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Trace Determination of Hg(II) in Human Saliva Using Disposable Electrochemically Pretreated Graphite Pencil Electrode Surfaces

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Abstract

An electrochemically pretreated graphite pencil electrode (PGPE) was designed to assay trace levels of Hg(II) in human saliva. The GPE was pretreated in 0.1 mol/L nitric acid by cycling the potential between -1.6 and -0.6 V for 60 cycles at a scan rate of 50 mV/s. The effects of pretreatment conditions, including media constituents, pH, and various electrochemical techniques and parameters, were analyzed and optimum conditions determined. Square wave anodic stripping voltammetry (SWASV) was used for the determination of Hg(II). The calibration curve obtained under optimum conditions showed that the linear range of the PGPE was from 10.0×10^{-9} mol/L to 175.0×10^{-9} mol/L with a detection limit of 3.0×10^{-9} mol/L ($S/N = 3$). Relative to non-pretreated GPE surfaces, electrochemical pretreatment improved the electrochemical performance of GPE surfaces in detecting Hg(II). The present analytical method was used to measure Hg(II) released from dental amalgam in human saliva.

Keywords: Pretreated graphite pencil electrode, Mercury (II), Square wave anodic stripping voltammetry, Human saliva

1. Introduction

Mercury is a long-standing occupational hazard, especially in dental offices and health care institutions, as well as in some homes.^{1,2} The major manifestations of mercury poisoning include nephrotoxicity, primarily proteinuria and tubular necrosis, and neurotoxicity, which can be profound with high exposure.³ The most important symptoms of mercury toxicity include tremors, nail changes, hair loss, oral and gingival inflammation, ataxia, excessive and uncontrollable salivation, anorexia and weight loss, labile affect and irritability, pathologic shyness and avoidance of people, and acrodynia (erythema and painful desquamative dermatitis of the hands and feet).^{4–14} Individuals with congenital mercury toxicity will have severe mental retardation and motor abnormalities, including disturbances in swallowing.^{15,16}

Various methods have been developed recently to determine mercury in body fluids, including urine,¹⁷ serum,¹⁸ and saliva.^{19,20} Trace levels of mercury can be measured by techniques including atomic absorption spectroscopy,²¹ cold vapor atomic emission,²² X-ray fluorescence,²³ mass spectrometry,²⁴ and ICP-OES.²⁵ However, all of these methods have limitations in the routine analysis of mercury, including high costs, complex instrumentation, long duration and poor selectivity.

Electrochemical methods are frequently used in analytical chemistry due to their high sensitivity, low cost, fast response, simple instrumentation and portability.^{26,27} The poor electrocatalytic properties of conventional electrodes, however, limit their use in measuring mercury concentrations. These electrocatalytic properties of electrodes can be improved by electrochemical pretreatment,²⁸ modifying the electrode with a suitable electrocatalyst or electron mediator,^{29,30} and using a solution that enhances electrochemical reactions. Various types of modified electrodes have been developed to detect and measure mercury concentrations. These include silica modified electrodes,³¹ bimetallic Au-Pt inorganic-organic hybrid nanocomposite-modified electrodes,³² mercaptoacetic acid modified gold microwire electrodes,³³ organic-inorganic pillared montmorillonite-modified electrodes,³⁴ electro-

des modified with 5-methyl-2-thiouracil, graphene oxide and gold nanoparticles,³⁵ carbon nanotube modified electrodes,³⁶ and DNA-modified electrodes.³⁷ Despite the selectivity of these voltammetric techniques, methods that are cheaper and/or more sensitive and selective are needed to detect mercury. Electrochemical pretreatment of pencil graphite electrodes is a simpler, less time consuming and more applicable strategy compared with other procedures. This method eliminates the use of some toxic compounds required in the modification of the electrode surface.

Trace metals in saliva may be biomarkers for exposure to and metabolism of trace metals.³⁸ Blood flow in salivary glands is high, with chemicals and metabolites distributed in saliva by several mechanisms, including passive diffusion, active transport, and ultrafiltration.³⁹ Previous studies on the use of saliva for biomonitoring have focused on herbicides,⁴⁰ lead,⁴¹ phthalate,⁴² and fluoride ions,⁴³ in humans, animals or artificial models. The concentrations of chemical contaminants in saliva have been shown to reflect their concentrations in plasma. Saliva sampling is non-invasive and has advantages over urine and blood collection, particularly from newborns and infants. The present study describes a simple sensor, based on an electrochemically pretreated graphite pencil electrode (PGPE), for the detection of trace levels of mercury (II) in human saliva. The analytical performance of this sensor was evaluated by anodic stripping square wave voltammetry.

2. Experimental

2.1. Reagents

All chemicals used in this study were analytical reagent grade and used without further purification. Hydrogen peroxide (30%), sodium hydroxide, lithium chlorate and sodium acetate buffer (3.0 mol/L, pH 5.2) were obtained from Sigma Aldrich® (USA). Nitric acid was obtained from AnalaR® (England). A standard stock solution of mercury (5.0×10^{-3} mol/L, plasma emission standard solution) was obtained from BDH, ARISTAR® (England) and diluted as required. Hi-polymer graphite pencil HB black leads were obtained from Pentel (Japan). All leads had a total length of 60.0 mm and a diameter of 0.5 mm and were used as received.

2.2. Apparatus and Procedures

A Jedo mechanical pencil (Korea) was used to hold both bare and pretreated graphite pencil leads. Electrical contact with the lead was achieved by soldering a copper wire to the metallic part that holds the lead in place inside the pencil. The pencil was fixed vertically with 15 mm of the lead extruded outside, and 10 mm of the lead immersed in the solution, corresponding to an electrode area of ca. 16 mm². An electrochemical analyzer (CHI 660C model, CH Instruments, USA), was used in all electroche-

mical experiments. The electrochemical cell contained a PGPE as a working electrode, a Pt wire counter electrode, and an Ag/AgCl (Sat. KCl) reference electrode. Saliva samples were analyzed using an ICP-OES (iCAP 6000 series) spectrometer (Thermo Scientific, USA).

2.3. Pretreatment of GPE

A 10.0 mm length of GPE extruded from the pencil, an Ag/AgCl reference electrode, and a Pt counter electrode were immersed in a cell containing HNO₃ or other solutions at different concentrations, and different potential ranges were applied to pretreat the GPE surface. The pretreated electrodes were washed by gently dipping them twice in deionized water, and all entire electrochemical measurements were performed right after preparation of the pretreated electrodes.

2.4. Saliva Collection

Saliva was collected according to the recommendations of the World Medical Association Declaration of Helsinki for International Health Research. Saliva samples were obtained from volunteers living in Dhahran, Saudi Arabia, at least one hour after food consumption and after participants rinsed their mouths with water at least three times to remove any food residue. The samples were spat into detergent washed collection vials and examined for the presence of food, blood or nasal discharge. Contaminated samples were discarded, and retained samples were stored at -20.0 °C until analyzed.

2.5. Digestion of Saliva

Prior to sample preparation, the saliva samples were thawed and allowed to equilibrate to room temperature before being rechecked for any trace contaminants. A 5.0 ml aliquot of saliva was placed in a beaker, to which 20.0 ml of 2% nitric acid and 5.0 ml of 10.0 mol/L hydrogen peroxide were added. This solution was filtered through a Whatman no. 42 filter paper into a 100.0 ml volumetric flask and diluted to a final volume with distilled deionized water (DDW). These samples were stored until analyzed.

2.6. Electrochemical Procedure

The GPE, a working electrode in a three electrochemical cell, was immersed in 0.1 mol/L HNO₃ solution and treated electrochemically using cyclic voltammetric technique at a scan rate of 50 mV/s, with a potential range of -1.6 to -0.6 V, for 60 segments. The SWASV measurements of Hg(II) were completed after a deposition time of 300 s at -1.6 V from a stirred 0.1 mol/L pH 5.5 acetate buffer solution. The electrode was stripped after a 10 s rest period (without stirring) at an amplitude of 0.06 V and a frequency of 100 Hz, the experimentally determined opti-

mal parameters for Hg(II) determination by the SWASV method using PGPE (Table 1).

3. Results and Discussion

3.1. Evaluation of the Electrode and Pretreatment Solution

The electrochemical oxidation of Hg(II) was assessed by recording SWASVs of different electrode materials, including glassy carbon, graphite pencil, carbon paste, Pt disc and gold disc electrodes, in acetate buffer (0.1 mol/L, pH 5.5) (Figure 1a). Carbon paste, Pt disc and gold disc electrodes did not respond well to 6.2×10^{-7} mol/L Hg(II) solution. This may have been due to differences among the various types of carbon electrodes (i.e. carbon paste, glassy carbon, and graphite electrodes), and to the effect of the electrochemical treatment in 0.1 mol/L HNO₃. Both GCE and GPE gave a relatively well-defined SWASV signal, with the highest obtained signal at the GPE (Figure 1b).

Figure 1: a) Square wave anodic stripping voltammograms (SWASVs) and b) corresponding histograms of 6.2×10^{-7} mol/L Hg(II) in acetate buffer (0.1 mol/L, pH 5.5) at 1) carbon paste, 2) Pt, 3 Au, 4) glass carbon and 5) graphite pencil electrode. Working conditions: deposition potential, -1.6 V; deposition time, 120 s; frequency, 100 Hz; scan rate, 100 mV/s; amplitude, 0.06 V

The differences in behavior among these five electrodes, in particular between the Pt- and Au-electrodes illustrated in Figure 1b, are observed. This may be due to amalgam formation with gold, as gold-mercury amalgam formation is well-known and even used in the extraction of gold from ore; platinum, by contrast, does not form such an amalgam. Various types of gold electrodes, including solid gold,⁴⁴ gold fiber,⁴⁵ and plated gold,^{46–48} electrodes have been used for the determination of mercury; however, to the best of our knowledge, no study to date has examined platinum electrodes for this purpose.

Because a high electrochemical oxidation signal is essential for the fabrication of an ultrasensitive electroanalytical sensor, GPE was chosen as the transducer material for the electroanalytical determination of Hg(II).

The effect of GPE pretreatment solution was evaluated in NaOH, LiClO₄, HNO₃, H₂O₂ and a mixture of H₂O₂ and HNO₃ (Figure 2a). The potential pretreatment range was between -1.6 to -0.6 V of cyclic voltammetry (CV) with 20 pretreatment scans at a scan rate of 100 mV/s, followed by detection of Hg(II) in acetate buffer (0.1 mol/L, pH 5.5). Pretreatment of the pencil graphite electrode in

Figure 2: a) Square wave anodic stripping voltammograms (SWASVs) and b) corresponding histograms of 6.2×10^{-7} mol/L Hg(II) in acetate buffer (0.1 mol/L, pH 5.5) at 1) unpretreated (6) and pretreated GPEs in 1) NaOH, 2) LiClO₄, 3) H₂O₂ and HNO₃ and 5) HNO₃, each at a concentration of 0.1 mol/L. Working conditions: deposition potential, -1.6 V; deposition time, 120 s; frequency, 100 Hz; amplitude, 0.06 V

LiClO_4 and NaOH did not increase the SWASV response of Hg(II) . In contrast, both H_2O_2 and HNO_3 prominently increased the peak current for Hg(II) , with 0.1 mol/L HNO_3 showing the highest peak current of Hg(II) . This may be attributed to an increase in surface roughness and a corresponding increase in electrode surface area. GPEs electrochemically pretreated with 0.1 mol/L HNO_3 were used in further experiments.

3. 2. Effect of Electrochemical Pretreatment

The main constituents of pencil graphite electrodes are graphite (65%), clay (nearly 30%) and an electro-inactive polymer acting as a binder (5%). Graphite is a form of carbon in which atoms are connected by weak bonds between planes. Clay is a naturally occurring aluminosilicate with ion exchange properties. However, the graphite part of a pencil in contact with the pretreatment solution, such as HNO_3 , is cleaned and linked to various oxygen-containing functional groups. An increased GPE signal after pretreatment can be attributed to an increase in the num-

ber of oxygen-containing groups on the electrode surface or to the formation of a graphite oxide film.

To determine the effect of the concentration of pretreatment solution (HNO_3) on the PGPE, GPEs were pretreated with different concentrations of HNO_3 , ranging from 0.05 mol/L to 0.8 mol/L, in a potential range of -1.6 to -0.6 V at a fixed scan rate of 100 mV/s, and Hg(II) concentrations were measured with the pretreated electrodes. Figure 3a shows SWASVs obtained using these electrodes in acetate buffer (0.1 mol/L, pH 5.5). As its concentration increased, the peak current for 6.2×10^{-7} mol/L Hg(II) also increased, with a maximum peak current obtained at 0.1 mol/L HNO_3 (Figure 3b).

To evaluate the number of CV segments, GPEs were potentiodynamically pretreated by altering the number of scans between -1.6 and -0.6 V, at a scan rate of 100 mV/s. The maximum i_p was observed after 60 pretreatment scans, making the optimum number of pretreatment scans 60 segments (Figure 4).

We also assessed the effect of potential scan range on GPE. Figure 5a shows the influence of scanning poten-

Figure 3: a) Square wave anodic stripping voltammograms (SWASVs) of 6.2×10^{-7} mol/L Hg(II) in 0.1 mol/L acetate buffer, pH 5.5 at GPE surfaces pretreated in 1) 0.05, 2) 0.1, 3) 0.2, 4) 0.4, 5) 0.6 and 6) 0.8 mol/L HNO_3 . Working conditions: Pretreatment CV segments, 20; pretreatment potential, -1.6 to -0.6 ; deposition potential, -1.6 V; deposition time, 120 s; frequency, 100 Hz; amplitude, 0.06 V. b) Corresponding plot of peak currents i_p (μA) vs. HNO_3 concentrations.

Figure 4: a) Square wave anodic stripping voltammograms (SWASVs) of 6.2×10^{-7} mol/L Hg(II) in 0.1 mol/L acetate buffer, pH 5.5 as a function of number of pretreated CV segments on GPE surfaces: 1) 20, 2) 40, 3) 60, 4) 80 and 5) 100 segments. Working conditions: Pretreatment potential, -1.6 to -0.6 ; pretreatment solution, 0.1 mol/L HNO_3 ; deposition potential, -1.6 V; deposition time, 120 s; frequency, 100 Hz; amplitude, 0.06 V. b) Corresponding plot of i_p vs number of pretreated CV segments.

tial range used during GPE pretreatment on SWV in a 0.1 mol/L solution of acetate buffer (pH 5.5) containing 6.2×10^{-7} mol/L Hg(II). The potential range of -1.6 to -0.6 V showed the highest peak current (Figure 5b).

Figure 5: a) Square wave anodic stripping voltammograms (SWASVs) and b) corresponding histograms of 6.2×10^{-7} mol/L Hg(II) in 0.1 mol/L acetate buffer, pH 5.5 at GPE surfaces and after pretreatment potential ranges of 1) -1.6 to -0.6 , 2) -0.6 to 0.6 and 3) 0.6 to 1.6 V. Twenty CV segments were pretreated; other working conditions were identical to those in Fig. 4a.

Figure 6: Histograms showing the effect of pretreatment scan rate on the detection of 6.2×10^{-7} mol/L Hg(II) in 0.1 mol/L acetate buffer (pH 5.5) at GPE surfaces. Pretreatment CV segments, 20; other working conditions are described in Fig. 4a.

Figure 6 shows the effect of scan rate on Hg(II) response at the electrochemically pretreated GPE, with a scan rate of 50 mV/s showing the maximum response.

3. 3. Optimization of SWASV Parameters

To select a suitable voltammetric technique for the detection of Hg(II) using the developed GPE, different voltammetric techniques were tested, including differential pulse, square wave, differential normal pulse, linear sweep, staircase, and normal pulse voltammetry. Of these methods, square wave voltammetry showed the highest peak current for the same concentration of Hg(II) (Figure 7).

Figure 7: Histograms showing the effect of various voltammetric techniques at GPE surfaces on the detection of 6.2×10^{-7} mol/L Hg(II) in 0.1 mol/L acetate buffer (pH 5.5). Working conditions: deposition potential -1.6 V, amplitude 0.06 V, frequency 100 Hz, deposition time 120 s.

To select the best medium for detecting Hg(II), various solutions were tested, including HNO_3 , NaOH, and acetate and phosphate buffers, all at the same concentration, 0.1 mol/L. NaOH and phosphate buffer showed no peak for 6.2×10^{-7} mol/L Hg(II), whereas HNO_3 and acetate buffer showed well-defined peaks (Figure 8a). Because the peak current for Hg(II) was the highest in 0.1 mol/L acetate buffer (Figure 8b), further optimizations were completed in acetate buffer solution.

The pH of the aqueous medium and the SWASV parameters can significantly influence the detection limit of any analyte. Thus, the effects of pH and SWASV parameters on Hg(II) electro-oxidation by PGPE were analyzed.

The SWV response to the electro-oxidation of 6.2×10^{-7} mol/L Hg(II) in acetate buffer at the PGPE was systematically studied over the pH range 3.2–6.5. As the pH increased, the electro-oxidation peak potential (E_p) of Hg(II) became less positive (Figure 9a). The highest electro-oxidation signal was obtained at pH 5.5 (Figure 9b), making this the optimum pH.

Figure 8: a) Square wave anodic stripping voltammograms (SWASVs) and b) corresponding histograms of 6.2×10^{-7} mol/L Hg(II) at GPE surfaces in a) 0.1 mol/L NaOH b) 0.1 mol/L phosphate buffer, c) 0.1 mol/L HNO₃ and d) 0.1 mol/L acetate buffer. Pretreatment CV segments, 20; other working conditions are identical to those in Fig. 4a.

To determine the effect of amplitude variation on the activity of the PGPE, Hg(II) was measured at different amplitudes. The SWV curves showed variations in peak current and peak potential, with an amplitude of 0.06 V being optimal for Hg(II) detection (Figure 10a).

To test the effect of frequency on PGPE activity, different frequencies were applied to detect of 6.2×10^{-7} mol/L Hg(II), while maintaining all other parameters con-

Table 1: Optimal parameters for Hg(II) determination by the SWASV method using PGPE.

Parameter	Optimum Parameter
Electrode type	PGPE
Pretreatment solution	0.1 mol/L HNO ₃
Pretreatment potential range, V	–1.6 to –0.6
Pretreatment CV scan segments	60
Pretreatment CV scan rate, V/s	50
Sensing solution, mol/L	0.1 acetate buffer
Sensing pH	pH 5.5
Amplitude, V	0.06
Frequency, Hz	100
Deposition time, s	300

Figure 9: a) Square wave anodic stripping voltammograms (SWASVs) of 6.2×10^{-7} mol/L Hg(II) at GPE surfaces in 0.1 mol/L acetate buffer at pH 1) 3.2, 2) 4.0, 3) 4.8, 4) 5.5, 5) 6.0 and 6) 6.5. Pretreatment CV segments, 20; other working conditions are identical to those in Fig. 2a. b) Corresponding plot of pH vs peak current.

stant. The highest peak current was obtained when a 100.0 Hz frequency was applied (Figure 10b), making 100.0 Hz the optimum frequency for Hg(II) detection.

We also attempted to optimize the deposition time required for the detection of Hg(II) at the PGPE. Peak current increased at deposition times of 0–300 s, but later became nearly constant (Figure 10c). Finally, we attempted to optimize the deposition potential for 6.2×10^{-7} mol/L Hg(II) at the PGPE. The deposition potential was varied from –1.4 V to –2.0 V, with the peak current highest for –1.6 V (Figure 10d). The optimal SWASV parameters are summarized in Table 1.

3. 4. Calibration

The dependence of Hg(II) peak currents on their concentrations are presented in Figure 11. Under the optimum conditions described in Table 1, the peak currents were linearly proportional to Hg(II) concentration ranging from 5.0×10^{-9} mol/L to 1.75×10^{-7} mol/L ($R^2 = 0.994$; Figure 11, inset). Thus the limit of quantification was 10.0×10^{-9} mol/L and the limit of detection was ($S/N = 3$) 3.0

Figure 10: Plots of peak current vs. a) amplitude, b) frequency, c) deposition time, and d) deposition potential of the square wave voltammograms of 6.2×10^{-7} mol/L Hg(II) solution in 0.1 mol/L acetate buffer (pH 5.5) at GPE surfaces. Other conditions are identical to those described in Fig. 2a.

$\times 10^{-9}$ mol/L for Hg(II) at the PGPE. The relationship between i_p and Hg(II) concentration can be represented by the equation $i_p = aC + b$, where a and b are the slope and

intercept of the straight line respectively. These results indicate that our method based on square wave adsorption stripping voltammetry using inexpensive and renewable graphite pencil electrodes is both convenient and efficient for quantitation of Hg(II).

3. 5. Determination of Hg(II) in Human Saliva

Because of their low electroactivity, non-pretreated GPEs cannot detect Hg(II) in human saliva. The ability of the PGPE to detect low Hg(II) concentrations in saliva was determined. PGPE yielded promising results (Table 2), similar to ICP-OES for the same Hg(II) concentrations.

4. Conclusions

This study showed that pretreatment of GPE electrode surfaces enhanced the electrochemical catalytic activity of these electrodes towards the oxidation of Hg(II). Comparison of non-pretreated GPEs and PGPEs showed that the latter are highly sensitive, with a low limit of detection ($S/N = 3$) of 3.0×10^{-9} mol/L. Moreover, PGPEs are sensors that

Figure 11: Square wave anodic stripping voltammograms (SWASVs) in 0.1 mol/L acetate buffer, pH 5.5, containing 1) 0.0, 2) 5.0×10^{-9} , 3) 1.0×10^{-8} , 4) 2.5×10^{-8} , 5) 5.0×10^{-8} , 6) 7.5×10^{-8} , 7) 1.0×10^{-7} , 8) 1.5×10^{-7} , and 9) 1.75×10^{-7} mol/L of Hg(II) at the pretreated GPE. Other working conditions were identical to those in Table 1. The inset shows the corresponding calibration curve from 1.0×10^{-8} to 1.0×10^{-7} mol/L Hg(II).

Table 2: Concentrations of Hg(II) in spiked saliva samples measured by PGPE and inductively coupled plasma (ICP-OES).

Saliva Sample	Hg(II) Concentration after Addition	Hg(II) Concentration Detected by ICP-OES	Hg(II) Concentration Detected by the Electrochemical Method	Recovery of the Electrochemical Method
1	25.0 nmol/L	25.0 ± 10.0 nmol/L	25.0 ± 4.0 nmol/L	100.0%
2	50.0 nmol/L	41.0 ± 6.0 nmol/L	47.0 ± 5.5 nmol/L	94.0%
3	100.0 nmol/L	96.0 ± 4.9 nmol/L	99.5 ± 3.5 nmol/L	99.5%

(where n = 3)

are both inexpensive and easy to manufacture. These sensors give satisfactory results when used to detect low concentrations of Hg(II) in human saliva samples. As saliva can be easily and non-invasively collected, the development of these sensors can allow the use of human saliva as a biomonitoring matrix to electrochemically measure Hg(II).

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6. References

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Povzetek

Pripravili smo elektrokemijsko obdelano elektrodo iz grafitnega svinčnika (PGPE) za analizo sledov Hg (II) v humani slini. Obdelavo GPE smo izvedli z uporabo 0,1 mol/L dušikove kisline in ciklanje potenciala med –1,6 in –0,6 V v 60 ciklih pri hitrosti skeniranja 50 mV/s. Optimalne pogoje smo določili na osnovi analize vplivov sestavin medija, pH in različnih elektrokemijskih tehnik in parametrov. Za določanje Hg (II) smo uporabili anodno inverzno voltometrijo s pravokotnimi pulzi (»square wave anodic stripping voltammetry«, SWASV). Pri optimalnih pogojih je bila kalibracijska krivulja linearna v območju od 10×10^{-9} mol/L do 175×10^{-9} mol/L, meja zaznave pa $3,0 \times 10^{-9}$ mol/L (S/N = 3). Metodo smo uporabili za merjenje Hg (II) v slini, kamor prehaja iz amalgamskih zalivk.