Voltammetric determination of mercury in biological samples using crown ether/multiwalled carbon nanotube-based sensor

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ABSTRACT

An electrochemical assay for sensitive and selective determination of mercury was suggested. Preparation, characterization, and testing of the electro-catalytic activity of Crown-ether/multi-walled carbon nanotubes (MWCNTs)/CPEs were performed. The voltammetric responses of modified electrodes with various sensing elements and nanomaterials were carefully investigated. Consequently, improving the sensitivity and selectivity were achieved using a combination of 12-crown-4-ether and MWCNTs (10% *w/w* for each). A linear response was observed from 5 to 110 μg/ml, with the detection limit (S/N = 3) of 0.25 μg/ml. The method was interference free from many species and successfully applied for the determination of mercury in blood, urine or tab-water samples with a high accuracy and precision comparable with the reference method (Atomic Absorption Spectroscopy AAS).

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1. Introduction

For sure, mercury contamination is a panic since it is a highly toxic element in all of its chemical forms [1–2]. This metal represents an important environmental risk because its compounds are not degraded under environmental conditions; therefore, they are easily bioaccumulated in the liver, brain, and bone tissues. Mercury can cause kidney failure, nervous-system disorders, intellectual impairment, and even death [3–4]. The sources of mercury pollution are natural or anthropogenic, and the latter being the most relevant with regard to environmental contamination. However, approximately 25% of mercury pollution results from fuel combustion, and approximately 30% originated from industrial sources [5].

From the analytical point of view, effective analytical procedures for determination of different mercuric species in various types of samples are needed. Classical methods for mercury determination were relied on atomic techniques, such as cold vapor atomic absorption spectroscopy (CV-AAS) [6–7], atomic emission spectroscopy (AES) [8] or inductive coupled plasma-mass spectroscopy (ICP-MS) [9]. Nevertheless, these methods require expensive instrumentation and it is very difficult to use them out of the laboratory, i.e. online measurements in the field directly without pre-treatments are not possible. Electrochemical methods are a good alternative to the classical techniques, in terms of simplicity, relative low cost of analysis and portability in addition to the online monitoring. Thus, numerous electrochemical methods have

been developed for mercury determination. Most of the reported electrochemical methods were based on the preconcentration of mercury on the working electrode and subsequent stripping, primarily using anodic stripping voltammetry (ASV) [10–11]. As a result, different types of working electrodes and electrode materials have been presented in the literature [12]. The use of bare-carbon electrodes was applied to the analysis, attributed to the low cost and low chemical reactivity [13–14]. However, the low selectivity (interference problems) as well as the low sensitivity (high detection limit) were great challenges. Therefore, further modifications and several electrochemical approaches were developed.

Gold surfaces exhibit a high affinity for mercury deposition, thereby improving the effects of pre-concentration. Strong interaction between the electrochemically reduced form of mercury(0) and the gold electrode surface results in the formation of a compacted and stable-adsorbed layer. Thus, there's a difficulty to achieve the complete stripping of the mercury (i.e. incomplete electrochemical oxidation process). As a result, the contaminated electrode surface will be kept [15].

Consequently, chemically modified electrodes provide advantages, such as high sensitivity and selectivity, when used for the electrochemical determination. In this regard, a selective interaction between the target analyte and the macromolecular sensing material enhances the sensitivity and selectivity of the working electrode. Glassy carbon electrode modified with Nafion-2-ferrocenyl-1,3,9-trithia-14-crown ether was developed for the determination of traces of mercury(II) by the anodic stripping voltammetry [16]. For the easy fabrication and surface modification, carbon paste electrodes (CPEs) have been modified with several species that were able to form a complex and capture the

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mercury on the electrode surface [17–19]. For example, cyclodextrins and other species that have been added into carbon paste matrix to improve the signals of mercury oxidation [20]. For further modification, nano-materials were implemented for enhancing the durability and sensor performance [21–23].

From the selectivity and sensitivity point of view, identification of a high performance recognition element in addition to exploiting the functions of nano-materials is needed for the development of effective method of mercury analysis. Thus, the main concern of this study is the preparation, characterization and testing of the activity of carbon paste electrodes modified with crown ether/MWCNTs composites for voltammetric determination of mercury in different environmental and biological samples. After a comprehensive electrochemical study, the target sensing element and the more efficient nano-structure have been identified.

2. Experimental

2.1. Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Chloride and nitrate salts of metals were provided from Merck or Fluka and were used without any purification. Different cyclic macromolecules were tested as sensing ionophores including; native α-, β- and γ-cyclodextrin (Sigma), 12-crown-4 ether (Fluka), 15-crown-5 ether (Fluka), 21-crown-7 ether (Fluka), 24-crown-8 ether (Fluka), 30-crown-10 ether (Fluka), calix[4]arene and calix[8]arene (Aldrich). Paraffin oil (Fluka), and synthetic carbon powder 1–2 μm (Aldrich) were used for the preparation of the carbon paste electrode. Different carbon nanomaterials including: multiwall carbon nanotubes (MWCNTs, Aldrich), and single wall carbon nanotubes (SWCNTs, Aldrich), were used.

2.2. Apparatus

All electrochemical measurements were performed using a computer controlled Gamry Potentiostat/Galvanostat/ZRA G750, connected to a three electrode system comprising a CNTs–CPE paste working electrode, a Pt disc auxiliary electrode and an Ag/AgCl/3 M KCl reference electrode.

Classical Kjeldahl apparatus, atomic absorption spectroscopy (Perkin-Elmer Model 372) and pH meter (Metrohm), were used for digestions of biological samples (blood and urine), measuring the mercuric content as a reference method and pH measurements, respectively.

2.3. Sensor construction

The Crown ether/MWCNTs modified carbon paste electrodes were prepared by thoroughly hand-mixing 75 mg of MWCNTs, 75 mg of 12-Crown-4, and 600 mg of synthetic graphite powder with 200 μl of paraffin oil in a small mortar. Then the prepared homogeneous-paste was packed into the electrode assembly with a surface area of 0.3 cm². Electrode surface regeneration was performed by polishing with a wet filter paper till a shiny electrode surface was obtained.

2.4. Electrochemical procedures

Prior to each electrochemical measurement, the prepared sensor was electrochemically activated in phthalic acid by 10-cyclic scans from −0.2–1 V with scan rates of 50 mV/s. Cyclic voltammetry (CV) and Linear Sweep Voltammetry (LSV) were used for sensor characterizations, assay optimizations, whereas the signals were recorded in the potential range from 0.2 to 1.0 V with scan rates of 50 mV/s without stirring at room temperature.

2.5. Blood and urine digestions using Kjeldahl method

The blood and urine samples were collected and treated with certain concentrations of mercuric nitrate (spiked samples). The Kjeldahl method was used for digesting and liberating the free metal-ions from the complex organic matrix [24]. Afterwards, the mercuric contamination in the biological samples was determined. In addition to the biological samples, Hg (II) was determined in tap water samples.

3. Results and discussion

3.1. Sensor constructions and characterizations

Since the main concern of this study is to convey a sensitive and selective electrochemical assay for Hg^{2+}, preparation, electrochemical characterization and testing of the electro-catalytic activity of a combination of chelating agents/CNTs nanocomposites were performed.

3.1.1. Effect of sensing ionophore

The principle of the proposed method involves the accumulation of Hg^{2+} ions onto the CPEs' surface via modification of the electrode with different macromolecules namely; cyclodextrins, crown ethers or calixarenes. The adsorbed mercuric concentration was then stripped producing specific oxidation peak current with height depending on the complexation of Hg with the applied electrode modifier.

Thus, the binding affinity of Hg^{2+} toward various derivatives of calixarenes, crown ethers and cyclodextrins has been investigated. In that end, due to the intramolecular interactions, the change in the electrochemical signals of all prepared sensors was obvious allowing mercuric ions to be coordinated/sorbed in their cavity forming inclusion complex, as shown in Fig. 1. However, 12-Crown-4 and 12-Crown-5 showed the greatest electrochemical responses, which may be attributed to the strong binding affinity with the mercuric ions. Therefore, the 12-Crown-4, as the best modifier, has been assigned for the sensor construction.

3.1.2. The impact of carbon nanomaterials

Incorporation of nanomaterials, particularly multi-walled carbon nanotubes (MWCNTs), enhances the sensor performance through improving the conductivity and transduction of the chemical signal to electrical signal [25]. Thus, the carbon nanotubes (CNTs) have attracted considerable attention due to their high electrical conductivity, strong adsorptive ability and good mechanical strength [26]. In order to select
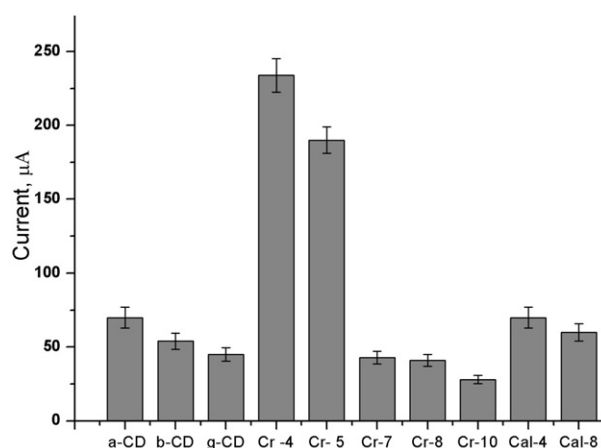
Fig. 1. Voltammetric responses of chemically modified carbon paste electrodes with different recognition elements. 10% of each ionophores (calixarenes, crown ethers or cyclodextrins) was used for the detection of Hg^{2+}. Acetate buffer (0.1 M) was the supporting electrolyte, scan rate 50 mV/s and 30 s for the equilibrium time were implied. The oxidation peak currents that are represented in this figure were subtracted from the baseline of each modified electrode.