Electrode modification based on electrochemical grafting with p-carboxybenzene diazonium couple with an in situ deposition of bismuth for detection of lead and cadmium using square wave anodic stripping voltammetry

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Abstract

Electrografting of 4-carboxybezene diazonium tetrafluoroborate (4-CBD) on glassy carbon electrode (GCE) with an in situ deposition of bismuth (Bi/CP/GCE) was fabricated. The modified electrode was used to detect cadmium and lead in tap water by using square wave anodic stripping voltammetry (SWASV). The parameters that have an impact on the current in SWASV were studied. Under the optimum conditions, lead has a linearity in the range of 25-500 μg L\textsuperscript{-1} with detection limit of 10 μg L\textsuperscript{-1} while cadmium provides linear range 50-500 μg L\textsuperscript{-1} and detection limit of 25 μg L\textsuperscript{-1}. The detection limit is calculated using S/N ≥ 3. The fabricated electrode shows good repeatability within 6 measurements, in which % RSD is 11 % (Pb\textsuperscript{2+}) and 4.9 % (Cd\textsuperscript{2+}). The reproducibility of 6 electrodes are also in the accepted level with the % RSD of 13 % (Pb\textsuperscript{2+}) and 16 % (Cd\textsuperscript{2+}). The electrode is applicable for trace analysis of Cd\textsuperscript{2+} and Pb\textsuperscript{2+} in tap water.
## List of abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectrophotometry</td>
</tr>
<tr>
<td>Ac buffer</td>
<td>Acetate buffer</td>
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<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>ASV</td>
<td>Anodic Stripping Voltammetry</td>
</tr>
<tr>
<td>Bi</td>
<td>Bismuth</td>
</tr>
<tr>
<td>Bi/CP/GCE</td>
<td>Bismuth and p-carboxybenzene diazonium film glassy carbon electrode</td>
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<tr>
<td>Bi/GCE</td>
<td>Bismuth film glassy carbon electrode</td>
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<tr>
<td>CP/GCE</td>
<td>Grafted p-carboxybenzene diazonium film glassy carbon electrode</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>$E_p$</td>
<td>Pulse height</td>
</tr>
<tr>
<td>$E_s$</td>
<td>Pulse step</td>
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<tr>
<td>FAAS</td>
<td>Flame atomic absorption spectrometry</td>
</tr>
<tr>
<td>GCE</td>
<td>Glassy carbon electrode</td>
</tr>
<tr>
<td>HAc</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>Inductively coupled plasma - optical emission spectrometry</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>LOD</td>
<td>Detection limit</td>
</tr>
<tr>
<td>LOQ</td>
<td>Quantitation limit</td>
</tr>
<tr>
<td>NaAc</td>
<td>Sodium acetate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NBu$_4$BF$_4$</td>
<td>Tetrabutylammonium tetrafluoroborate</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>S</td>
<td>Slope of calibration equation</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Standard deviation of blank signal</td>
</tr>
<tr>
<td>SWASV</td>
<td>Square wave anodic stripping voltammetry</td>
</tr>
<tr>
<td>SWV</td>
<td>Square wave voltammetry</td>
</tr>
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</table>
$\tau$  

Pulse period

4-CBD  

4-Carboxybenzene diazonium tetrafluoroborate

**Author contribution**

All experiments were conducted by Thi Ngoc Huyen Nguyen under the instruction of Sereilakhena Phal, PhD student.
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1. Introduction

1.1 Significance of lead and cadmium detection

Lead and cadmium are two heavy metals which show a serious threat to ecological system and public health due to their toxic and non-biodegradable\(^1\) nature. Low concentrations of these ions can cause serious health problems such as teratogenic, nervous, reproductive and carcinogenic toxicity to humans\(^2\). Young children are vulnerable to the toxic effects of lead, and can suffer from the deep and permanent adverse health effects, particularly the development of the brain and nervous system. Lead can also lead to increased risk of high blood pressure and kidney damage in adults\(^3\). Pregnant women exposed to high levels of lead can suffer from miscarriage, stillbirth, premature birth and low birth weight\(^3\). Long-term exposure to cadmium through air, water, soil, and food leads to cancer and organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central and peripheral nervous, and respiratory systems\(^4\). World Health Organization set a threshold limit values of 10 µg L\(^-1\) for Pb\(^{2+}\), 3 µg L\(^-1\) for Cd\(^{2+}\) in drinking water\(^5\). Therefore, it is extremely important to develop a highly sensitive, rapid, and simple methods for the detection of these pollutants in the environment.

1.2 Current detection methods

At present, the techniques available for the detection of heavy metals, include inductively coupled plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (FAAS) and atomic absorption spectrophotometry (AAS). These techniques are adequately sensitive, specific and accurate for the determination heavy metals at trace levels\(^6\). However, all of them require expensive and bulky equipment, trained personnel, and laborious operation\(^6\). Therefore, researchers have been contending to develop cheap, simple, sensitive, specific, accurate, user-friendly, and environmental-friendly detection methods, and square wave anodic stripping voltammetry (SWASV) is one of the most promising solutions.

1.3 Stripping voltammetry

Electroanalytical methods have attracted extensive attention because they offer advantages of quick analysis, simple instrumentation, low cost, and high sensitivity. Currently various electroanalytical methods, including cyclic voltammetry, square wave voltammetry, linear sweep voltammetry, staircase voltammetry, stripping voltammetry have been studied and applied. Stripping voltammetry is considered the most sensitive electroanalytical and suitable for trace-level determination of many metals and compounds in environmental samples, clinical and industrial. The procedure consists of two main steps\(^7\):

**Pre-concentration:** Pre-concentration can be done either through adsorptive deposition\(^8\) or by applying a constant reduction potential. The species concentrate in a much smaller volume compared to the volume of the solution which explains the remarkable sensitivity of the technique.

**Stripping:** During this step, the deposited analyte is determined by stripping, through a potential sweep. This sweep can be performed by various techniques such as square wave (most commonly used), differential pulse, linear sweep or staircase.
1.3.1 Square wave voltammetry (SWV)

Figure 1 shows the waveform of SWV, which consists of a square wave superimposed on a staircase. During each cathodic pulse, analyte is reduced at the electrode surface. During the anodic pulse, analyte that was just reduced is re-oxidized. Electrons flow from the electrode to analyte at point 1 and in the reverse direction at point 2. Since the two currents have opposite signs, their difference is larger than either current alone. The signal is increased because reduced product from each cathodic pulse is right at the surface of the electrode waiting to be oxidized by the next anodic pulse.9

![Waveform for SWV](image)

Figure 1. Waveform for SWV. Typical parameters are pulse height ($E_p$), step height ($E_s$), and pulse period ($t$). Current is measured in regions 1 and 2.

1.3.2 Square Wave Anodic Stripping Voltammetry (SWASV)

Square wave anodic stripping voltammetry (SWASV) is now widely recognized as a powerful tool for the analysis of metal ions and certain organic compounds in solutions. In the pre-concentration step, the analytes are collected by reduction in certain time, normally assisted by convection, forming a composite or alloy (bismuth electrodes and mercury). In stripping step, the potential is changed to a more positive potential which results in oxidation of the metal in the amalgam or alloy and stripping of the ions back into the electrolyte solution and recording a current peak due to this process. The current peak height reflects the concentration of the analyte at the electrode surface, which is proportional to the amount of analyte dissolved in the solution.7

1.3.3 GCE modified by 4-CBD couple with an in situ Bi^{3+}

Traditional electrochemical techniques usually involve a three-electrode system, including a working electrode, a counter electrode, and a reference electrode. The choice of the working electrode is a key issue in the stripping voltammetry detection of heavy metal ions. Mercury-based electrodes are commonly used as working electrode because of the unique ability of mercury to pre-concentrate heavy metals. However, because of its serious toxicity and difficult handling, new and more environmentally friendly mercury-free electrodes have replaced these electrodes.10 Carbon electrode is one of the most attractive alternatives to mercury electrode which has the advantages of chemical
inertness, broad useful potential range, low residual current, low cost, easy modification, and great versatility. The modification of carbon through the electrochemical or chemical reduction of aromatic diazonium derivatives has been extensively used for modification of various carbon materials. This modification method is well-recognized as a very versatile and simple way to graft a wide variety of functional groups onto carbon surfaces for various applications\textsuperscript{11}. There are two steps in electrode modification using diazonium (scheme 1): 1) reduction of aryl diazonium ion to aryl radical and 2) attachment of aryl radical with electrode surface through covalent bond. In step 2, multilayers or monolayer can be created based on the concentration of diazonium or other parameters relevant in electrochemical process.

Bismuth is also a very attractive alternative material due its ability to form a “fused alloy” with heavy metals. Recently, there has been considerable interest in the study of bismuth-modified electrodes to improve the sensitivity of electrochemical detection methods.

In this project, the grafted electrode with 4-CBD will be used for determination of Cd\textsuperscript{2+} and Pb\textsuperscript{2+} by using SWASV in presence of Bi\textsuperscript{3+}.

\begin{center}
\textbf{Scheme 1. Electrochemical grafting of diazonium salt on GCE surface}
\end{center}

In this case, R is -COOH.

\textbf{Aim of the diploma work}

To modify GCE using 4-CBD salt with an in situ Bi\textsuperscript{3+} plating for detection of Cd\textsuperscript{2+} and Pb\textsuperscript{2+} by SWASV.
2. Popular scientific summary including societal and ethical aspects

2.1 Popular scientific summary

Lead and cadmium are considered to be highly toxic and hazardous to human health even at trace levels. The detection and quantification of these metal ions in natural and drinking water is of paramount importance. The spectroscopic techniques, which are used for detection of these metals, are very expensive and require trained personnel to work with the equipment and the analytical procedures. In this research, a rapid, low cost, simple and reliable electrochemical technique (SWASV based on GCE modified by the reduction of 4-CBD couple with an in situ Bi$^{3+}$ deposition) is studied for the detection of lead and cadmium in tap water sample.

2.2 Societal and ethical aspects

The Institute for Health Metrics and Evaluation (IHME) estimated that in 2016 lead exposure accounted for 540,000 deaths and 13.9 million years of healthy life lost (disability-adjusted life years (DALYs)) worldwide due to long-term effects on health. IHME also estimated that in 2016, lead exposure accounted for 63.8% of the global burden of idiopathic developmental intellectual disability, 3% of the global burden of ischaemic heart disease and 3.1% of the global burden of stroke. The International Agency for Research on Cancer (IARC) classifies cadmium in Class 1 ‘The agent (mixture) is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans.’ Because of their toxicities, it is extremely important to develop methods for the detection of these pollutants in the environment.

3. Experimental

3.1 Chemical

Alumina powder (1.0, 0.3, and 0.05 µm) were purchased from Buehler, USA. Acetonitrile; ACN (HPLC grade), NBu$_4$BF$_4$ were purchased from Sigma-Aldrich. Mili-Q water used for chemical preparation and electrode cleaning was obtained from Q-POD’s Milipore water system. 4-CBD was prepared by Sereilakhena Phal, PhD student in my group. 4-CBD solution was freshly prepared in blank solution containing 50 mM NBu$_4$BF$_4$ and ACN. Acetate (Ac) buffer solutions 0.1 M were prepared from 0.1 M HAc and 0.1 M NaAc, then adjusted by HCl 5 M and NaOH 5 M to required pH. Bi$^{3+}$ stock solution (1000 mg L$^{-1}$) was obtained by dissolution of bismuth (III) nitrate pentahydrate in Mili-Q water (added 2.0 mL acetic acid). Stock solution of Cd$^{2+}$ and Pb$^{2+}$ (1000 mg L$^{-1}$) were obtained from CPI international (Amsterdam, Netherlands) and diluted as required. The working solutions of Cd$^{2+}$ and Pb$^{2+}$ were prepare by diluting stock solution with Ac buffer containing Bi$^{3+}$.

3.2 Apparatus

Cyclic voltammetry (CV) and SWASV were performed using three-electrode system with ModuLab electrochemical system, ECS (Solartron Analytical, UK). A three-electrode system consists of a glassy carbon disk working electrode (GCE, 3 mm diameter), an Ag/AgCl (saturated KCl) reference electrode and a Pt counter electrode. Mettler Toledo was used for measuring pH.
All electrochemical measurements were carried out with 50 mL solution in a cell with exception of diazonium solution where 20 mL was used. Prior to the measurements, the solutions were degassed with nitrogen gas for about 5 minutes.

### 3.3 CV of 4-CBD grafting on GCE and characterization of the grafted surface

Prior to the modification, the GCE was polished with 1.0, 0.3, and 0.05 µm alumina slurries and rinsed with Mili-Q water after polishing with each particle. It was then sonicated with ACN for 1 min to remove adsorbed particles. The cleaned GCE was modified using CV under optimized conditions: 2.5 mmol L\(^{-1}\) 4-CBD solution for 5 cycles from +0.7 to -0.7 V at a scan rate of 100 mV s\(^{-1}\). The grafted GCE (CP/GCE) was then sonicated in ACN for 5 minutes and used for further measurement.

The bare GCE and CP/GCE were characterized by CV measurement in blank solution, scanning from +0.7 to -0.7 V at 100 mV s\(^{-1}\). This was done to assure that the CP layer was successfully grafted on GCE.

### 3.4 Cd\(^{2+}\) and Pb\(^{2+}\) measurement

The SWASV measurements were performed using CP/GCE in Cd\(^{2+}\) and Pb\(^{2+}\) solution with the presence of 1.0 mg L\(^{-1}\) Bi\(^{3+}\) in 0.1 M Ac buffer solutions (pH 4.5). The CP/GCE was immersed in solution for 15 s, then SWASV was measured by scanning potential from -1.0 V to +0 V under the optimized parameters: pulse amplitude (25 mV), step potential (1 mV), pulse frequency (25 Hz) and integration period (20 % of pulse width).

### 3.5 Preparation of tap water sample

Hot tap water was collected in our laboratory and was cooled to room temperature. For analysis, the sample was prepared in the same way as the preparation of Ac buffer solution with Mili-Q water. Standard addition was performed for the determination and for the recovery test.

### 4. Results and Discussion

#### 4.1 Cyclic voltammograms (CV) of 4-CBD grafting on GCE and characterization of the grafted surface

CV of 2.5 mmol L\(^{-1}\) 4-CBD is displayed in figure 2A. On the first scan, two broad reduction peaks were observed with peak potential values at 0.27 V (peak 1) and -0.07 V (peak 2). On the next scans, the peaks disappeared. Peak 1 corresponded to reduction of 4-CBD on bare GCE surface, which led to the formation of first layer based on attachment of the CP radical to the electrode surface\(^{12}\). The layer continued developing by binding 4-CBD on monolayer GCE, related to peak 2. The reduction current decreased dramatically in the next cycles indicated that electron transfer to 4-CBD was completely inhibited by the multilayers formed in the first scan\(^{12}\).

The grafted surface was characterized by CV measurements in 50 mmol L\(^{-1}\) NBu\(_4\)BF\(_4\) on bare GCE and grafted GCE, scanning from +0.7 to -0.7 V at 100 mV s\(^{-1}\). As shown in figure 2B, the CP/GCE provides lower current compared to bare GCE. This is an
indication of electron transfer blocking behavior of CP layer that was already grafted on GCE.

Figure 2.
(A) CV of 2.5 mmol L\(^{-1}\) 4-CBD + 50 mmol L\(^{-1}\) NBu\(_4\)BF\(_4\) + ACN.
(B) CVs of 50 mmol L\(^{-1}\) NBu\(_4\)BF\(_4\) + ACN at the bare GCE and the CP/GCE.
Scan rate 100 mV s\(^{-1}\) and voltage range +0.7 to -0.7 V.

4.2 Stripping responses of Pb\(^{2+}\) and Cd\(^{2+}\)
Initially, the SWASV measurements were done with 500 µg L\(^{-1}\) Cd\(^{2+}\) and Pb\(^{2+}\) in 0.1 M Ac buffer solution (pH = 4.5) in two conditions: (1) preconcentration (applied potential at -1.2 V for 120 s) with stripping and (2) preconcentration (immersed 15 s) with stripping. In stripping, the initial setups were: pulse frequency (25 Hz), potential step (4 mV) and pulse amplitude (25 mV). Four types of electrodes (GCE, Bi/GCE, CP/GCE and Bi/CP/GCE) were involved in these measurements to check their performance. To get the CP layer in CP/GCE or Bi/CP/GCE, 2.5 mmol L\(^{-1}\) 4-CBD was used to graft on GCE by running CV for 10 cycles from +0.7 to -0.7 V at 100 mV s\(^{-1}\). The electrodes modified with Bi was obtained during SWASV measurement by adding 1 mg L\(^{-1}\) Bi\(^{3+}\) into a target solution.

Figure 3A represents the voltammograms of Pb\(^{2+}\) and Cd\(^{2+}\) with different electrodes measured by condition (1). The given current by each electrode are demonstrated in figure 3B. As can be seen in figure 3B, the response at the bare GCE was very weak with only one broad peak of lead. With the modification, the signal at the CP/GCE and Bi/GCE was remarkably enhanced and showed two sharp and separate stripping peaks corresponding to Pb\(^{2+}\) and Cd\(^{2+}\). The Bi/CP/GCE electrode produced the highest signal with well-defined peak.

Figure 3C is the voltammograms of Pb\(^{2+}\) and Cd\(^{2+}\) detected using condition (2) with the same kinds of electrodes as condition (1). The current of each metal was plotted against different electrodes in figure 3D. The current for both metals (figure 3D) shows similar trend as observed in figure 3B. Both results indicate that the Bi/CP/GCE has highest performance among other electrodes. However, current obtained from Bi/CP/GCE in condition (2) is higher than what gotten from condition (1), figure 3E. Moreover, the fluctuation of the result confirming by % RSD, 3 % (Pb\(^{2+}\)) and 2 % (Cd\(^{2+}\)) is less compared to % RSD given by condition (1), 13 % (Pb\(^{2+}\)) and 8 % (Cd\(^{2+}\)). Therefore, the measurement by condition (1) which consists of deposition and stripping steps cannot be applied in this study. The preconcentration could be just based on the adsorption of metal ions on electrode surface (condition (2)) as suggested in scheme 2. The condition (2) is selected in this study.
Scheme 2. Suggested mechanism of adsorption and stripping steps

![Diagram showing adsorption and stripping steps]

Figure 2.

(A), (B) SWASVs of Cd$^{2+}$ and Pb$^{2+}$ at bare GCE, Bi/GCE, CP/GCE and Bi/CP/GCE. Experimental conditions (1). Test solution: 0.1 M pH 4.5 acetate buffer solutions containing 500 µgL$^{-1}$ Cd$^{2+}$ and Pb$^{2+}$ (1 mgL$^{-1}$ Bi$^{3+}$); deposition time and potential: 120 s, -1.2 V; SWASV settings: frequency 25 Hz, potential step 4 mV, and pulse amplitude 25 mV.

(C), (D) SWASVs of Cd$^{2+}$ and Pb$^{2+}$ at bare GCE, Bi/GCE, CP/GCE and Bi/CP/GCE. Experimental conditions (2). Experimental conditions: Test solution: 0.1 M pH 4.5 acetate buffer solutions containing 500 µgL$^{-1}$ Cd$^{2+}$ and Pb$^{2+}$ (1 mgL$^{-1}$ Bi$^{3+}$); immersion time: 15s; SWASV settings: frequency 25 Hz, potential step 4 mV, and pulse amplitude 25 mV.

(E) SWASVs of Cd$^{2+}$ and Pb$^{2+}$ Bi/CP/GCE. Experimental conditions: Test solution: 0.1 M pH 4.5 acetate buffer solutions containing 500 µgL$^{-1}$ Cd$^{2+}$ and Pb$^{2+}$, 1 mgL$^{-1}$ Bi$^{3+}$; deposition time and potential: 120 s, -1.2 V; SWASV settings: frequency 25 Hz, potential step 4 mV, and pulse amplitude 25 mV.

4.3 Optimization of SWV conditions

The SWV set up conditions (step potential, pulse amplitude, pulse frequency) was considered to get optimum conditions for the simultaneous determination of trace levels of Cd$^{2+}$ and Pb$^{2+}$. 

7
Figure 4A shows the effect of step potential on SWASVs of Cd$^{2+}$ and Pb$^{2+}$ in the range from 1 to 10 mV. The anodic peak currents of both metal ions dropped with increasing step potential. The best step potential was determined to be 1 mV.

The dependence of the peak Cd$^{2+}$ and Pb$^{2+}$ currents on the pulse amplitude in range from 10 to 35 mV was described in figure 4B. The currents of both Cd$^{2+}$ and Pb$^{2+}$ increased with increasing pulse amplitude first and then decreased with increasing pulse amplitude further (ideal value was 25 mV).

The effect of pulse frequency on peaks current Cd$^{2+}$ and Pb$^{2+}$ was depicted in figure 4C. When pulse frequency increased, peak current increased; however, the level of noises also changed. Thus, signal to noise ratio (S/N) was considered to select the best frequency. Figure 4D shows the relationship between pulse frequency and S/N. As shown, 25 Hz gave the significant high S/N when compared to other values. Considering both peak current and S/N, 25 Hz was chosen for next study.

![Figure 4](image.png)

**Figure 4.** The effects of (A) step potential, (B) pulse amplitude, (C) pulse frequency on the peaks current for Cd$^{2+}$ and Pb$^{2+}$ ions. Error bar: standard deviation for n=3. (D) The effect of pulse frequency on signal to noise ratio of the peaks current for Cd$^{2+}$ and Pb$^{2+}$ ions.

**Experimental conditions:** Test solution: 0.1 M pH 4.5 acetate buffer solutions containing 500 µgL$^{-1}$ Cd$^{2+}$ and Pb$^{2+}$ (1 mgL$^{-1}$ Bi$^{3+}$); immersion time: 15s.

### 4.4 Optimization of grafting condition

#### 4.4.1 Number of cycles

The number of cycles required for grafting of p-carboxybenzene diazonium tetrafluoroborate (4-CBD) on the GCE surface using CV was varied to get optimal current for Cd$^{2+}$ and Pb$^{2+}$. A concentration of 2.5 mmol L$^{-1}$ 4-CBD was chosen for this study and the CV was scanned between -0.7 and +0.7 at scan rate of 100 mV s$^{-1}$ (1 – 12 cycles), figure 5A. The current for lead increased when the number of cycles increased from 1-5 cycles. The increase in current for lead has therefore to do with the increase of the thickness of the diazonium film on the GCE surface. However, the signal of lead gradually decreased with an increase of the number of cycles from 5-12 cycles, which
may be due to the diazonium layer film at GCE surface becoming too thick. This caused inhibition of electron transfer on the surface. For cadmium, the current started to drop slightly after 1 cycle. It could be explained that reduction potential was enough to reduce Cd\(^{2+}\) at thinner film, however at a thicker film the resistance increased so the potential was not sufficiently reduced Cd\(^{2+}\). Combine both metal ions, 5 was the optimum number of cycles.

### 4.4.2 Concentration of 4-CBD

The effect of the concentration of 4-CBD between 1.0 and 4.0 mmol L\(^{-1}\) was studied with 5 scans at the scan rate of 100 mV s\(^{-1}\) from -0.7 and +0.7 V for the drafting of diazonium on the GCE surface (figure 5B). The signal of lead initially increased with the concentrations from 0.5 to 3.0 mmol L\(^{-1}\) due to the increased thickness of modified layer thereby enhance the binding site for metal ion. Meanwhile, there was a decreasing trend for cadmium in this range of concentrations because at thick film the resistance increased so the potential was not sufficiently to reduce cadmium. When the concentrations of diazonium were higher than 3.0 mmol L\(^{-1}\), the signals of lead and cadmium detection became stable. This was probably because the diazonium layer saturated. To choose the best concentration of 4-CBD for modification of GCE, both Cd\(^{2+}\) and Pb\(^{2+}\) signals were considered. Because 2.5 mmol L\(^{-1}\) gave high currents for both metal ions, it was chosen as optimal condition.

**Figure 5.** The effect of number of grafting cycles (A) and 4-CBD concentration (B) on the peaks current for Cd\(^{2+}\) and Pb\(^{2+}\). Error bar: standard deviation for n=6.

**Experimental conditions:** Test solution: 0.1 M pH 4.5 acetate buffer solutions containing 500 µg L\(^{-1}\) Cd\(^{2+}\) and Pb\(^{2+}\) (1 mg L\(^{-1}\) Bi\(^{3+}\)); immersion time: 15s; SWASV settings: frequency 25 Hz, potential step 1 mV, and pulse amplitude 25 mV.

### 4.5 Optimization of the operational conditions

#### 4.5.1 Effect of the bismuth concentration

The Bi\(^{3+}\) concentration used for in situ formation of the bismuth film has strong influence on the current of target metals. Figure 6A shows peak current for a solution containing 500 µg L\(^{-1}\) each Cd\(^{2+}\) and Pb\(^{2+}\) with different Bi\(^{3+}\) concentrations in the range between 0.1 and 2.0 mg L\(^{-1}\). Clearly, the optimal concentration of bismuth was 1.0 mg L\(^{-1}\) which gave the highest current and the lowest standard deviation for both metal ions. When the concentration of Bi\(^{3+}\) was lower than the optimal concentration, it could not form multi-component “fusing” alloys with the metals completely\(^{13}\). In contrast, when the concentration of Bi\(^{3+}\) was larger, thick bismuth layer on the electrode surface hampered the target metal ions for diffusing out of the bismuth film during the stripping step\(^{13}\).
4.5.2 Effect of pH

Figure 6B demonstrates the influence of pH which varied in the range of 3.5 to 5.5 on the stripping responses of 500 μg L⁻¹ Cd²⁺ and Pb²⁺. It is clear that, the peak currents of both metals decreased rapidly upon the decline of pH from 4.5 to 3.5. At lower pH CP layer on the electrode are mostly has the form of -COOH (CP on GCE has pKₐ ≈ 2.8)¹⁴. Thus, attachment of metal ion with modified layer was declined lead to the decline of current. When the pH was higher than 4.5 the current levels went down, this can be due to the hydrolysis of Bi³⁺ in the medium¹⁵. As an evidence the stripping signal of Bi was decreased (pH 5.0) and disappeared (pH 5.5). Therefore, pH of acetate buffer was kept at 4.5 for next steps.

4.5.3 Immersion time

Immersion time is expressed as the period between placing the electrode in the test solution and pressing the Run button. To study the effect of the immersion time on the peak current for 500 μg L⁻¹ of each Cd²⁺ and Pb²⁺, experiments were done with and without stirring for different periods of time: varied in the range of 0.25-10 minutes (figure 6C). With stirring, the current for both metal ions declined when the immersion time is increased. The decrease in current was less when the experiment was repeated without stirring. It seems that the adsorption of the metal ions to the grafted GCE is faster than the time interval used for this study (0.25-10 minutes). Therefore 15 seconds (without stirring) was chosen as optimum immersion time.

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**Figure 6.** The effects of Bi³⁺ concentration (A), pH (B) and immersion time (C) on the stripping peaks current of 500 μg L⁻¹ Cd²⁺ and Pb²⁺. Error bar: standard deviation for n=6.

**Experimental conditions:** Test solution: 0.1 M acetate buffer solutions containing 500 μg L⁻¹ Cd²⁺ and Pb²⁺. SWASV settings: frequency 25 Hz, potential step 1 mV, and pulse amplitude 25 mV.

In summary, the optimum conditions for this method were shown in table 1.
### Table 1. Optimized results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial condition</th>
<th>Optimization range</th>
<th>Optimal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWV set up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step potential (mV)</td>
<td>4</td>
<td>1-10</td>
<td>1</td>
</tr>
<tr>
<td>Pulse amplitude (mV)</td>
<td>25</td>
<td>10-35</td>
<td>25</td>
</tr>
<tr>
<td>Pulse frequency (Hz)</td>
<td>25</td>
<td>25-65</td>
<td>25</td>
</tr>
<tr>
<td>Modification GCE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cycles</td>
<td>10</td>
<td>1-12</td>
<td>5</td>
</tr>
<tr>
<td>4-CBD concentration (mmol L(^{-1}))</td>
<td>2.5</td>
<td>1.0-4.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Operational conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi(^{3+}) concentration (mg L(^{-1}))</td>
<td>1</td>
<td>0.1-2.0</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>4.5</td>
<td>3.5-5.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Immersion time (minute)</td>
<td>0.25</td>
<td>0.25-10</td>
<td>0.25</td>
</tr>
</tbody>
</table>

### 4.6 Analytical performance

#### 4.6.1 Calibration curve, detection limit, and limit of quantification

Under the optimum conditions, simultaneous determination of Cd\(^{2+}\) and Pb\(^{2+}\) was performed on Bi/CP/GCE electrode using SWASV. Figure 7A shows a series of stripping responses for the target metal ions from 25 to 500 µg L\(^{-1}\) in 0.1 M acetate buffer containing 1 mg L\(^{-1}\) Bi\(^{3+}\) solution. The peak currents increased with increasing concentrations for both Pb\(^{2+}\) and Cd\(^{2+}\). A plot of peaks current against concentrations displayed linear relationships for both Pb\(^{2+}\) and Cd\(^{2+}\) in the concentration range 25–500 µg L\(^{-1}\), and 50-500 µg L\(^{-1}\) respectively, figure 7B. The LOD and LOQ were calculated based on S/N ≥ 3 and S/N ≥ 10, respectively. The LOD was found to be 10 µg L\(^{-1}\) (Pb\(^{2+}\)) and 25 µg L\(^{-1}\) (Cd\(^{2+}\)) where the LOQ was 25 µg L\(^{-1}\) (Pb\(^{2+}\)) and 50 µg L\(^{-1}\) (Cd\(^{2+}\)).

#### 4.6.2 Repeatability

For the repeatability study, three sets of measurements with standard solutions containing 50, 300, and 500 µg L\(^{-1}\) of each Pb\(^{2+}\) and Cd\(^{2+}\) were studied (n=6 for each concentration) by comparing the current responses as shown in figure 7C. The relative standard deviation (RSDs) of all concentrations are shown in table 2, which are much better than the acceptable RSD value according to AOAC guideline\(^{16}\). These results showed that the Bi/CP/GCE electrode has a good repeatability.

<table>
<thead>
<tr>
<th>Concentration (µg L(^{-1}))</th>
<th>RSD Cd (%)</th>
<th>RSD Pb (%)</th>
<th>Acceptable RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>11</td>
<td>4.9</td>
<td>12</td>
</tr>
<tr>
<td>300</td>
<td>1.4</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td>500</td>
<td>1.3</td>
<td>2.3</td>
<td>9</td>
</tr>
</tbody>
</table>

#### 4.6.3 Reproducibility

The modified electrode was evaluated by repetitive measurements of 50, 300 and 500 µg L\(^{-1}\) heavy metal ions under optimized working conditions to study its reproducibility.
(figure 7D and 7E). The relative standard deviation from 6 measurements using different sensors prepared independently are shown in table 3. These values are much better than the acceptable RSD value according to AOAC guideline indicating that the reproducibility for Bi/CP/GCE electrode is good.

Table 3. Reproducibility of six different electrode preparations

<table>
<thead>
<tr>
<th>Concentration (µg L$^{-1}$)</th>
<th>RSD Cd (%)</th>
<th>RSD Pb (%)</th>
<th>Acceptable RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>13.4</td>
<td>15.9</td>
<td>25</td>
</tr>
<tr>
<td>300</td>
<td>12.5</td>
<td>9.5</td>
<td>19</td>
</tr>
<tr>
<td>500</td>
<td>10.4</td>
<td>5.9</td>
<td>18</td>
</tr>
</tbody>
</table>

Figure 7.

(A) SWASVs of the mixture containing Pb$^{2+}$ and Cd$^{2+}$ at the Bi/CP/GCE electrode in acetate buffer of pH 4.5. Concentration of Pb$^{2+}$ and Cd$^{2+}$ (from a to g, µg L$^{-1}$): 25, 50, 100, 200, 300, 400 and 500.

(B) Calibration curves for Pb$^{2+}$ and Cd$^{2+}$.

(C) The repeatability of the responses of the Bi/CP/GCE from the same electrode.
(D) and (E) The reproducibility of the responses of Pb²⁺ and Cd²⁺ at the Bi/CP/GCE from six different electrode preparations.

Experimental conditions: Test solution: 0.1 M pH 4.5 acetate buffer solutions containing Cd²⁺ and Pb²⁺(1 mgL⁻¹ Bi³⁺); immersion time: 15s; SWASV settings: frequency 25 Hz, potential step 1 mV, and pulse amplitude 25 mV.

4.7 Tap water analysis

To evaluate the practical use of the proposed method, Pb²⁺ and Cd²⁺ ions were analyzed in tap water using standard addition method. Standard addition curve was constructed based on current of three standard concentrations (70, 90 and 120 µg L⁻¹) and the sample, figure 8A. There were no metal ions detected in tap water which was possibly due to their presence in low level than LOD of the method. Additionally, the detection results were compared with those obtained by ICP-OES. With ICP-OES the linear ranges for Pb²⁺ and Cd²⁺ were 48.9-496.0 µg L⁻¹ and 3.75-496.0 µg L⁻¹, respectively, figure 8B. LODs and LOQs were calculated from the SD of blank signals (σ) and slope of the calibration equation (S). The LODs (3×σ/S) was found to be 75.8 µg L⁻¹ and 1.86 µg L⁻¹ Pb²⁺ and Cd²⁺, respectively. LOQ (10×σ/S) was 252.7 µg L⁻¹ for Pb²⁺ and 6.20 µg L⁻¹ for Cd²⁺. Standard addition was used for determination Pb²⁺ and Cd²⁺ in tap water, figure 8C. Similar to our proposed method, the metal ions were not detected.

Accuracy of both methods was verified by recovery test. The recoveries were calculated and were summarized in table 4. These good recoveries were within the acceptance criteria (70 % to 110 % recoveries at the level). This demonstrates that our proposed method can be used to detect the metals ion in real sample with accurate results.

<table>
<thead>
<tr>
<th>Added (µg L⁻¹)</th>
<th>Found by SWASV (µg L⁻¹)</th>
<th>Recovery (%)</th>
<th>Found by ICP-OES (µg L⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd²⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>80</td>
<td>77.4 ± 5.0</td>
<td>96.8 ± 6.2</td>
<td>81.6 ± 0.6</td>
<td>102.0 ± 0.8</td>
</tr>
<tr>
<td>100</td>
<td>96.2 ± 5.4</td>
<td>96.2 ± 5.4</td>
<td>102.2 ± 0.8</td>
<td>102.2 ± 0.8</td>
</tr>
<tr>
<td>110</td>
<td>111.0 ± 6.1</td>
<td>100.9 ± 5.6</td>
<td>97.5 ± 0.8</td>
<td>88.6 ± 0.7</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>80</td>
<td>83.0 ± 1.6</td>
<td>103.7 ± 2.0</td>
<td>72.7 ± 2.3</td>
<td>90.9 ± 5.3</td>
</tr>
<tr>
<td>100</td>
<td>101.0 ± 2.0</td>
<td>100.9 ± 2.0</td>
<td>94.9 ± 12.3</td>
<td>94.9 ± 12.4</td>
</tr>
<tr>
<td>110</td>
<td>108.0 ± 2.9</td>
<td>98.1 ± 2.6</td>
<td>92.3 ± 7.8</td>
<td>83.9 ± 7.0</td>
</tr>
</tbody>
</table>
Figure 8.

(A) Standard addition curves Pb\(^{2+}\) and Cd\(^{2+}\) from SWASV.

Experimental conditions: Test solution: 0.1 M pH 4.5 acetate buffer solutions containing Cd\(^{2+}\) and Pb\(^{2+}\) (1 mgL\(^{-1}\) Bi\(^{3+}\)); immersion time: 15s; SWASV settings: frequency 25 Hz, potential step 1 mV, and pulse amplitude 25 mV.

(B) Calibration curves for Pb\(^{2+}\) and Cd\(^{2+}\) from ICP-OES.

(C) Standard addition curves Pb\(^{2+}\) and Cd\(^{2+}\) from ICP-OES.

Experimental conditions: Test solution: 0.1 M pH 4.5 acetate buffer solutions containing Cd\(^{2+}\) and Pb\(^{2+}\) (1 mgL\(^{-1}\) Bi\(^{3+}\))

6. Conclusions and Outlook

In this work, the modification of GCE by the reduction of 4-CBD couple with an in situ bismuth were studied for simultaneous determination Pb\(^{2+}\) and Cd\(^{2+}\) using SWASV. Parameters (SWV set up conditions, numbers of cycles for grafting of 4-CBD on the GCE surface, the concentration of 4-CBD, Bi\(^{3+}\) concentration, pH, immersion time) were optimized to develop this method. The modified GCE provides wide linear ranges, satisfactory LODs, LOQs, repeatability and reproducibility. This method appropriates for determination of metal ions in tap water. Although the concentrations of metals could not be specified, the method is still possible to use for analysis of Pb\(^{2+}\) and Cd\(^{2+}\). Moreover, it is proven to be accurate, cheap, simple, user-friendly, and environmental-friendly.
Acknowledgement

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References