Bismuth/hydroxyapatite-modified carbon screen-printed electrode for heavy-metal ion detection in aqueous media

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Abstract. Square-wave voltammetric stripping analysis is attractive for environmental monitoring and trace metal ion determination. The sensitivity is a result of analytes preconcentration steps on the electrode and advanced measurement procedures, where metal analytes are stripped away from the electrode at appropriate potential scan. Screen-printed electrode (SPE) has great advantages for *in situ* assays of heavy metal ions. Modification of SPE with bismuth (Bi) film improves the amalgamation of metal ions and the addition of hydroxyapatite (HA) increases the ion sorption, and enhances the current response due to the large porous structure and surface active sites for the metal ion binding. The ionization of the functional groups on the electrode surface upon contact with the aqueous system further assists the cation binding. The analytical performance of Bi and HA-modified SPE for simultaneous detection of Cd(II) and Pb(II) ions by square wave anodic stripping voltammetry (SWASV) was evaluated. Under the optimized electrochemical working conditions, calibration graph is linear for 240 s deposition time, in 0.1 M acetate buffer at pH 7.6 with the detection limit of 16.8 ppb for Pb(II). Two peaks corresponding to Cd(II) at –0.8 V and Pb(II) at –0.6 V can be discerned suggesting that Bi-HA modification had increased the current responses.

1 Introduction

Heavy metals are non-biodegradable and follow a cycle in the environment which include water (surface, ground, precipitation), air (particulate matter), soil (bottom sediment) and biosphere (plants, animals, fish, human). Among the priority heavy metal pollutants are lead, cadmium, and mercury [1]. These may be retained indefinitely in the eco-systems and food chain. Lead accumulation has harmful effects on soil microflora and crop growth which will eventually affect the food safety [2]. Lead exposure has caused numerous health effects such as lifelessness, tetchiness, reduced attention span and memory loss, and decreased glutathione in red blood cells which may lead to hallucination, convulsions, paralysis, coma and death [3,4]. Excessive cadmium can lead to hypertension, immuno-suppression, emphysema, kidney failure, bone demineralization, neurotoxicity and induce cancer [5,6].

The maximum allowable limit for heavy metals in water set by the US Environmental Protection Agency is (ppm) 0.002 (mercury), 0.005 (cadmium) and 0.015 (lead) [7]. Environmental monitoring is therefore crucial to evaluate the disturbances to environmental cycles so that strategies can be devised to allow control and mitigation of any potential disaster. One of the Principal of Green Chemistry is to promote analytical methodology development for real-time analysis, *in-situ* monitoring and management before hazardous substances formation [8,9]. Screen-printed electrode (SPE) is promising for this *in situ* assay due to its versatility, disposability, ease of preparation, tailored capability and affordability through electronic, optical, or micro-gravimetric signal transduction. Screen-printing technology enables mass production of portable systems in the market with the development of disposable SPE as a new dimension in electrochemical-analysis for real-time, non-invasive analysis of a sample [10–14]. The attractiveness is boosted by the possibility of fabricating SPE into the microelectrodes and incorporation into highly sensitive biosensors [15–18]. SPEs are versatile through preparation in machine-controlled manufacturing, for low cost production of carbon ink-based electrodes of small dimensions, and planar configuration [15] and suitable as single-use sensors.

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Modification of sensor surface is one of the methods to enhance the sensitivity and selectivity of detection. Bismuth (Bi) has low toxicity with widespread pharmaceutical use [19]. Different types of Bi electrodes have been developed for analysis of inorganic ions and organic compounds [20] such as Bi-film and Bi-bulk film electrodes [2121,22], Bi-microelectrode and microelectrode arrays [2323,24], Bi-nanoparticle modified electrodes [25], graphene/Bi nanocomposite film-modified glassy carbon electrode [2626], Bi/electrochemically reduced graphene/ionic liquid composite modified SPE [2727], Bi-film/crown ether/Nafion modified SPE [28], Bi-film SPE [29] and micro-patterned reduced graphene oxide/carbon nanotube/Bi composite electrodes [30]. The analytical properties are attributed to the property of Bi to form "fused alloys" with heavy metals, analogous to the amalgams that mercury forms [19].

Hydroxyapatite (HA), the main constituent of bones and teeth, is a bioactive ceramic material with high bio-affinity and biocompatibility. HA consists mainly of calcium phosphate, with sorption capacity for divalent heavy metal ions. The metal ions interact with HA modified electrode by pre-concentration with surface complexation, followed by simultaneous adsorption on HA, and calcium ion substitution coupled with diffusion on the electrode surface. Bi nanoparticles [31], HA [32] and Bi-HA [33] have been used as modifiers, with resulting higher sensitivity. We have recently reported the Limit of Detection (LOD) and Limit of Quantification (LOQ) of the carbon-modified electrode based on cellulose-HA composite which achieves 0.11 ppb LOD and 0.36 LOQ for trace Pb(II) ion detection in blood serum [3434]. To the best of our knowledge, the Bi-HA SPE has not yet been reported for simultaneous detection of Pb(II) and Cd(II) ions.

In this study, Bi film and HA was applied on the SPE and the effects were compared with the carbon paste electrodes (CPE) for the detection of Pb^{2+} and Cd^{2+} ions in aqueous media. The main objectives were to develop robust, portable and user-friendly detection methods based on BiHA-modified SPE.

2 Materials and methods

2.1 Chemicals and SPE

HA (Sigma-Aldrich), $Bi(NO_3)_2$ (Merck) and all the chemicals used were of analytical grade.

The commercial SPE (Figure 1) was a 4 mm diameter probe from DropSens (Asturias, Spain), connected to the DropSens potentiostat (µStat400) by a flexible cable (Connector, DropSens). A complete electrochemical cell with three electrodes was in one single strip consisting of graphite working electrode (WE) for modifier application, a graphite counter electrode (CE) and a silver pseudo-reference electrode (RE).

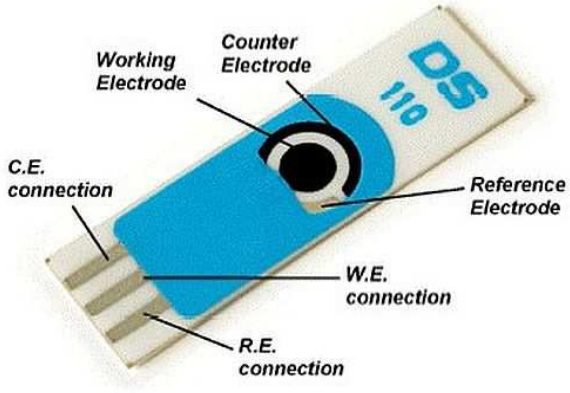

Fig. 1. Dropsens carbon SPE [35].

2.2 *Ex situ* modification

The SPE was immersed in 20 mL of 0.2 M acetate buffer solution (pH 4.5) containing 500 ppb Bi(II) for modification of WE. The solution was degassed under vacuum for 10 min, and the deposition potential of –1.0 V was applied for 240 s with solution stirred, followed by a rest period (without stirring) of 20s. This allowed Bi(II) film to be deposited and the modified SPE was rinsed carefully with deionized water. The Bi solution was then replaced in the cell by analyte solution. For comparison, an Hg film deposition was also carried out, only that the SPE was immersed in 20 mL of 0.2 M acetate buffer solution (pH 4.5) containing 500 ppb Hg^{2+}. Once the Hg film was deposited, SPE was rinsed carefully with deionized water and the Hg^{2+} solution replaced.

2.3 *In situ* modification

One common technique of applying HA modification is a simple layer application by mixing HA with graphite in the form of paste, before applying on the SPE surface. HA-graphite was applied to the 4 mm diameter working area surface and left to dry at room temperature. In another technique, 10 mg HA was dissolved in 10 mL deionized water, followed by ultrasonication for 20 min. Subsequently, 5 µL of HA was dropped on the electrode surface and left to dry for 1 hr.

2.4 SPE analysis

Electrochemical measurements (cyclic voltammetry (CV) and square-wave voltammetry (SWV)) were performed using a portable, handheld, µStat400 battery-operated bipotentiostat/galvanostat (DropSens, Spain). It was connected to a computer via USB and Bluetooth and controlled by Dropview software version 2.9 for Windows Vista and Windows 7 (DropSens, Spain).

For analyte measurement, 100 µL of test solution was placed on the WE area (4 mm diameter) of a modified SPE, ensuring that the area was completely covered by the drop. This was followed by the application of SWV using a square-wave potential ramp with a frequency of 25 Hz, a potential step of 4 mV, and amplitude of 25 mV. The electrochemical accumulation step was at −1.0 V for 120 s, an equilibration period was 15 sec, and the SWV stripping scan was from −1.0 to –0.2 V.

2.5 Process parameters

Heavy metal analyses of modified electrodes depend mainly on the process conditions. SWV was applied to modified electrodes under different electrolytes (HCl, KCl, NaCl, NaH_2PO_4 and acetate buffer), pH, deposition potential and deposition time to investigate enhanced conditions for voltammetric analysis.

2.6 Calibration, validation and data analyses

Calibration was obtained by a standard addition method by first measuring the peak area/peak height of current response curve of the sample with an unknown concentration. The 1000 mg/L calibration standard solutions of Cd and Pb in 0.05 M HNO_3 were used as standards. Standard solutions for calibration were prepared by diluting appropriate volume of 1000 mg/L calibration standard solutions with 1 % KNO_3.

To calibrate heavy metal analysis in environmental water sample, results obtained with modified electrodes were compared with the atomic absorption spectrometer (AAS) (Hitachi Z-5000, Japan) with a graphite lamp. The AAS was calibrated with standard solutions prior to the determination of the detection and quantification limits. Standard method for analyzing heavy metal ions in trace quantities was employed using an AAS. Five standard solutions were prepared ranging from 4 ppb to 50 ppb in 10 mL volumetric flasks.

The calibration curve was established as follows:

$$\Delta\mu A = b\ (\text{Pb conc.}) + a \qquad (1)$$

where $\Delta\mu A = I_p - I_b$, I_p is the peak current (μA), I_b is the base-line current (μA), a is the intercept, and b is the slope.

The LOD and LOQ were calculated as follows [36,37]:

$$\text{LOD or LOQ} = k\ (\text{S.D.}a)/b \qquad (2)$$

where k = 3 (for LOD) ; and k = 10 (for LOQ); S.D.a is the standard deviation of the intercept; and b is the slope.

3 Results and discussions

3.1 Electrode synthesis

The SEM, FESEM, FTIR and the XRD analyses have been reported before to elucidate the interaction of the material with the substrate [33]. The SEM and FESEM micrographs suggest that the powdered layer of the HA exhibits meso-porous particles of 150 ± 50 nm in length, and the EDX spectra of HA demonstrates the presence of P and Ca peaks. The HA distribution in between the graphite flakes of the HA-modified carbon electrode (HA-CME) surface was disorderly, suggesting higher surface area for metal ions sorption. The IR spectra of the HA powder demonstrates the typical absorption bands related to the bands of phosphate, hydroxyl, water, and hydrogen phosphate. It is possible that the heavy metal ion sorption in HA may take place at the hydroxide and carbonate groups [3333]. The XRD pattern show the broad diffraction peaks corresponding to the Ca-HA [3838], which indicate the low crystallinity of HA with mesoporous structure and the *d*-spacing of 1.227 Å. The small *d*-spacing may actually highlight the potential of the metal ions being sorbed and held within the sorption sites, and not easily leaked out [33].

3.2 Voltammetric characteristics

Electrochemical interactions at the SPE surface were examined with CV to evaluate the changes in electron transfer at the surface of the modified electrode. Figure 2 shows the voltammograms for HA-modified SPE from CV at the scan rate of 25 mV/s – 1.0 V/s using 5.0 mM $K_3Fe(CN)_6$ as the test probe in 0.1 M KCl. Increase in scan rate had resulted in an increase in the electron transfer as evident in the increased peak potential difference (ΔE_p) between anodic and cathodic potential.

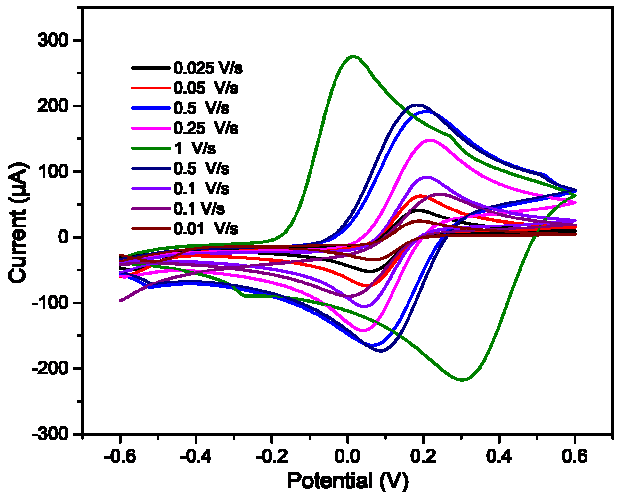

Fig. 2. Cyclic voltammogram of BiHA-SPE in supporting electrolyte 0.1 M KCl with pH 5.6 at the scan rate of 25 mV/s to 1.0 V/s.

3.3 Improvement of the BiHA-SPE conditions

Improvement studies were carried out using square-wave anodic stripping voltammetry (SWASV) measurements. In theory, when the potential reaches its characteristic oxidation potential, unique for each metal [3939], the metal gets stripped out from the electrode surface with the generation of oxidation signal. The I_p is the measuring signal that transforms into the anodic current at these potentials which is proportional to the analyte concentration in the sample [4040,41]. The reaction of interest occurs at the WE which behaves as a cathode, where metal ions such as Pb^{2+} are reduced into metals in their metallic forms, Pb, as shown in the following mechanism:

$$Pb^{2+} + 2e^- \rightarrow Pb \text{ (Reduction)} \quad (3)$$

$$Pb \rightarrow Pb^{2+} + 2e^- \text{ (Oxidation)} \quad (4)$$

For Pb^{2+} to be reduced, the potential should be low enough, called the deposition potential. After the period of deposition, the potential is increased linearly (linear potential scan) and Pb starts to be oxidized at the same WE (serves as an anode) causing a sharp increase in current. As the deposited Pb is consumed, the current drops at low level (to give the first peak of Pb).

The supporting electrolyte and the pH effects on the Bi-HA SPE were investigated by adding 0.1 M HNO_3, 0.1 M $HClO_4$, 0.1M HCl and 0.1 M acetate buffer at various pHs. Higher I_p of $Pb^{2+}$and Cd^{2+} were achieved in 0.1 M acetate buffer at pH 7.6. Acidic medium is suitable for relegation of Pb as shown in Equation 5 and 6 where Pb(II) species leach out from the HA at the electrode/solution interface and can be detected directly by reduction [42].

$$HA–Pb(II) \rightarrow HA + Pb(II) \quad (5)$$

$$Pb(II) + 2e^- \rightarrow Pb(0) \quad (6)$$

The decreased I_p of $Pb^{2+}$and Cd^{2+} in nitric acid and perchloric acid may probably be due to their oxidizing properties, resulting in the reduction of gaseous electro-inactive products, NO_2 and ClO_2, during deposition step. This is attributed to the kinetic polarization, thus shifting the reduction potentials of ions to the more negative potentials [43]. It is important to fix the accumulation potential (E_{acc}) and the time (t_{acc}) during the SWV studies. The influences of the E_{acc} on the reduction peaks of Pb^{2+} and Cd^{2+} were studied over the range of –1.4 to –0.6 V. The plot of stripping I_p as a function of E_{acc} suggested the maximum I_p at –1 V (data not shown). Thus, the accumulation potential of –1 V was chosen for subsequent experiments. Variation of the t_{acc} from 1–3 min showed that the I_p increased with the increasing t_{acc}. The peak height reached plateau after 240 s, and the higher current response was obtained at the potential lower than –0.8V (data not shown). This was however achieved with high background current, presumably due to the saturation of the electrode surface with the adsorbed layer of metals. Thus, deposition time of 240 s was used throughout, as it combined good sensitivity and relatively short analysis time.

3.4 Performance enhancement of the BiHA-SPE

HA-added SPE was modified by Bi film through *in situ* deposition with Pb^{2+} and Cd^{2+} ions. The stripping peak current was affected by the thickness of the Bi film, and this was controlled by the concentration of the Bi film. The effect of the concentration of the Bi film on the peak currents of Cd^{2+} was investigated at the concentration range of 100 to 1000 ppb (Figure 3). As the Bi concentration was increased, the stripping peak current of Cd^{2+} was increased up to 500 ppb and then remaining constant with small increment at 1000 ppb. Therefore, the Bi concentration of 500 ppb was chosen for the determinations of $Pb^{2+}$and Cd^{2+} using BiHA-SPE.

Further enhancement of the BiHA-SPE performance involved varying the square-wave parameters such as the frequency, pulse amplitude and step potential. The frequency of 150 Hz, pulse amplitude of 25 mV, and the step potential of 4 mV were chosen as the step potential in the analysis for enhanced performance. In SWV, interferences may be attributed to competitive ions and complexes on the electrode surface. The effects of these ions were analyzed using a fixed amount of Pb^{2+} and Cd^{2+} and different concentrations of other ions/complexes. Interfering ions (Cu^{2+} and Zn^{2+}) at 100 ppb produced analyte error less than 5 % for 20 ppb of Pb^{2+} and Cd^{2+}, are deemed acceptable. However, the presence of Fe^{2+} at 100 ppb affected the Cd^{2+} determination but was tolerable for Pb^{2+}.

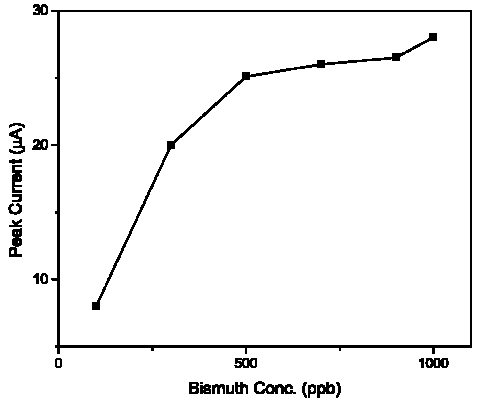

Fig. 3. Stripping voltammogram for Cd^{2+} with Bi-HA SPE in acetate buffer at pH 7.6, 240 s deposition time.

3.5 Calibration and validation

BiHA-SPE was utilized to evaluate different concentrations of Pb^{2+} in the presence of Cd^{2+} under enhanced conditions (Figure 4). Two peaks corresponding to Cd^{2+} at –0.8V and Pb^{2+} at –0.6V can be discerned suggesting that the application of BiHA modifier had increased the current response for BiHA-SPE for Pb^{2+}detection. The response of the proposed electrode was linear for Pb^{2+} (R^2 = 0.9777). The R^2 of

0.9959 was obtained for Cd^{2+} but only based on 3 points and therefore the calibration curve is not shown.

The LOD of Pb^{2+} was 16.8 µg/L, and the LOQ was 56.2 µg/L. The LOD and LOQ for Cd^{2+} was not considered due to the lack of valid points on the standard curve. The values were within suitable range for the detection of ultratraces of Pb^{2+} in water samples. The LOD and LOQ of BiHA SPE can be further optimized with better composite material such as that based on cellulose-HA composite [34]. This signifies the importance of high sensitivity and selectivity of the method that will be employed for analyses of water samples.

Both the interaction of HA and alloy formation of Bi at the SPE surface with metal ions resulted in good reproducibility and selectivity. HA interacts with Pb^{2+} and Cd^{2+} at the electrode surface and the addition of Bi film along with HA film at the SPE surface enhanced the peak current response and can be explored further within the mechanical manufacturing set-up for sensor fabrication. SPE has the advantages of compact size, *in situ* modification and simplicity. The performance of Bi-HA SPE can be expected to improve with further optimization on electrode modification preparation. Electrochemical characterization and good response for heavy metal ions detection proved the feasibility of BiHA-SPE as a potential low cost, disposable electrodes for robust and on-site environmental monitoring.

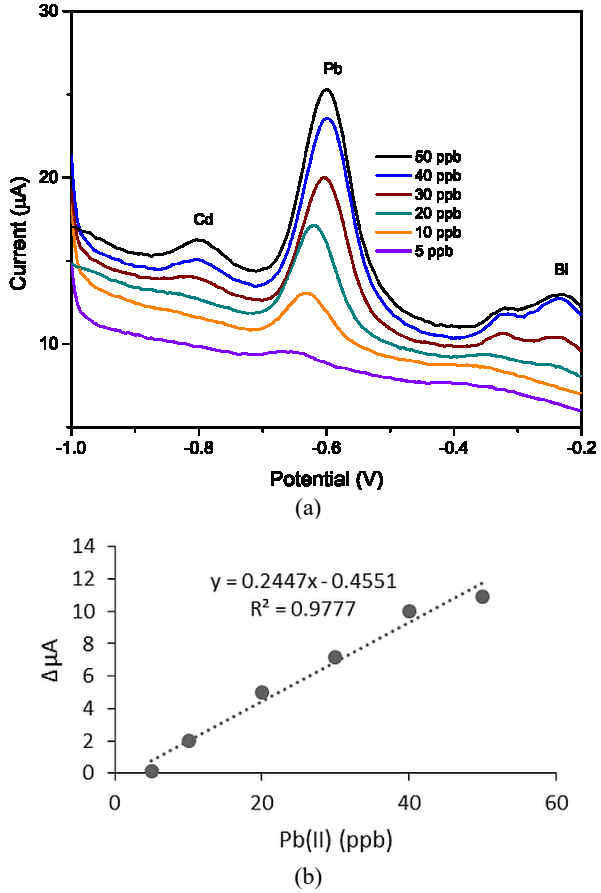

Fig. 4. a) Stripping voltammogram for different $Pb^{2+}$and Cd^{2+}concentrations, b) calibration plot for Pb^{2+}concentration, with Bi-HA SPE in acetate buffer at pH 7.6, 240 s deposition time, and Bi at 500 ppb.

In order to test the validity of the developed BiHA-SPE, the electrochemical determination of Pb^{2+} ions in different lake water samples was carried out at enhanced conditions. The results were compared with the analyses from the AAS (Table 1). It was clear that the Pb^{2+} ions detection by the BiHA-SPE was comparable to AAS. Bi solid particles have been the main component of the modified SPE surface [20,44]. The particles incorporate and form deposits of metallic bismuth at the SPE surface, followed by the reduction at the oxidation/deposition potential. Comparison of BiHA-modified Carbon Electrode (BiHA-CME) and BiHA-SPE however suggested that the BiHA-CME demonstrated better recovery for $Pb^{2+}$and Cd^{2+} [33] as compared to the Bi-HA SPE. Further optimization is required with regards to BiHA SPE fabrication to improve sensitivity and selectivity. However, Bi film with HA deposition in the carbon matrix offers a good stripping peak having favorable signal-to-background ratio, and relatively free from oxygen interferences during SWV measurements. Well-defined peaks corresponding to Pb^{2+} and Cd^{2+} can be discerned for the developed BiHA-SPE.

Table 1. Determination of Pb(II) ions from the spiked lake water sample.

Sample	Spiked sample (µg/L)	Bi-HA SPE* (µg/L)	AAS* (µg/L)
A	80	71±4	79±1
B	50	45±2	48±1

* Electrode analysis consists of mean for 3 replicates with standard errors

4 Conclusion

The application of BiHA modifier had increased the current response for Pb^{2+} as low as 16.8 µg/L. SPE had the advantages for *on site* application due to its compact, small size, *in situ* modification, without complicated pre-cleaning. Electrochemical characterization and good response for heavy metal ion detection confers BiHA-SPE as a superior alternative to mercury based electrodes as economical, disposable electrodes for environmental monitoring.

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