercury, without distinguishing between inorganic and organic forms.

Voltammetric techniques have not been the method of choice for the determination of CH_3Hg^+ because the reduction of methylmercury, as well as of other organomercury compounds, is a relatively complex process [6, 30, 31]. Few papers concerning the speciation between inorganic mercury and methylmercury by voltammetry have been published. Heaton and Laitinen determined CH_3Hg^+ at a dropping mercury electrode (DME), but this method obviously offers low sensitivity [31]. Carbon electrodes coated with Nafion or with thiolic resins have been used for the preconcentration and determination of traces of methylmercury [32] and for the separation between the signals of MM and Hg_{inorg} adopting different deposition potentials [6]. Ireland and Ripert proposed a method of double standard additions for determining methylmercury in the presence of mercury (II) ions by differential pulse anodic stripping voltammetry at a gold film electrode [33]. Lai et al. combined a simple flow-injection system with fast-scan voltammetry in which methylmercury was detected using a Pt microelectrode coated with a thin mercury film. The oxidation peak of inorganic mercury was found to occur at a different potential from that of MM [5].

In previous works we developed two procedures for the determination of aqueous Hg(II) with ASV using a solid gold electrode [29] and a gold nanoparticles-modified glassy carbon electrode (AuNPs-GCE) [34]. The nanostructured electrode permitted to greatly improve the sensitivity of the determination in comparison to the solid gold electrode and ensured ease of maintenance and long term repeatability owing to its renewable surface. The aim of the present paper is to test the possibility of applying the procedure and the electrode optimised for the determination of inorganic mercury to the determination of methylmercury and to devise strategies to differentiate between organic and inorganic mercury.

2 Experimental

2.1 Apparatus and reagents

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

Scanning electron microscopy (SEM) images were obtained using a LEICA-Stereo scan 410 SEM. A 1 KW UV lamp, connected to a fan and a timer, was adopted for the irradiation of the test solutions. Microwave treatments of the test solutions were performed in polytetrafluormethoxyl (TFM) bombs, with a Milestone MLS-1200 Mega microwave laboratory unit (Milestone, Sorisole, Italy). High purity water (HPW) obtained from a Milli-Q (Millipore, Bedford, MA, USA) apparatus was used throughout. HCl was purified by sub-boiling distillation. Analytical grade reagents were used. A 1,000 mg L^{-1} standard solution of mercury was prepared from $HgCl_2$ in 0.012 M HCl. More diluted Hg_{inorg} standard solutions were prepared from the concentrated standard in the supporting electrolyte. MM standard solutions were prepared from CH_3HgCl in HPW acidified with HCl to pH 2 [33], unless otherwise stated. The concentrations of MM are expressed as μg L^{-1} of Hg throughout the text. A 10^{-2} M Sn(II)

solution was obtained by dissolution of $SnCl_2 \cdot 2H_2O$ in 3.5 M HCl. A more diluted (10^{-4} M) solution was prepared at the moment of the analysis and 20 μL were added into the cell to reduce the inorganic mercury.

2.2 Procedures

2.2.1 *Deposition of gold nanoparticles on the electrode*

Gold nanoparticles were obtained starting from a 100 mg L^{-1} $HAuCl_4 \cdot 3H_2O$ solution (corresponding to 50 mg L^{-1} of Au) prepared in previously filtered HPW and deaerated with a nitrogen stream. The GCE was polished with a suspension of 0.3 μm alumina in HPW for 1 min, rinsed three times with ethanol and HPW, alternatively, and dried using a nitrogen stream. The electrode was dipped into the $HAuCl_4$ solution and a potential of −0.8 V was applied for 6 min to obtain modification with gold nanocrystals. The modified electrode was washed with HPW and kept in 0.1 M NaOH until use [34, 35].

The presence of gold nanoparticles, visible through a colour change of the glassy carbon surface from black to red-orange, was confirmed by SEM analysis. Figure 1 reports an example of a SEM image, which shows the regularity of the deposition. The Au nanoparticles appear as circular bright spots and their average diameter is 125 ± 25 nm. The nanoparticle layers obtained in different depositions showed the same morphological features; in particular, from the SEM images we observed that probably two layers of gold nanoparticles are formed during the deposition: we suppose that the second layer is formed on the first one and this permits to obtain a final gold surface with uniform features in subsequent depositions, apart from the roughness of the glassy carbon surface. This is confirmed by the repeatability of the signal obtained after different depositions.

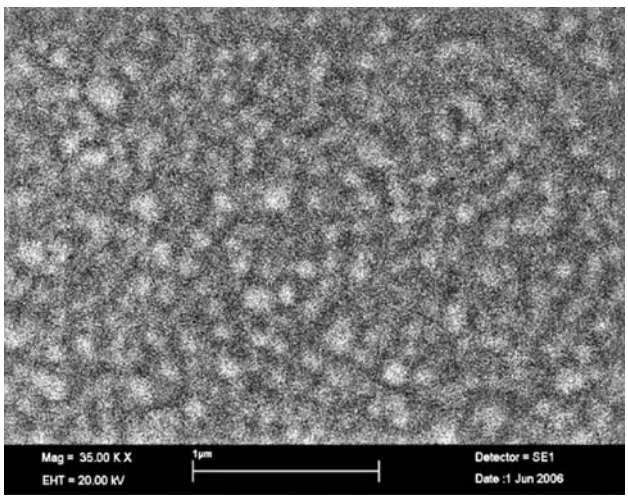

Fig. 1 SEM image of the gold nanoparticles electrochemically deposited on the glassy carbon electrode

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].

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 dissolution of the gold layer was performed by varying the potential from 0 to 1.6 V in 6 M HCl whilst stirring the electrode [34]. The same accuracy level was obtained with different gold depositions.

2.2.2 *Voltammetric analysis*

Twenty millilitre test solutions of supporting electrolyte (60 mM HCl) were delivered into the voltammetric cell.

After 120 s of deposition a voltammetric scan was performed in the square wave mode with these parameters: frequency: 150 Hz, amplitude: 0.03 V, step potential: 0.004 V. In all determinations the working electrode was stirred (2,000 rpm). After recording the voltammogram of the blank, aliquots of analyte (Hg_{inorg} or Hg_{MM}) 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 other researchers [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 type of electrochemical cleaning procedures is well know in the literature and it permits to clean the solid electrodes surface avoiding to damage or modify them. In our experiments this procedure was necessary to remove residues of mercury from the active surface of the electrode; this treatment does not seem to have any effect on the determination since the sensitivity of the response remains unvaried before and after the cleaning step. The experiments were performed in triplicate.

2.2.3 *Pre-treatments*

For the determination of total mercury three pre-treatments were tested: (a) UV irradiation for 3 h; (b) UV irradiation for 3 h after addition of 0.01 M H_2O_2; (c) acid digestion. For this last pre-treatment the test solutions were added with 3 mL^{-1} of HNO_3 and 3 mL^{-1} of 30% H_2O_2 and heated in a microwave oven according to this programme: 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^{-1} with HPW. Aliquots of 5 mL^{-1} of these solutions were transferred into the voltammetric cell and added with 15 mL^{-1} of 60 mM NaCl.

3 Results and discussion

3.1 Determination of methylmercury

In our previous study on the determination of inorganic mercury by ASV with the AuNPs-GCE, we optimised all the parameters affecting the analytical determination. The optimal conditions found are reported in Sect. 2.2.2 and permitted to obtain a very low detection limit (0.15 ng L^{-1}) and to quantify very low concentrations of Hg_{inorg} (10 ng L^{-1}) with good accuracy (relative error 3%) and repeatability (relative standard deviation 2.8%) using a-short deposition time (60 s). The possible interference of some cations and anions present in solution was also studied. The procedure was applied for the analysis of different samples (water, sediment and pharmaceutical), and a very good agreement between the results obtained and those expected was found in all cases [34].

It is known that mercury deposited on gold causes structural changes [40, 41] of the gold surface thus affecting the reproducibility of Hg determinations on gold electrodes. A great advantage of the AuNPs-GCE is its renewable surface, which permits to avoid memory effects and deterioration of the electrode surface as it happens with solid gold electrodes.

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 (μg L^{-1} level) analyte concentrations [34, 39, 42]. In fact, the presence of chloride ions results in the formation of Hg_2Cl_2 which is scarcely soluble in water (pKs = 17.9) and precipitates onto the electrode surface [43]. On the other hand we observed that the chloride-free supporting electrolytes, such as nitric or perchloric acid, did not give satisfactory results in terms of sensitivity and linearity [29]. We obtained well defined peaks by subtracting the blank signal from the voltammograms of the sample solutions in 60 mM HCl.

In this study we determined methylmercury applying the same conditions of analysis. Works concerning the application of gold nanoparticle-based electrodes for the quantification of methylmercury have never been published.

Figure 2 shows the voltammograms of 3 μg L^{-1} of Hg_{inorg} and 3 μg L^{-1} of Hg_{MM} after blank subtraction. As can be seen, in the conditions adopted the oxidation peak potential of both species is 0.58 V.

Many researchers hypothesized that the reduction mechanism of CH_3Hg^+ on gold [44] is similar to that proposed at the mercury electrode [33]. A widely accepted mechanism is that of Heaton and Laitinen who studied the reduction of methylmercury at a DME [31]. They proposed the following mechanism:

$$CH_3Hg^+ + e^- \rightleftarrows CH_3Hg^\bullet$$
$$2CH_3Hg^\bullet \rightleftarrows (CH_3Hg)_2$$
$$(CH_3Hg)_2 \rightleftarrows (CH_3)_2Hg + Hg$$
$$CH_3Hg^\bullet + H^+ + e^- \rightarrow CH_4 + Hg^0$$

According to this mechanism during the deposition step CH_3Hg^+ is reduced to elemental mercury, and this causes the formation of a peak at the same potential as that of Hg_{inog} during the stripping step.

The analytical features of the MM determination were evaluated with 3 μg L^{-1} Hg_{MM} test solutions. Two standard additions (3 μg L^{-1}) of Hg_{inorg} were made and the concentration of the test solution was estimated with the standard addition method as 2.91 ± 0.15 μg L^{-1}, in very good agreement (−3%) with the expected value. The standard addition plot obeyed the equation y (μA) = 5.6 (μg L^{-1}) + 3.67; the linearity was very good (R^2 = 0.999). The detection limit, estimated as three times the standard deviation of the blank, was found to be 0.2 μg_{MM} L^{-1}. These results are different from those of Ireland and Ripert, who obtained a poor repeatability (RSD = 20–40%) and ascribed it to an incomplete reduction of methylmercury to mercury at the gold electrode in acidic medium [33]. They used a different deposition potential, i.e. −0.5 V instead of 0 V as used in our experiments. Actually we verified that, using our procedure with the AuNPs-GCE and −0.5 V as deposition potential, the background is higher and the peaks are lower and less reproducible than the ones obtained at 0 V.

Many different solvents are used to prepare MM standard solutions: CH_3OH [45], HPW [46], diluted HCl [32], diluted HNO_3 [33], etc. We tested three different matrices: HPW, 10^{-2} M HCl and CH_3OH/HPW mixtures (10:1 v/v). The peak heights obtained for different concentrations of MM, prepared from the three different standard solutions, in the range 0.6–10 μgHg L^{-1}, were evaluated and compared with the intensities measured for the same concentrations of inorganic mercury. The results are shown in Fig. 3 and the equations of the curves obtained from the data are reported in Table 1.

The peak heights for Hg_{MM} are higher than those observed for Hg_{inorg}, as we have seen before (Fig. 2). Also Agraz et al. [6] found the same trend and suggested that the preconcentration rate of MM was greater than that of Hg^{2+} during the first few minutes of deposition. In particular CH_3Hg^+ solution prepared in CH_3OH/HPW gave rise to the highest intensity and slope values. This is probably due

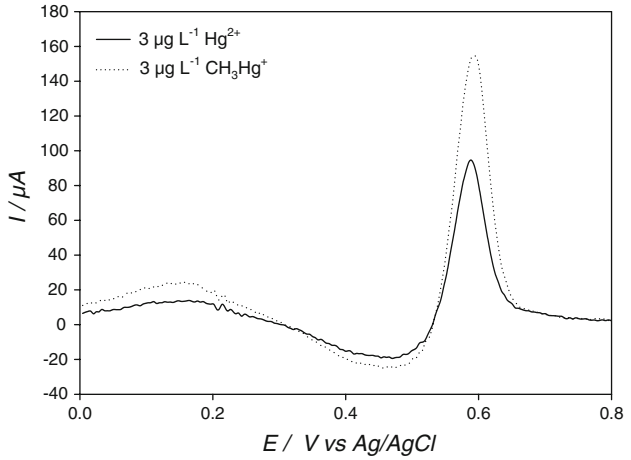

Fig. 2 Voltammograms of 3 µg L^{-1} Hg_{inorg} and 3 µg L^{-1} Hg_{MM} after blank subtraction

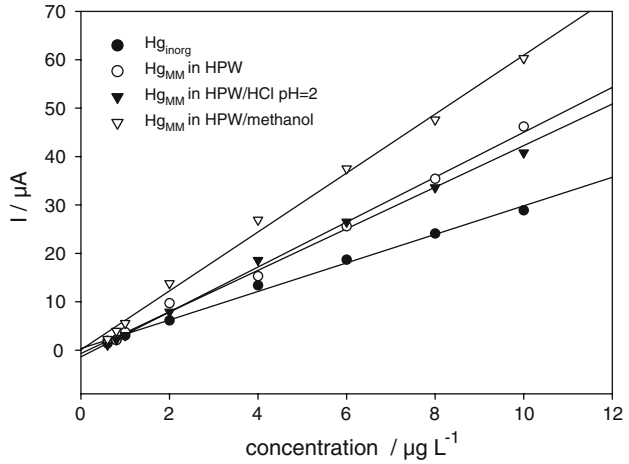

Fig. 3 The peak intensities obtained for different concentrations of ● Hg_{inorg}, ○ Hg_{MM} in HPW, ▼ Hg_{MM} in HPW/HCl, Δ Hg_{MM} in HPW/CH_3OH

to a more efficient solubilization of the salt. The linearity observed in water/HCl is slightly lower than in the other investigated media (see Table 1); however we decided to prepare methylmercury standard solutions in HPW/HCl we obtained more defined peaks, a better repeatability in the considered range than we starts from the other standard solutions and because it is the most extensively used in literature. The electrode response (in terms of µA/µg L^{-1}) tends to decrease as MM concentration increases, because at low analyte levels the reduced competition for electrode surface ensures a more efficient deposition, whereas at higher concentrations more than one layer of mercury on the electrode surface is probably formed, which gives rise to a lower peak. This feature indicates that it is convenient to perform the calibration with standard solutions having concentrations close to the ones present in the samples.

3.2 Determination of total mercury

To determine the total amount of mercury, the sample solutions need to be pretreated in order to convert all mercury into inorganic form. Agraz et al. obtained a complete transformation with an acid digestion [6], whereas Suda et al. [47] treated the samples by UV-irradiation after addition of H_2O_2.

We tested three treatments, namely UV-irradiation, UV-irradiation after addition of H_2O_2 and acid digestion, for the conversion of MM to Hg_{inorg}. Then the concentration of mercury in the solution was quantified with standard additions of inorganic mercury. We found large positive errors when the samples were irradiated without hydrogen peroxide. This seems to be due to the incomplete conversion of CH_3Hg^+ to Hg^{2+}, because the inorganic mercury used for the standard additions caused a lower increment in the peak intensity than MM. The errors were lower if H_2O_2 was added before irradiation, but the results were not yet satisfactory.

Literature data on the decomposition of CH_3Hg^+ are controversial. Some researchers found that the use of a 15 W UV lamp was more than sufficient to ensure efficient conversion to inorganic mercury in simple solutions [24] and also other studies report that UV treatment permits the release of mercury from CH_3Hg^+ [38, 48]. Leermakers et al. observed that it is possible that UV irradiation does not release CH_3Hg^+ quantitatively from complexing substances and an acidification would eliminate this drawback [48].

We then performed experiments with microwave digestion with a mixture of nitric acid and hydrogen peroxide. Before the voltammetric determination we added chloride ions to the test solutions in order to enhance the sensitivity of the mercury stripping signal [49]. We used

Table 1 The equations of the curves, standard deviations of the slope and of the intercept, and R^2 values

Standard solution	Equation of the curve	Std. Dev. of slope	Std. Dev. of intercept	R^2
Hg_{inorg}	I (µA) = 2.95 µg L^{-1} + 0.36	±0.078	±0.412	0.998
Hg_{MM} in HPW	I (µA) = 4.64 µg L^{-1} − 1.36	±0.131	±0.689	0.998
Hg_{MM} in HPW/HCl	I (µA) = 4.29 µg L^{-1} − 0.66	±0.134	±0.707	0.997
Hg_{MM} in HPW/CH_3OH	I (µA) = 6.09 µg L^{-1} + 0.09	±0.163	±0.861	0.998

Table 2 Determination of total mercury after acid digestion of mixtures of Hg_{inorg} and Hg_{MM} using 120 or 60 s as deposition time

Test solution	$[Hg]_{tot}$ found (μg L^{-1}) Dep. time = 120 s	Recovery (%)	$[Hg]_{tot}$ found (μg L^{-1}) Dep. time = 60 s	Recovery (%)
1 μg L^{-1} Hg_{inorg}/1.9 μg L^{-1} Hg_{MM}	3.3 ± 0.2	114	2.7 ± 0.2	93
2 μg L^{-1} Hg_{inorg}/1.9 μg L^{-1} Hg_{MM}	4.1 ± 0.2	105	3.6 ± 0.2	92
4 μg L^{-1} Hg_{inorg}/1.9 μg L^{-1} Hg_{MM}	6.9 ± 0.3	117	5.6 ± 0.3	95

NaCl instead of 60 mM HCl to avoid the formation of nitrosyl chloride which attacks gold electrodes [50].

Table 2 shows the results obtained with mixtures of Hg_{inorg} and Hg_{MM} in different proportions. The concentrations found were always greater than expected with 120 s of deposition time. When the deposition time was reduced to 60 s, in order to reduce the amount of analyte deposited on the electrode surface, the recoveries were higher than 90% for all the considered sample solutions. We can conclude that microwave oven digestion is a suitable treatment for the release of mercury from MM. Moreover the advantages of microwave digestion are the shorter treatment times in comparison to UV irradiation and the minimization of contamination problems, because operations are carried out in closed vessels. Microwave oven digestion is also currently adopted for preparing environmental and biological samples for the determination of total mercury by CVAAS [51–53].

3.3 Discrimination of inorganic and organic mercury

We investigated the possibility to distinguish between Hg_{inorg} and Hg_{MM} by quantifying one species and determining the other by difference. We exploited their different reactivity with stannous chloride, which reduces inorganic mercury only. This procedure is utilised also for speciation using CVAAS: inorganic mercury is determined after reduction to elemental mercury with $SnCl_2$ and the total mercury content is measured after sample mineralization [51, 54]. Organomercury is determined by difference.

In our experiments a simple addition of 20 μg L^{-1} of 10^{-4} M $SnCl_2$ to the sample solution permitted to determine the CH_3Hg^+ concentration in the presence of different amounts of inorganic mercury obtaining an average recovery of 117%, as shown in Table 3.

The reduction of inorganic mercury is not immediate; we recorded seven voltammograms after the addition of $SnCl_2$: in the first five scans we observed a progressive decrease of the mercury peak height, due to the progressive reduction of Hg_{inorg}, afterward the signal was stable and the actual determination could be done. We can conclude that the sample must be prepared 15 min before measurements to ensure a complete reduction. We also verified that the addition of $SnCl_2$ does not influence the response of methylmercury which remains unvaried in the presence of the reducing agent.

Table 3 Determination of Hg_{MM} after acid digestion using $SnCl_2$ to reduce Hg_{inorg} to elemental Hg

Test solution	$[Hg]_{MM}$ found (μg L^{-1})	Recovery (%)
1 μg L^{-1} Hg_{inorg}/1.9 μg L^{-1} Hg_{MM}	2.1 ± 0.2	111
2 μg L^{-1} Hg_{inorg}/1.9 μg L^{-1} Hg_{MM}	2.4 ± 0.1	126
4 μg L^{-1} Hg_{inorg}/1.9 μg L^{-1} Hg_{MM}	2.2 ± 0.1	116

The high recoveries observed for MM are presently unexplained, but they can be considered acceptable if compared to the results of other researchers about analysis of mixtures of Hg^{2+} and CH_3Hg^+. Ireland-Ripert et al. determined CH_3Hg with a gold film electrode with a recovery of 130% and a large relative standard deviation (%RSD ≈ 30%) [33]; Agraz et al. with carbon paste electrode modified with thiolic resin obtained a positive error of 6–10% for relatively high concentrations, ranging from 10 to 50 μg L^{-1} [6]. Moreover, they obtained a relatively high detection limit of 2 μg L^{-1} with long preconcentration times (10–15 min).

Our results confirm the difficulties in the voltammetric determination of MM. In literature we read about different procedures adopted by other researchers to overcome some of these difficulties. Ireland and Ripert recognized that the normal standard addition method would give unsatisfactory results for the determination of methylmercury in the presence of inorganic mercury, and developed the so-called double addition method [33]. With this method we were not able to quantify CH_3Hg^+ or Hg^{2+} simultaneously present in solution, with a few fortunate exceptions (in the presence of particular CH_3Hg^+/Hg^{2+} ratios). Also Lai et al. observed that the simultaneous quantification of Hg^{2+} and CH_3Hg^+ with the method of double standard additions was unsuitable [5]. As described before, we found the same peak potential (0.58 V) for both Hg_{inorg} and MM. Therefore the differentiation of the two species based on a different position of the peaks, as it happens with inorganic tin and organotin compounds [55], is impossible, at least with our procedure. Several works in literature report the overlap of the peaks of the two mercury species [6, 33, 45].

Only Ribeiro et al. and Lai et al. observed well separate oxidation potentials for methylmercury and inorganic mercury, using carbon microelectrode and a Pt microelectrode modified with a Hg film respectively [5, 46].

Other researchers [6, 33, 46] observed that Hg_{inorg} and MM had the same peak potential for oxidation of Hg^0, but that their reduction potential are different; they affirmed that it was possible to deposit Hg_{inorg} at the electrode surface at $0 \div -0.3$ V, whereas more negative potentials ($-0.5 \div -1$ V) are necessary for the reduction of MM.

We suppose that the use of AuNPs-GCE favours the reduction of MM on the electrode, since it is well known that the nanoparticles facilitate the electron transfer between the solution and the electrode surface; indeed our results demonstrated that MM is completely reduced at the gold nanostructured electrode at 0 V. Therefore this feature does not permit to differentiate the two species exploiting different deposition potentials.

Korolczuk and Rutyna described a novel procedure for the selective determination of CH_3Hg^+ in the presence of Hg^{2+} at a gold film electrode. Hg^{2+} ions were complexed with DTPA to move their reduction potential to the metallic state to more negative values than the potential of CH_3Hg^+ reduction to elemental mercury [45]. This procedure may be a suitable and simple way to differentiate between mercury species. We can compare our results with theirs only for methylmercury, because data on inorganic and total mercury are not reported. The detection limit found with the AuNPs-GCE (0.2 μg L^{-1}) was slightly lower than that reported by Korolczuk and Rutyna (0.49 μg L^{-1}), probably thanks to the effect of nanoparticle surface. The recovery of methylmercury found by the authors was 106%, confirming the trend observed in our data and in literature on the excess recoveries for MM.

4 Conclusions

The interest in the development of procedures for the quantification and differentiation of the inorganic and organic forms of mercury derives from their different toxicities, because alkyl mercury derivatives, and mainly methylmercuy, have a higher tendency to bioaccumulation and biomagnification than inorganic mercury.

The determination of CH_3Hg^+ can be carried out at the AuNPs-GCE with good performance using the optimized ASV procedure. The determination of total mercury concentration requires the decomposition of methylmercury, which can be performed by microwave digestion with nitric acid and hydrogen peroxide. The differentiation between inorganic and organic mercury can be obtained by reducing the former to the elemental state with the aid of a selective reducing agent. After determining the total mercury concentration, the amount of Hg_{inorg} can be computed by difference.

ASV coupled to the AuNPs-GCE can also be used for the determination of other elements, e.g. arsenic and copper, or of single species after their separation with suitable pretreatments, such as liquid–liquid extraction. The use of the AuNPs-GCE has two main advantages: (1) the high sensitivity due to the large surface area of gold nanoparticles, which improves the analytical performance (lower detection limits and/or shorter deposition times); (2) the renewable surface which permits to eliminate the problem of irreversible contamination of the gold layer, to minimize memory effects, and to avoid frequent time-consuming and dangerous mechanical cleaning necessary with solid bulk electrodes. This feature permits to attempt to work in more drastic conditions, e.g. with very positive potentials or with aggressive or complex matrices, since in the worst of the hypothesis only the gold surface layer would be damaged, and a new deposition of nanoparticles would be possible.

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