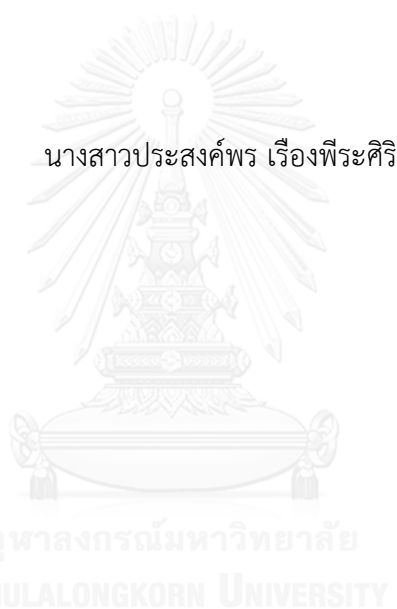การพัฒนาวิธีตรวจวัดโลหะหนักปริมาณน้อยมากด้วยซีเคว็นเชียลอินเจ็กชัน-แอโนดิกสทริปปิง
โวลแทมเมตรีโดยใช้ขั้วไฟฟ้าดัดแปรด้วยแอนติโมนี-แกรฟีนออกไซด์

นางสาวประสงค์พร เรืองพีระศิริ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

METHOD DEVELOPMENT FOR DETERMINATION OF TRACE HEAVY METALS BY

SEQUENTIAL INJECTION-ANODIC STRIPPING VOLTAMMETRY USING

ANTIMONY-GRAPHENE OXIDE MODIFIED ELECTRODE

Miss Prasongporn Ruengpirasiri

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2015

Thesis Title	METHOD DEVELOPMENT FOR DETERMINATION OF TRACE HEAVY METALS BY SEQUENTIAL INJECTION-ANODIC STRIPPING VOLTAMMETRY USINGANTIMONY-GRAPHENE OXIDE MODIFIED ELECTRODE
By	Miss Prasongporn Ruengpirasiri
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Suchada Chuanuwatanakul, Ph.D.
Thesis Co-Advisor	Professor Orawon Chailapakul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑Dean of the Faculty of Science

(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑Chairman

(Associate Professor Vudhichai Parasuk, Ph.D.)

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑Thesis Advisor

(Assistant Professor Suchada Chuanuwatanakul, Ph.D.)

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑Thesis Co-Advisor

(Professor Orawon Chailapakul, Ph.D.)

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑Examiner

(Assistant Professor Pakorn Varanusupakul, Ph.D.)

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑External Examiner

(Wanida Wonsawat, Ph.D.)

ประสงค์พร เรืองพีระศิริ : การพัฒนาวิธีตรวจวัดโลหะหนักปริมาณน้อยมากด้วยซีเคว็นเชียลอินเจ็กชัน-แอโนดิกสทริปปิงโวลแทมเมตรีโดยใช้ขั้วไฟฟ้าดัดแปรด้วยแอนติโมนี-แกรฟีนออกไซด์ (METHOD DEVELOPMENT FOR DETERMINATION OF TRACE HEAVY METALS BY SEQUENTIAL INJECTION-ANODIC STRIPPING VOLTAMMETRY USINGANTIMONY-GRAPHENE OXIDE MODIFIED ELECTRODE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.สุชาดา จูอนุวัฒนกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.อรวรรณ ชัยลภากุล, 60 หน้า.

งานวิจัยนี้ได้พัฒนาวิธีวิเคราะห์ปริมาณโลหะหนัก 4 ชนิดในคราวเดียวกันด้วยซีเคว็นเชียลอินเจ็กชันร่วมกับเทคนิคโวลแทมเมตรีโดยใช้ขั้วไฟฟ้าคาร์บอนพิมพ์สกรีนดัดแปรด้วยแอนติโมนี – แกรฟีนออกไซด์ ซึ่งแกรฟีนออกไซด์ได้จากการสังเคราะห์ขึ้นผ่านวิธีของ Hummer และยืนยันเอกลักษณ์ด้วยเทคนิค ATR-FTIR และเครื่องวัดการดูดกลืนแสง (UV-vis spectrometry) ฟิล์มของแอนติโมนีได้จากการเกาะติดด้วยกระแสไฟฟ้าภายในระบบของไหล จากขั้วไฟฟ้าดัดแปรสามารถทดลองหาภาวะที่เหมาะสมในการวิเคราะห์ปริมาณแคดเมียม(II) ตะกั่ว(II) ทองแดง(II) และปรอท(II) ในคราวเดียวกันด้วยซีเคว็นเชียลอินเจ็กชัน-สแควร์เวฟแอโนดิกสทริปปิงโวลแทมเมตรี ด้วยเทคนิคนี้พบว่าความสัมพันธ์ระหว่างสัญญาณกับความเข้มข้นของโลหะหนักมีความเป็นเส้นตรงในช่วง 0.1 – 1.5 ไมโครโมลาร์ และขีดจำกัดต่ำสุดของการตรวจวัดแคดเมียม(II) ตะกั่ว(II) ทองแดง(II) และปรอท(II) เท่ากับ 0.054, 0.026, 0.060 และ 0.066 ไมโครโมลาร์ ตามลำดับ ภายใต้ภาวะที่เหมาะสมสามารถวิเคราะห์ปริมาณโลหะหนักแต่ละชนิดได้ทั้งแบบในคราวเดียวกันและแบบแยกชนิดกันวิเคราะห์ ได้ศึกษาเกี่ยวกับตัวรบกวนการวิเคราะห์ในตัวอย่างน้ำ รวมทั้งสามารถวิเคราะห์ปริมาณโลหะหนักในน้ำตัวอย่างด้วยวิธีการเติมสารมาตรฐานได้ โดยมีค่าร้อยละการคืนกลับอยู่ในช่วงที่ยอมรับได้ (94.3 – 113)

ภาควิชา	เคมี	ลายมือชื่อนิสิต	....
สาขาวิชา	เคมี	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2558	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

5672012323 : MAJOR CHEMISTRY

KEYWORDS: HEAVY METAL IONS / SEQUENTIAL INJECTION / VOLTAMMETRY / SCREEN-PRINTED ELECTRODE / ANTIMONY / GRAPHENE OXIDE

PRASONGPORN RUENGPIRASIRI: METHOD DEVELOPMENT FOR DETERMINATION OF TRACE HEAVY METALS BY SEQUENTIAL INJECTION-ANODIC STRIPPING VOLTAMMETRY USINGANTIMONY-GRAPHENE OXIDE MODIFIED ELECTRODE. ADVISOR: ASST. PROF. SUCHADA CHUANUWATANAKUL, Ph.D., CO-ADVISOR: PROF. ORAWON CHAILAPAKUL, Ph.D., 60 pp.

A sequential injection, an automated flow-based system, coupled with voltammetric method has been developed for the simultaneous determination of heavy metals using antimony-graphene oxide modified electrode (SbF-GO-SPCE). Graphene oxide was synthesized via Hummer's method and was characterized by ATR-FTIR and UV-vis spectrometry. Antimony film was obtained by on-line in situ electrodepositing at constant potential. The determination was done with square wave anodic stripping voltammetry (SWASV) because of high sensitivity and low-cost technique. Optimal conditions to determine four heavy metals; which are Cd(II), Pb(II), Cu(II), and Hg(II) ions by sequential injection-SWASV, has been investigated. Using sequential injection-SWASV, the relationship between peak height and concentration of all heavy metal ions was linear in the range of 0.1-1.5 µM. Calculated limits of detection (LOD = 3.3σ/s) were 0.054, 0.026, 0.060, and 0.066 µM for Cd(II), Pb(II), Cu(II) and Hg(II), respectively. The individual determination of all metal ions could be performed under the optimal conditions with a great sensitivity and reproducibility. The interfering species in real water sample were studied. In addition, practical water samples were analyzed using standard addition method and calibration method. This technique could be used for determination of heavy metal ions in real samples with a 94.3 – 113% recovery.

Department:	Chemistry	Student's Signature	....
Field of Study:	Chemistry	Advisor's Signature	....
Academic Year:	2015	Co-Advisor's Signature	....

ACKNOWLEDGEMENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

A	ampere
CV	cyclic voltammetry
E	potential
GO	graphene oxide
L	liter
LOD	limit of detection
LOQ	limit of quantification
mg	milligram
mL	milliliter
mV	millivolt
PVC	polyvinylchloride
RSD	relative standard deviation
SbF	antimony film
SEM	scanning electron microscope
SD	standard deviation
SIA	sequential injection analysis
SPCE	screen-printed carbon electrode
SWASV	square wave anodic stripping voltammetry
SWV	square wave voltammetry
V	volt
µg	microgram
µL	microliter
°C	degree Celsius

CHAPTER I

INTRODUCTION

1.1   Introduction

Human have been widely used heavy metals for a long time ago, for example, metal plating plant, batteries, lead based paint, oil rig and sewage from steel mill. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues. Some of the heavy metal ions cause important environmental problems. The major threats to human health from heavy metals, such as lead, copper, cadmium, and mercury, are connected with exposure to them and accumulated in human body. Especially, a large amount of these toxic compounds are present in some surface and tap water due to the misuse of industrial wastes. World Health Organization (WHO) also concerned about this problems and studied their effects to human recently. Thus, it is important to look for a rapid, selective, sensitive, low-cost and simple technique for detection and monitoring all of these polluted substances in water.

There are various conventional techniques to analyze heavy metal contaminated in water, for instance, atomic absorption spectroscopy [1-3], inductively coupled plasma optical emission spectrometry [4-6], and inductively coupled plasma mass spectrometry [7, 8]. However, these spectrometric instruments still have some drawbacks which are bulky, expensive, low sensitive and not suitable for on-site analysis [9, 10]. To overcome these disadvantages, the electrochemical techniques became an alternative technique to detect heavy metal ions due to high sensitivity, short analysis time, relatively low cost, and portability [11]. Square Wave Anodic Stripping Voltammetry (SWASV) is one of the highest potential electrochemical methods for detection of trace heavy metal ions in nature. To improve sensitivity of the detection, chemical modified working electrode is chosen in this work.

Conventional working electrodes, such as glassy carbon electrode, have some drawbacks. A screen-printed electrode was introduced to solve former problems. The

benefits of a Screen-Printed Electrode (SPE) are relatively low cost, feasible in-house fabrication, and small compared to original solid electrode. The platform of electrode can be designed in various shape and size according to purpose. Its simple fabrication and modification, SPE is selected as a chemical modified electrode which can use many kinds of materials, such as multi-walled carbon nanotube [12], antimony film [13], and bismuth [14-16] for heavy metals determination.

Recently, graphene and its family gained more attention since the discovery of these new 2D material. Graphene is a relatively new material discovered in 2004. It is actually a monolayer of carbon atoms which are covalently linked and arranged into a two dimensional honeycomb-like lattice [17]. Graphene has many excellent characteristics such as high optical transparency, electrical conductivity, and catalytic activity making it a very interesting material [18, 19]. Graphene can be obtained from the reduction of graphene oxide (GO) which is a sheet of graphene that is partially oxidized, containing of both sp^2- and sp^3- hybridized carbon atoms, hydroxyl functional groups on each side of the carbon sheet, and decorated by carboxyl groups [20, 21]. Graphene oxide has functional groups such as hydroxyl and carbonyl, which are advantageous for adsorbing heavy metal ions. Therefore, graphene oxide can preconcentrate target analytes at surface of electrode.

In the past, conventional working electrode for metal ions detection was made of mercury owing to its excellent electroanalytical performance. However, mercury is a very toxic substance thus avoid using mercury would be better for environment. Therefore, searching for new material to replace mercury with remain great properties of mercury was done in many research. Alternative materials which have the same capability to mercury were bismuth and antimony due to intermetallic compounds of analytes and these materials. Deposited bismuth film onto the screen-printed CNT electrode for simultaneous determination of lead, cadmium and zinc was performed by SWASV. The deposited Bi film could improve the sensitivity; however, bismuth exhibited a large oxidation peak at around -0.1 V in stripping step [22]. Thus, bismuth film was not suitable for the detection of copper and mercury because these two metal ions showed the peaks at -0.2 V and +0.1 V, respectively. Because the bismuth

film modified electrode was unsuitable for determination of copper(II) and mercury(II) ions, antimony film modified electrode [23] became an attractive material for detection of cadmium(II), lead(II), copper(II), and mercury(II), simultaneously. In acid solution, there is no peak of antimony exist in the range of working potential window. Antimony film was proved to be potential for detection of heavy metal ions in the same way as mercury did with lower toxicity. In addition, it can form intermetallic compounds which can decrease the deposition potential.

To make automatic system, Sequential Injection Analysis (SIA) coupled with electrochemical detection was introduced in present work. SIA is an automated approach to sample handling that allows automating in situ antimony modification of electrode and SWASV procedures in a rapid, precise, and efficient manner. Small sample usage and accuracy were achieved by this automated procedure. Moreover, the advantages of SIA system are reducing health problem to researchers, minimizing human's errors and increasing sample throughput (samples/hour). Under this system, we can control sample volume in syringe pump, flow rate, and number of solutions in one detection system which is easier to operate by unskilled users.

Intentions of this work are fabrication of new working electrode platform by the combination of antimony film and graphene oxide and using SIA to operate electrode modification and heavy metal ion determination system. Moreover, this novel electrode platform will be an alternative material for detection of all these mixed target ions which has less studies on their amount present in water. The developed method will decrease analysis time both individual and simultaneous detections of Cd(II), Pb(II), Cu(II) and Hg(II) in real water samples. Many experimental parameters were optimized. After that, the analytical performance of developed technique was evaluated. Interferences study of cations and anions was obtained under optimized conditions. Finally, this method was applied to real water sample such as sewage, fertilizer waste and sea water successfully.

1.2   Objectives of the research

The objectives of this research are three main goals as follows:

1. To use the combination of graphene oxide and deposited antimony film for fabrication of an electroanalytical platform

2. To determine the amount of cadmium(II), lead(II), copper(II), and mercury(II) ions simultaneously by sequential injection analysis (SIA) coupled with square wave anodic stripping voltammetry

3. To apply the purposed method for detection of all target metal ions in real samples

CHAPTER II

THEORY AND LITERATURE SURVEY

2.1   Electrochemical technique

The electrochemical techniques provide information on the processes taking place when an electric potential is applied to the system under study. Electrochemistry can be used to studies the loss of electrons (oxidation) or gain of electrons (reduction) that a material undergoes during the electrical stimulation. These reduction and oxidation reactions are commonly known as redox reactions and can give information about the concentration, kinetics, reaction mechanisms, chemical status and other behavior of a species in solution. Generally, there are two kinds of electrochemical cells which are galvanic cell and electrolytic cell. Galvanic cell produces an electric current from energy released by a spontaneous redox reaction. On the other hand, electrolytic cell requires an external source of electrical energy to induce a chemical reaction. Electrolytic method have many advantages, for instance, high sensitivity with a wide linear dynamic range of concentration for both inorganic and organic species, simplicity, rapid analysis time and simultaneous detection of various target analytes. The selection of the electrochemical techniques depends on the nature of ions or compounds of interest and its interferences in surrounding environment. The following details in this section will focus on the electrochemical techniques that were used in this work.

2.2.1   Voltammetry technique

Voltammetry is an electrochemical technique which is based on the application of a potential to an electrode surrounded with an electrolyte containing electro-active species and measuring the signal as a current flowing through that electrode. Voltammetric electrochemical cell consists of three electrodes which are working electrode (WE), counter electrode (CE) and reference electrode (RE). Each electrode have their specific characteristics. Working electrode is the most important

electrode of electrochemical cell because the interested reaction will occur at the surface of this electrode. Next, counter electrode or auxiliary electrode is an electrode used in a three electrode electrochemical cell for voltammetric analysis or other reactions in which an electric current is expected to flow. This electrode will make the potential of reference electrode remain constant since no electron flow through. Lastly, reference electrode is an electrode which has a constant and well-known electrode potential. The high stability of the electrode potential is usually achieved by using a redox system with constant (buffered or saturated) concentrations of each species of the redox reaction. Occasionally, the potential that applied in the system changed as a function of time was controlled.

In this work, voltammetric methods consisted of Cyclic Voltammetry (CV), Square Wave Voltammetry (SWV), and Stripping Voltammetry (SV) were utilized for the detection of Cd(II), Pb(II), Cu(II) and Hg(II) individually and simultaneously. All of these methods will be explained more in the following section.

2.2.1.1 Cyclic Voltammetry

Cyclic Voltammetry (CV) is the most commonly known technique for studying qualitative information of substances, for instance, redox processes, reaction intermediates, and stability of reaction products. Thus, cyclic voltammetry is the first step to perform when electrochemical technique is applied. This technique is based on changing the applied potential of the working electrode in both forward and backward direction (opposite direction) at constant scan rate while monitoring the signal current. Electrochemical behavior of the system can be obtained from this simple technique that requires relatively small experimental attempt. Unfortunately, it is difficult to get quantitative information from this technique. Cyclic voltammetry experiment comprises of linear scan potential of a working electrode in an equilibrium unstirred solution by a triangular potential waveform shown in Figure 2.1. The potential waveform illustrates the forward scan and then backward scan. The measured current at working electrode is plotted versus the applied potential called

cyclic voltammograms. Normally, a voltammogram of reversible redox couple during one cycle shows cathodic current (i_{pc}) in the forward scan (from positive potential to negative potential) and anodic current (i_{pa}) in the reverse scan (from negative potential to positive potential) at the applied potential approached to the standard potential E^0 for that redox process. The corresponding peak potential occurring at i_{pc} and i_{pa} named cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}), respectively.
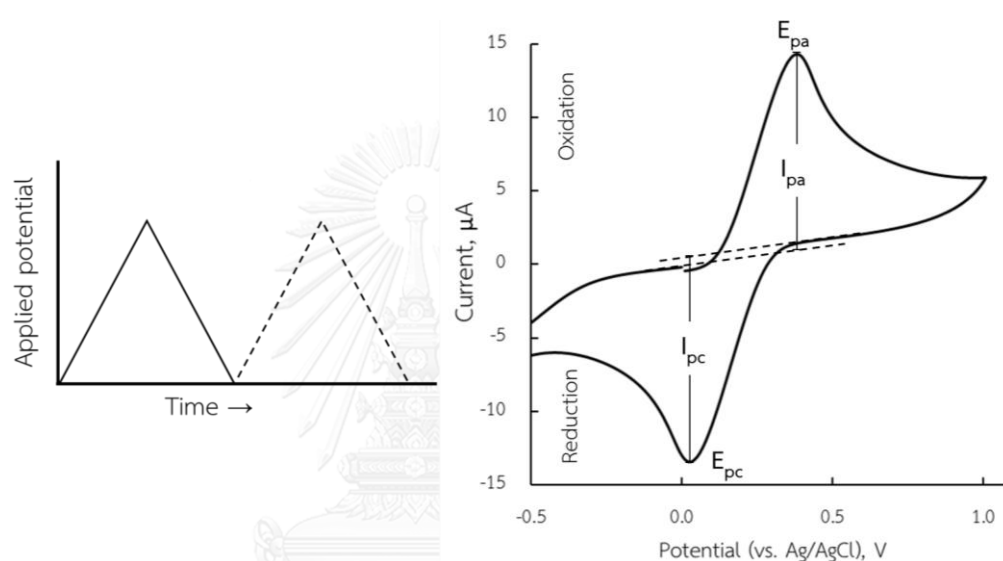
Figure 2.1   Cyclic voltammetric excitation signal (left) and cyclic voltammogram of a reversible reaction (right).

2.2.1.2 Square Wave Voltammetry

Square Wave Voltammetry (SWV) is one of the most powerful voltammetric methods because of high sensitivity and short analysis time.   The excitation signal is a symmetrical square-wave form of a large-amplitude differential technique.   The working electrode is applied with this potential waveform and measured the current twice during each square wave cycle once at the end of forward pulse and once at the end of the backward pulse.   Because the square-wave modulation amplitude is very large, the reverse pulses cause the reverse reaction of the product of the forward pulse. The difference between the forward and backward currents achieved the net current (i_{net}), is plotted versus the applied potential.

The peak height is directly proportional to the concentration of the electroactive species. The great sensitivity increases because the final current is larger than either the forward or backward compositions. The advantages of SWV is its speed. This rapid analysis, coupled with computer control and signal averaging, allows experiments to be operates repetitively and increases the signal-to-noise ratio. The sensitivity of this technique can be enhanced by increasing the amplitude of the frequency of the square wave. The kinetic aspects of the redox processes would be the limitation of current enhancing. Figure 2.2 shows the potential waveform and the voltammogram of square wave voltammetry
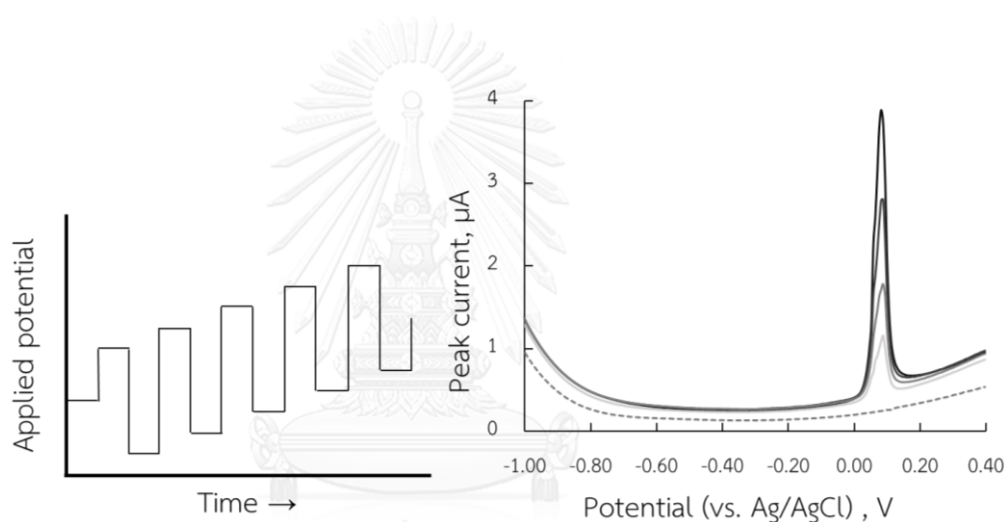
Figure 2.2   Square wave voltammetric excitation signal (left) and square wave voltammogram of Hg(II) at different concentration (right).

2.2.1.3 Stripping Voltammetry

The stripping technique is a highly sensitive electroanalytical technique which have the lowest limits of detection of the widely known for metal analysis. Stripping voltammetry consists of two steps as follow.

1) Deposition step

The deposition step involves the electrolytic deposition of a small part of the target metal ions in the solution on the working electrode for the preconcentration of analytes. Anodic stripping voltammetry is commonly known for

the stripping analysis. In this case, preconcentration step is performed by cathodic deposition as a controlled time and applied potential. The deposition potential is more negative than standard potential (E^0) to completely and easily reduce the metal ion in solution. The metal ions reach the working electrode by diffusion and convection.

Before starting the stripping step, a quiet period or equilibration time is set between the deposition and stripping step to cease the forced convection, maintain a uniform concentration distribution, and confirm that the solution is in quiescent solution before the stripping step.

2) Stripping step

Next step is stripping step or measurement step which related to the dissolution or stripping of the deposit. Any number of potential waveforms can be used for the stripping step. The most widely used of the potential waveforms are the differential pulse and the square wave owing to the discrimination against charging current. The stripping step is beginning with the scan potential in the anodic direction. In the meantime, the electrodeposited metals are reoxidized when the potential reaches the standard potential of each metal and stripped out of the electrode surface. The peak potential position are arranged in order of each metal standard potential. In addition, the stripping peak current is directly proportional to the concentration of the metal. Hence, the method is capable for simultaneous detection of several metal ions. Figure 2.3 illustrates the summary of both deposition or preconcentration and stripping step in anodic voltammetry.
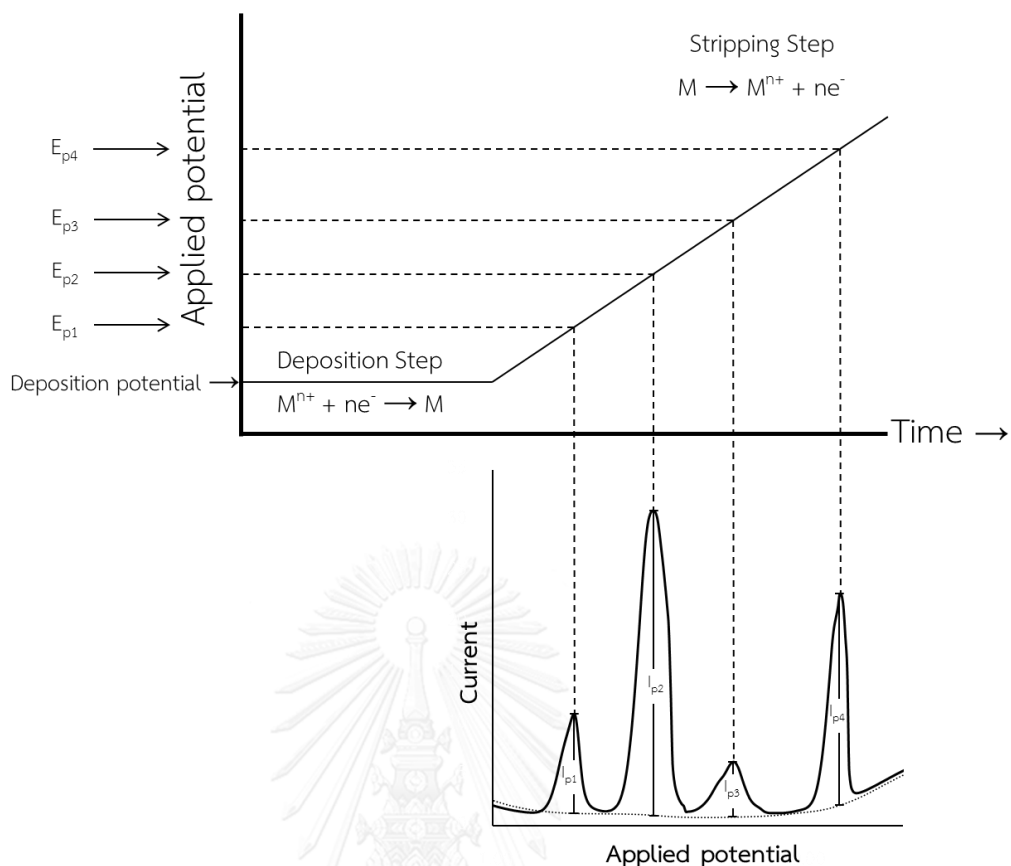
Figure 2.3 Stripping voltammetry for the determination of metals: the potential-time waveform (top) and voltammogram (bottom).

2.2.2 Electrodes

The electrochemical cell, where the voltammetric experiment is performed comprised of a working electrode, a reference electrode and a counter electrode. Usually, the electrode provides the interface between solid phase and electrolyte which charge can be transferred. At the suitable applied potential, the reduction or oxidation of analytes occur at the surface of electrode and the current is generated.

2.2.2.1 Screen-printed electrodes

New technology for fabrication of disposable electrochemical platform is screen-printed method. A screen-printed electrode is a flat device based on appropriate layers of a graphite powder based ink printed on polyvinyl chloride substrate. The benefits of the screen-printed electrode are ease of fabrication, simplicity, portability, low-cost, small size and mass production capabilities. Hence, this thesis selected screen-printed method for fabrication of electrode for simultaneous detection of heavy metal ions, which are Cd(II), Pb(II), Cu(II), and Hg(II).

2.2.2.2 Working electrode

The working electrode is the electrode at which the interesting reaction of target analytes occur. The reaction that occurred at the working electrode can be referred to as either anodic or cathodic. The applied potential of working electrode was varied in a specific value with the variations in the concentration of target analyte. In voltammetric technique, the small surface area of the working electrode is applied to increase polarization and to reduce the diminution of the analyte. The electrode performance seriously depends on the material of the electrode. The ideal working electrode should give a high signal-to-noise ratio of the interesting analytes, a reproducible electrode surface, a wide potential window, high electrical conductivity, low cost, low toxicity and availability.

2.2.2.3 Reference electrode

Reference electrode is an electrode having known electrode potential that remain constant at specific temperature and is independent of the concentration or composition of the analyte solution. This electrode acts as reference point along the potential axis by which the oxidizing or reducing power of the working electrode is evaluated. Moreover, the ideal reference electrode should be simple to fabricate and to use practically. The commonly used reference electrode is silver/silver chloride reference electrode because of simplicity, inexpensiveness,

stability, and low toxicity. As a result, silver/silver chloride reference electrode was selected as a reference electrode for the simultaneous detection of heavy metal ions.

2.2.2.4 Counter electrode

Counter electrode normally used to minimize errors from the cell resistance while controlling the potential of working electrode. This kind of electrode is generally made of a chemically inert conducting material with immense electrode surface area. Platinum wire or graphite rods are the most widely counter electrode in the stripping analysis.
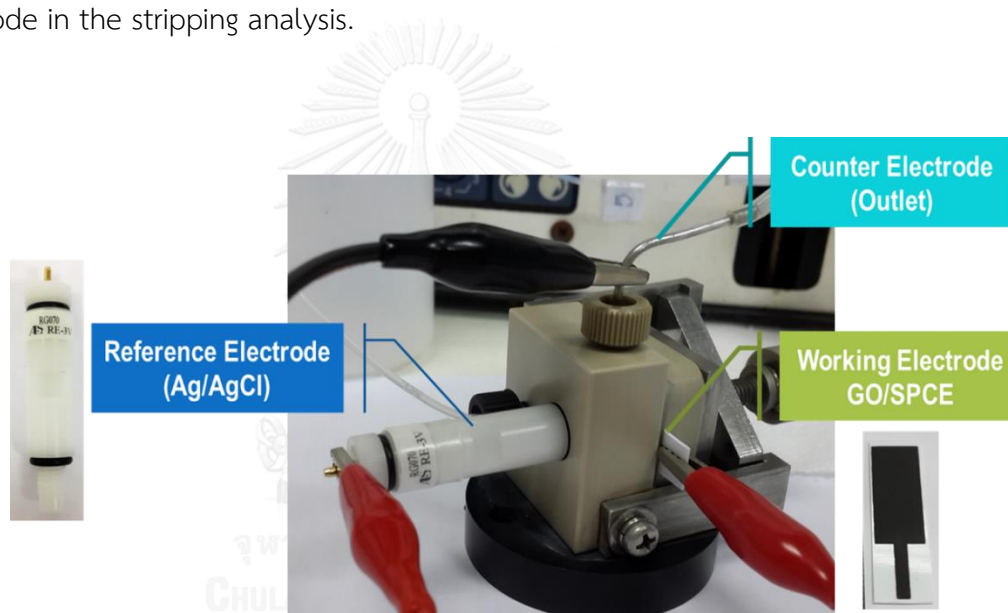
Figure 2.4   Electrochemical cell consisted of working electrode: graphene oxide modified screen printed electrode, reference electrode: silver/silver chloride electrode, and counter electrode: stainless steel outlet tube.

2.2.2.5 Graphene oxide

Graphene is a relatively new material discovered in 2004. It is actually a monolayer of carbon atoms which are covalently linked and arranged into a two dimensional honeycomb-like lattice. Graphene is a basic building block of other graphitic materials depending on how it is manipulated (Fig. 2.5). The study of graphene has been made for many years even before its physical discovery. In fact,

other carbon graphitic allotropes such as '0D fullerene', '1D carbon nanotube' and '3D graphite' are theoretically modeled as a wrapped, rolled, and stacked graphene, respectively [17].
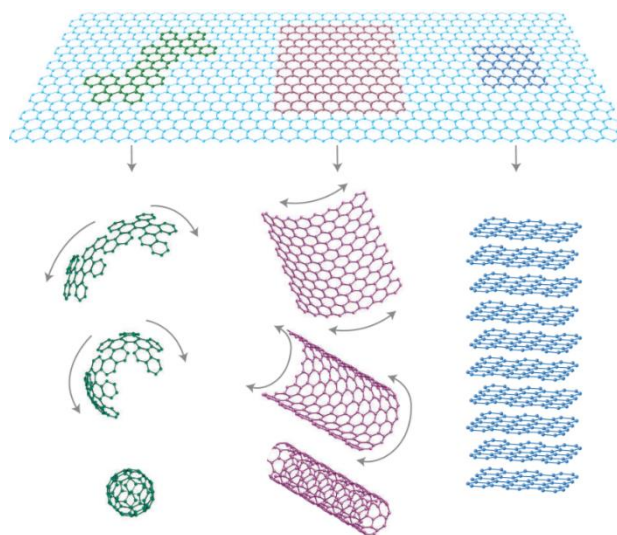
Figure 2.5 Graphene as a building block for other carbon materials [17].

Graphene has many excellent characteristics such as high optical transparency, electrical conductivity, and catalytic activity making it a very interesting material [18, 19]. The incredible electrical natures of graphene have been widely studied and used in electrical devices, composites, and sensors [24]. Graphene can be obtained from the reduction of graphene oxide (GO) which is a sheet of graphene that is partially oxidized, containing of both sp^2- and sp^3- hybridized carbon atoms, hydroxyl functional groups on each side of the carbon sheet, and decorated by carboxyl groups [20, 21]. Graphene oxide has functional groups such as hydroxyl and carbonyl, which are advantageous for adsorbing heavy metal ions. Moreover, it is stable under both acidic and basic conditions and heat cannot destroy its structure. The decorated oxygen groups allowed GO to produce stable dispersion in various non polar and polar solvents such as water which is commonly used as solvents for supporting electrolyte [21]. Interestingly, very few reports on the graphene oxide

decorated with metal film or metal oxides nanoparticles could be found in electrochemical detecting of heavy metal ions.
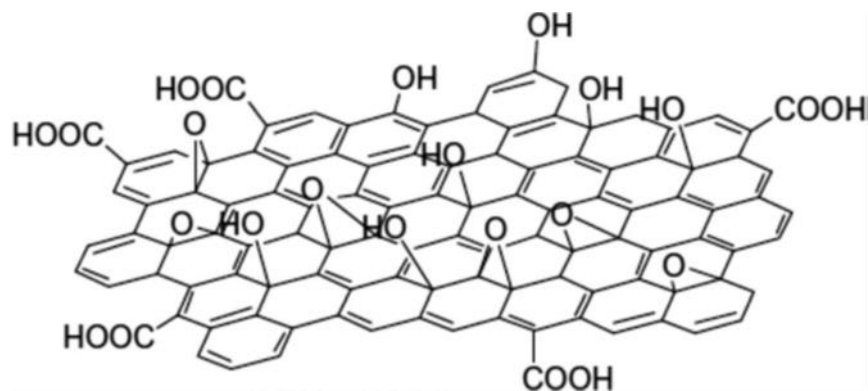
Figure 2.6   Structure of graphene oxide (GO) [25].

2.2.2.6 Antimony

The traditional material that is very popularly used for determination of heavy metal ions was mercury because of superior electroanalytical performance. Unfortunately, mercury is very toxic. Therefore it is hard to handling, storage, and disposal. Alternative materials which have the same capability to mercury were bismuth and antimony due to intermetallic compounds of analytes and these materials [22]. Because the bismuth film modified electrode was unsuitable for determination of copper(II) and mercury(II) ions, antimony film modified electrode [23] became an attractive material for detection of cadmium(II), lead(II), copper(II), and mercury(II), simultaneously. The comparison of different modified electrodes showed that the antimony film modified electrode has a lower baseline and the better peak shape. Moreover, the lower current at very negative potential illustrated that the hydrogen evolution would be low as well. This modified electrode revealed a great stripping current responses of Cd(II) and Pb(II) ions using SWASV. The SbF-CPE could be practically applied to a real water sample analysis. Antimony film was proved to be potential for detection of heavy metal ions in the same way as mercury did with lower toxicity [13, 26, 27]. In addition, it can form intermetallic compounds which can decrease the deposition potential.

2.2   Sequential injection analysis (SIA)

To make the system more automatic, sequential injection analysis (SIA) is used as an automated approach to sample handling that allows automating in situ antimony film modification of electrode and SWASV procedures in a rapid, precise, and efficient manner. SIA is an important analytical tool in the area of discontinuous flow methods. The system is controlled by computer software. Small solution zones are manipulated under controlled dispersion conditions in narrow bore tubing. This system will reduce human's error, glassware and risk of health problems of the operator on direct contact with toxic metal ions. A diagram of basic SIA instrument composed of a bi-directional syringe pump used to aspirate or dispense small samples and reagent solutions, a multi-position selection valve used to choose solutions, reaction or holding coil, pump tubes, and a detector flow cell. In figure 3.1, the sample reservoir is connected to the ports of the multi-position selection valve. Sample and reagent solutions are consecutively aspirated into holding coil connected to the common port of the multi-position selection valve by monitoring the pump in the reverse direction. The advantages of SIA are small volume of solutions (μL level), accuracy of solution handling, simplicity, durability, short time analysis, reduction of user's health problem, and great performance of simultaneous detection.

2.3   Literature survey

In 2008, Hwang *et al.* [22] reported the use of bismuth-modified carbon nanotube electrode (Bi-CNT electrode) for the determination of lead, cadmium and zinc. Bismuth film was deposited onto the screen-printed CNT electrode. The simultaneous determination of lead, cadmium and zinc was performed by SWASV. The deposited Bi film could improve the sensitivity; however, bismuth exhibited a large oxidation peak at around -0.1 V in stripping step. Thus, bismuth film was not suitable for the detection of copper and mercury because these two metal ions showed the peaks at -0.2 V and +0.1 V, respectively.

In 2009, Tesarova *et al.* [28] reported the in situ antimony film carbon paste electrode (SbF-CPE) as an electrochemical sensor. The comparison of different modified electrodes showed that one of antimony film modified electrode has a lower baseline and the better peak shape. Moreover, the lower current at very negative potential illustrated that the hydrogen evolution would be low as well. This modified electrode revealed a great stripping current responses of Cd(II) and Pb(II) ions using SWASV. The SbF-CPE could be practically applied to a real water sample analysis. Antimony film proved to be potential for detection of heavy metal ions in the same way as mercury did with lower toxicity. In addition, it can form intermetallic compounds which can decrease the deposition potential.

In 2010, Guzsvány *et al.* [29] reported the alternative applying of antimony-film modified glassy carbon electrode in SIA for determination of Pb(II) and Cd(II) by ASV. The antimony film was electrodeposited from an antimony(III) solution onto the electrode surface after that analyte metals were deposited from a sample solution. Square wave voltammetry was chosen as a stripping technique to observe the heavy metal ions. This modified electrode performed a great reproducibility. The developed SIA-ASV process was practically used in a tap water sample.

In 2011, Wei *et al.* [9] reported the detailed study of the SnO_2/reduced graphene oxide nanocomposite modified glassy carbon, which could be used for the simultaneous and sensitive electrochemical detection of ultratrace Cd(II), Pb(II), Cu(II), and Hg(II) in drinking water. This modified electrode showed a very great sensitivity and selectivity upon heavy metal ions using SWASV. Graphene oxide has functional groups such as hydroxyl and carbonyl, which are advantageous for adsorbing heavy metal ions.

In 2012, Gao *et al.* [10] reported the preparation, characterization, and electrochemical behavior toward heavy metal ions of the AlOOH-reduced graphene oxide nanocomposites. This new material was synthesized through a green one-pot hydrothermal method. Due to the strong affinity of AlOOH to heavy metal ions and the fast electrontransfer kinetics of graphene, the combination of solid-phase extraction and stripping voltammetric analysis allowed fast and sensitive determination

of Cd(II) and Pb(II) in drinking water, making these new nanocomposites promising candidates for practical applications in the fields of detecting heavy metal ions. Most importantly, these new nanocomposites may possess many unknown properties waiting to be explored.

In 2012, Wonsawat *et al.* [30] reported an environment friendly electrode for determining Cd^{2+} and Pb^{2+} levels in an automated flow system. Cyclic voltammetry and square wave anodic stripping voltammetry (SWASV) coupled with sequential injection analysis (SIA) were employed to study the electrochemical behavior of the electrode. The in situ bismuth-modified graphene-carbon paste electrode (Bi-GCPE) exhibited excellent electrooxidation of Cd^{2+} and Pb^{2+} in the automated flow system with a significantly higher peak current for both metal ions compared with the unmodified CPE.

In 2013, Punrat *et al.* [31] reported an automated method for determining the concentration of inorganic arsenic by sequential injection/anodic stripping voltammetry with a long-lasting gold-modified screen-printed carbon electrode. The long-lasting gold film was electrochemically deposited onto a screen-printed carbon electrode at a potential of -0.5 V vs. Ag/AgCl in a supporting electrolyte solution of 1 M hydrochloric acid. Under optimal conditions and applied potentials, the electrode demonstrated that it can be used for a long time without a renewal process. This method was used to determine the concentration of arsenic(III) in water samples with satisfactory results. The proposed method is an automated system that offers a less expensive alternative for determining trace amounts of inorganic arsenic.

Even though some of the publications had reported the highly sensitive methods for the detection of heavy metal ions, these methods still have disadvantages, for example, time consuming, complication of modifier synthesis. In addition, these heavy metal ions could be found together at significant amount which were difficult to detection in real samples. Therefore, the fast and ease methods for the simultaneous detection of heavy metal ions in real water sample is developed.

CHAPTER III

EXPERIMENT

In this chapter, the information of the chemicals, instruments, modification of the electrode, electrochemical measurements and sample preparations in this work are described.

3.1 Instruments and apparatus

The instruments and apparatus used in this research were listed in Table 3.1.

Table 3.1 List of instruments and apparatus.

Instruments and apparatus	Suppliers
PGSTAT 100 instrument	Eco Chemie, The Netherlands
Ag/AgCl reference electrode	BAS, Japan
Stainless steel tube counter electrode	Home made
Screen-printed blocks	Chaiyaboon, Thailand
Analytical balance, Mettler Toledo	Mettler, Switzerland
Hot air oven	Memmert, USA
Sequential analysis system (2.5 mL syringe pump)	MGC, Japan
PTFE tubing	Upchurch
Milli-Q water system (18 MΩ cm^{-1})	Millipore, Bedford, USA
Scanning electron microscope (SEM)	JEOL, Japan
FTIR spectrometer Nicolet iS10	Thermo Fisher Scientific, USA
UV-Vis spectrofluorometer	Agilent Technologies, USA

Instruments and apparatus	Suppliers
Centrifuge (Universal 320R)	Hettich, Germany

3.2 Chemicals and Reagents

The chemicals and reagents used in this research were analytical grade and were used as purchased without any further purification. The chemicals and reagents are listed in Table 3.2.

Table 3.2 List of chemicals and reagents.

Chemicals	Suppliers
Antimony (Sb(III)) standard solution, 1,000 mg L^{-1}	BHD, England
Cadmium (Cd(II)) standard solution, 1,000 mg L^{-1}	BHD, England
Lead (Pb(II)) standard solution, 1,000 mg L^{-1}	BHD, England
Copper (Cu(II)) standard solution, 1,000 mg L^{-1}	BHD, England
Mercury (Hg(II)) standard solution, 1,000 mg L^{-1}	BHD, England
Hydrochloric acid (HCl), 37 %w/w	Merck, Germany
Sulfuric acid (H$_2$SO$_4$)	Merck, Germany
Sodium nitrate (NaNO$_3$)	Sigma-aldrich, USA
Potassium permanganate (KMnO$_4$)	Sigma-aldrich, USA
Hydrogen peroxide (H$_2$O$_2$)	Sigma-aldrich, USA
Carbon ink	Electrodag PF-407C, Acheson, USA
Graphite	Sigma-Aldrich, Switzerland

3.3   Chemicals and reagents preparation

3.3.1   Preparation of 100 μM of Cd(II), Pb(II), Cu(II), and Hg(II) in 0.50 M HCl

The stock solutions of Cd(II), Pb(II), Cu(II), and Hg(II) were prepared by diluting 1000 mg L^{-1} of standard solution of Cd(II), Pb(II), Cu(II), and Hg(II) with 0.50 M HCl.

3.3.2   Preparation of 500 μg L^{-1} of Sb(II) in 0.50 M HCl

The stock solution of Sb(III) were prepared by diluting 1000 mg L^{-1} of standard solution of Sb(III) with 0.50 M HCl. These solutions can be stored for at least one month.

3.3.3   Working standard solutions of Cd(II), Pb(II), Cu(II), and Hg(II)

The working standard solutions of Cd(II), Pb(II), Cu(II), and Hg(II) were prepared daily by appropriate diluting the stock solutions in 0.50 M HCl.

3.3.4   Supporting electrolyte: 0.50 M HCl

A HCl solution was prepared daily by diluting 20.83 mL of 37% w/w HCl to 500 mL with Milli-Q water to appropriate concentration.

3.3.5   Synthesis of graphene oxide

According to Hummers' method [32], concentrated H_2SO_4 (34.5 mL) was added to a mixture of graphite flakes (1.5 g, 1 equivalent weight) and $NaNO_3$ (0.75 g, 0.5 equivalent weight), and the mixture was cooled to 0°C, then $KMnO_4$ (4.5 g, 3 equivalent weight) was added slowly in portions to keep the reaction temperature

below 20°C. Next, the reaction was adjusted to 35°C and the mixture was stirred for 30 mins, after that water (69 mL) was added slowly, producing a large amount of heat that ramped the temperature up to 98°C. External heating was supplied in order to maintain the reaction temperature at 98°C for 15 mins, then the reaction mixture was cooled down using a water bath for 10 mins. Additional water (210 mL) and 30% H_2O_2 (1.5 mL) were added, producing another heat up. The suspension was dispersed again in Milli-Q water and the mixture was centrifuged (4000 rpm for 1 h), then the liquid above solid particles was poured off. The remaining solid material was then washed with 60 mL of water, 60 mL of 30% HCl; for each wash, the mixture was filtered. The suspension was centrifuged (4000 rpm for 1 h) and the liquid above solid was poured off. The solid obtained on the filter paper was dried at room temperature, and 0.6 g of solid remained. Characterization of graphene oxide was carried out by ATR-FTIR and UV-vis spectroscopy.

3.4 Electrode preparation

3.4.1 Graphene oxide modified screen-printed carbon electrode

The graphene oxide modified screen-printed carbon electrodes (GO-SPCEs) were fabricated in house by screen-printing carbon ink (Electrodag PF-407C, Acheson, USA) mixed with graphene oxide at appropriate amount on PVC substrate. After that, electrodes were kept in an oven at 55°C for 1 hour.

3.4.1 *In-situ* antimony film modification of GO-SPCE

The Sb(III) solution were prepared to the optimized concentration by diluting the Sb(III) standard solution with the appropriate volume of supporting electrolytes. After that, the Sb(III) solution dispensed directly and continuously through the flow cell following by the analytes ions solution. Next, the Sb-film was electrodeposited on the electrode simultaneously with the analytes ions.

3.5   Sample preparation

For real sample analysis, water samples were prepared by mixing 0.05 mL of sample solution and specific volume of metal standard solutions. Then, the solutions were diluted with the supporting electrolyte which is 0.5 M HCl to the final volume of 10 mL. After that, the concentration of analytes were determined by standard addition method and the percent recovery from spiked sample solutions were determined by calibration method.

3.6   Experimental Procedure for SbF-GO-SPCE

The *in-situ* Sb film modification of the GO-SPCES and SWASV measurement steps are shown in Table 3.3. The sample solution and the Sb(III) solution were sequentially aspirated into the holding coil (Step 1 and Step 2). Next, the deposition potential was set at -1.4 V while solutions were flowed directly to the flow cell as electrodeposition of Sb film and the preconcentration of analytes (Step 3). Then, solution was stop for 10 second as equilibration (Step 4). Square wave voltammograms with a frequency of 50 Hz, a step potential of 6 mV, and an amplitude of 40 mV were recorded from -1.4 V to +0.4 V vs. Ag/AgCl (Step 5). Lastly, the electrode was cleaned to remove any remaining analyte metals and antimony film in flowing supporting electrolyte at flow rate of 30 μL s^{-1} for 17 seconds without any applied potential (Step 6 and Step 7). One measurement cycle for 4 analytes including Cd(II), Pb(II), Cu(II), and Hg(II) is about 3 min. The electrode was ready for a new measurement. A 0.5 M HCl solution was used as a supporting electrolyte for all steps. All experiments were performed without removing oxygen from the solution.

Table 3.3 Step sequence for the Sb film modification of the GO-SPCE and determination of heavy metal ions by SIA-SWASV.

Step	Description	Valve position	Flow rate (μL s^{-1})	Duration (s)	Electrode potential (V)
1	Aspirate sample solution into holding coil	5	200	5	+ 0.4
2	Aspirate Sb(III) solution into holding coil	6	200	2.5	+0.4
3	Dispense Sb(III) solution and sample solution into flow cell	8	10	150	-1.4
4	Equilibration	8	0	10	-1.4
5	Stripping and recording of voltammogram	8	0	5	-1.0 to +0.4
6	Aspirate 0.5 M HCl into holding coil	4	200	2.5	-
7	Dispense 0.5 M HCl into flow cell for electrode cleaning	8	30	17	-
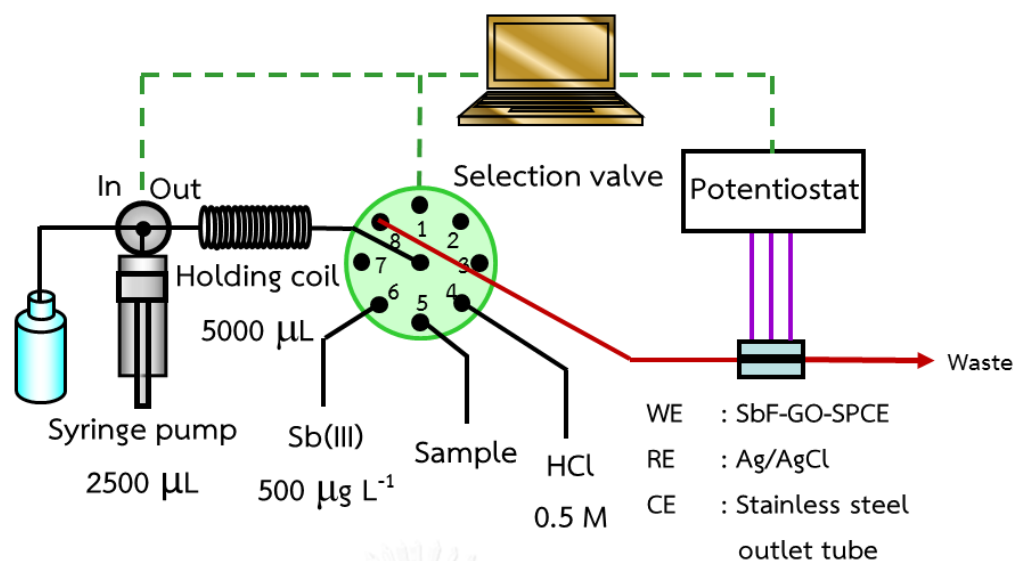
Figure 3.1   Schematic diagram of sequential injection analysis (SIA) in this work

3.7   Optimization of the parameters for SbF-GO-SPCE measurement

3.7.1   Percentages of graphene oxide to carbon ink

In order to achieve the highest signal-to-noise ratio for heavy metal detection, the percentages of GO to carbon ink with 0%, 1%, 2%, 3%, and 4% (w/w) were investigated by SIA-SWASV. Square wave anodic stripping voltammograms of 100 μg L^{-1} Cd(II), Pb(II), Cu(II), and Hg(II) on SbF-GO-SPCEs at various percentages of GO were recorded.

3.7.2   Effect of antimony(III) concentration

The current response was affected by the thickness of Sb-film on SbF-GO-SPCE. The influence of Sb(III) concentration, which controlled the film thickness, was evaluated from 0 to 1000 μg L^{-1}. The optimized concentration was obtained from the plot of the peak current and the Sb(III) concentration.

3.7.3   Effect of concentration of supporting electrolyte

There has been widely reported that HCl is a good supporting electrolyte for heavy metal analysis. The optimization of concentration of HCl as supporting electrolyte was carried out at different concentrations from 0.01 to 1.00 M. The anodic peak current behavior of heavy metal ions in various concentrations of HCl was achieved from the plotting of the peak current and the HCl concentration.

3.8   Optimization of electrochemical parameters

In order to obtain the best detection and good reproducibility, SWASV parameters were studied including deposition potential, SW frequency, amplitude and increment potential. First of all, the influence of deposition potential or accumulation potential was performed in the range of 1.6 to -1.0 V. The highest current response was gained from the plotting of the peak current with the accumulation potential. Secondly, the frequency affected to the scan rate of determination system and was evaluated from 10 to 90 Hz. Thirdly, the effect of amplitude was studied from 20 to 100 mV. Finally, the influence of increment potential was investigated from 2 to 10 mV. All the SWASV parameters were investigated under previous optimized SbF-GO-SPCE, 0.50 M HCl as supporting electrolyte and 100 µg L^{-1} of Cd(II), Pb(II), Cu(II), and Hg(II).

3.9   Optimization of SIA operation parameters

The factors that influence the sensitivity and analysis time of each SWASV detection in flow-based analysis are sample volume and flow rate of the solution in the electrodeposition step. These parameters are related to deposition time, changing the sample volume and/or the flow rate will affect to the deposition time. Hence, the sample volume was set at 1.0 mL. The effect of flow rate was studied in the range of 5 to 40 µL s^{-1} and at different deposition times. After finish each analysis, the Sb film and all deposited metals was cleaned by flowing 0.5 M HCl which was the

supporting electrolyte. To reduce waste, the cleaning solution volume was investigated.

3.10   Analytical performance

Under the optimized analytical conditions, the calibration data of Cd(II), Pb(II), Cu(II), and Hg(II) peak current related with different concentrations were evaluated. The analytical concentration ranges of analytes were 0.1 to 1.5 µM for all metal ions. The detection limits (LOD) were achieved from $3\sigma/S$ and $10\sigma/S$, where σ is the standard deviation of the blank measurement (n=10) and S is the slope of calibration curve.

3.11   Interference study

To analyze the target heavy metal ions in real water sample, various ions in such water can be found. Hence, the interference of many cations (Na^+, K^+, Mg^{2+}, Ca^{2+} and Al^{3+}) and some of anions (F^-, Br^-, NO_3^-, HCO_3^-, CO_3^{2-} and SO_4^{2-}) that might be found in sewage or sea water were investigated. Their concentrations were 100-fold higher than analytes concentration.

3.12   Analytical application

To evaluate the proposed method, this system was applied under optimized conditions for the simultaneous quantification of all target ions in sewage, fertilizer waste and sea water. All sample solutions were prepared by mixed 50 µL of each filtered water sample with 0.5 M HCl which was a supporting electrolyte to final volume of 10 mL. Prepared samples were introduced to determination system without any further pretreatment.

For sewage and fertilizer waste from a metal-plating factory in Thailand, preliminary testing found that only Cu(II) was detected. Thus, standard addition method was used to quantify concentration of copper solely. To validate the proposed method, the results were compared with standard method obtained by AAS.

A t-test at the 95% confidence level with degrees of freedom of 4 was achieved on the data obtained.

For sea water from Hadsangchan, Rayong Province, Thailand, the concentration of target ions was studied by calibration method without pretreatment. No anodic peak current of heavy metal ions was obtained in the unspiked sea water sample. The recovery of Cd(II), Pb(II), Cu(II), and Hg(II) were tested by addition of 0.30, 0.50, 1.00 µM standard stock solutions into the sea water. The obtained results were done based on three repetitions (n=3) of all analytes.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results and discussion of the modification screen-printed electrodes and comparison of the electrochemical response, optimization of the SbF-GO-SPCE, square wave stripping voltammetric conditions, analytical parameters and analytical applications. All of the results were obtained from SWASV techniques coupled with the SIA.

4.1 Characterization of synthesized graphene oxide

Graphene oxide was synthesized from graphite powder via Hummer's method [32]. The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of graphite and graphene oxide are shown in Fig. 4.1. It can be seen that a characteristic peaks of graphene oxide are O–H stretching vibration at 3208 cm^{-1}, C=O stretching vibration at 1717 cm^{-1} and the remaining sp^2 stretching vibration at 1617 cm^{-1} [20, 33-35].
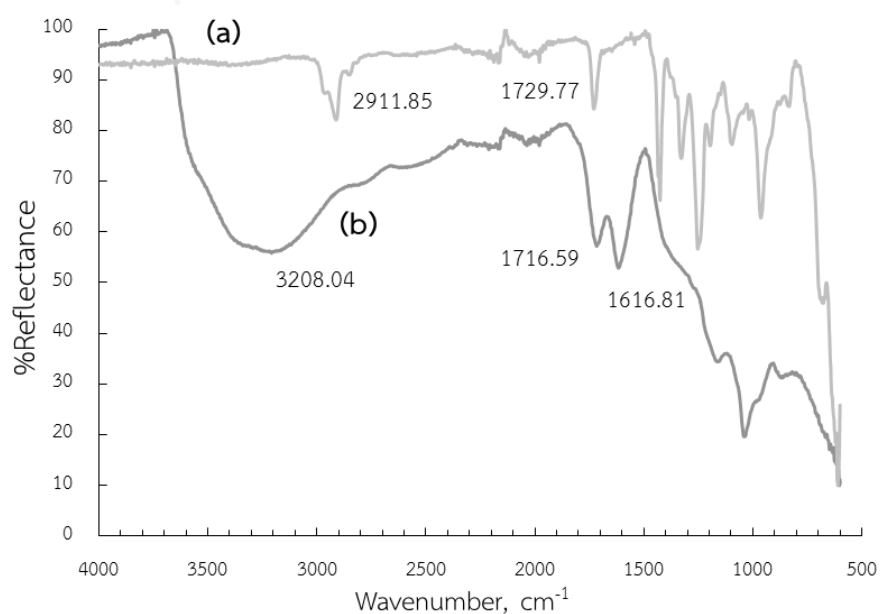
Figure 4.1   ATR FT–IR spectra of graphite (a) and graphene oxide (b).

UV-visible spectroscopy was also used to characterize graphene oxide. Fig. 4.2 shows ultraviolet-visible spectrum of graphene oxide. The spectrum of graphene oxide has an absorption peak at 232 nm. This peak is attributed to $\pi \rightarrow \pi^*$ transition of aromatic C-C bond, and a shoulder at about 300 nm can be assigned to the $\pi \rightarrow \pi^*$ transition of C=O bonds. Also, this result, which is consistent with the result of ATR-FTIR, confirms the existence of oxygen-containing functional groups in graphene oxide [36, 37].
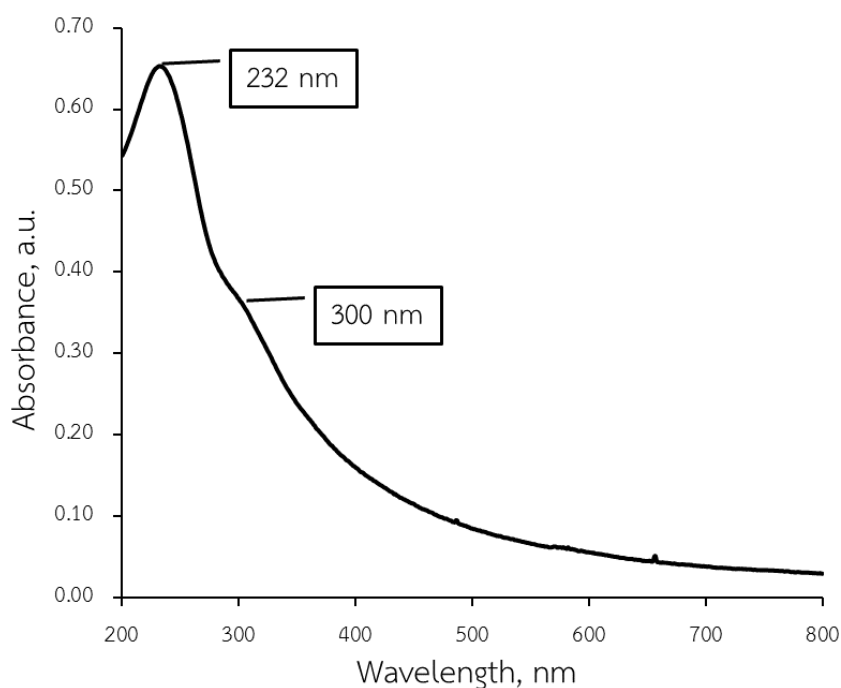
Figure 4.2 UV-vis spectrum of GO in water.

4.2 Square wave anodic stripping voltammetric detection using SbF-GO-SPCE

In this research, the performance of working electrodes were enhanced by adding graphene oxide powder into the commercial carbon ink before fabricating GO-SPCE with by screen-printing process.

The SWASV responses toward metals ion detection using various modified electrodes was compared including bare electrode (SPCE), GO-SPCE, SbF-SPCE, and SbF-GO-SPCE as shown in Fig. 4.3. The results showed that, when using SbF-GO-SPCE,

the anodic current was raised by 5 to 7 times higher than SPCE. We believed that the increase of electrochemical response is probably due to the combine influence of GO and SbF. These electrode modifiers were an interesting combination because GO can increase active electrode surface of working electrode and SbF can improve SWASV performance due to the intermetallic formation between antimony and these metals of interest during accumulation step of SWASV. Therefore, the proposed SbF-GO-SPCE was chosen as an electrochemical detector in sequential injection system for the further experiments.
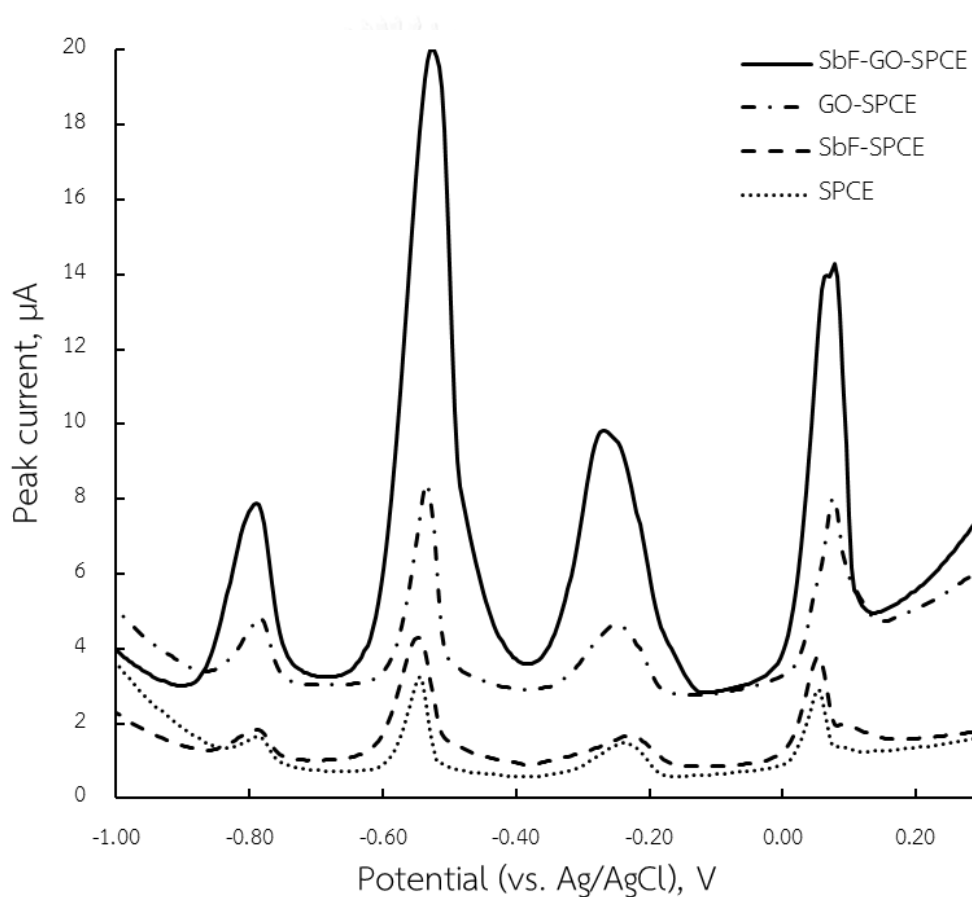
Figure 4.3   Comparison of SPCE (dot line), SbF-SPCE (dash line), GO-SPCE (dash dot line) and SbF-GO-SPCE (solid line) upon the stripping voltammetric responses at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II).

To investigate the morphology of the electrode, scanning electron microscopy (SEM) was performed. The SEM images illustrated that the electrodeposited antimony film was successfully covered the area of the electrode (Fig. 4.4b) compared to GO-SPCE (Fig. 4.4a). Moreover, the SEM images of antimony film present the different shape from GO-SPCE. The results indicate that the SbF and GO can be an alternative electrode modifier which can enhance the electrochemical performance of the detector in sequential injection system.
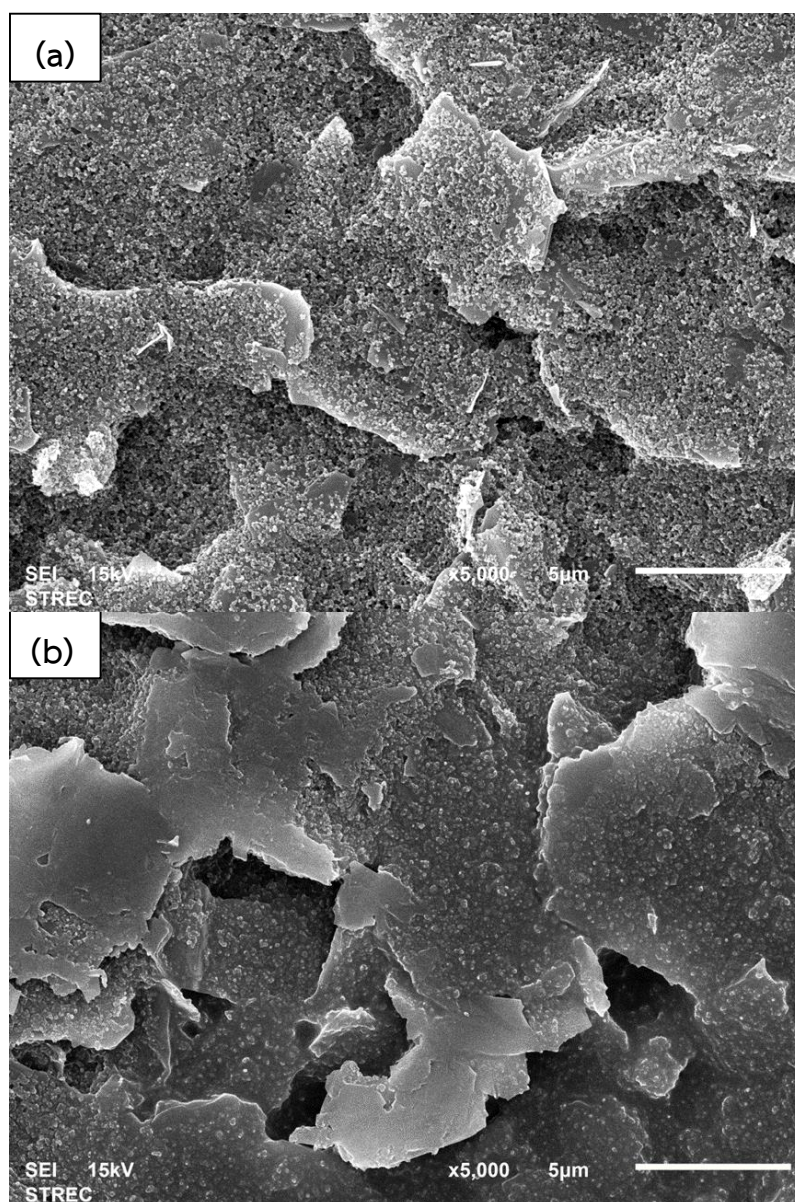
Figure 4.4   SEM images of GO-SPCE (a) and SbF-GO-SPCE (b)

4.3   Optimization of the parameters for SbF-GO-SPCE measurement

4.3.1   Percentages of graphene oxide to carbon ink

GO-SPCEs were fabricated from mixtures of graphene oxide and carbon ink. Graphene oxide (GO) was chosen as an electrode modifier because of their high electrical conductivity and high surface area which provide greater potential for heavy metal analysis. However, GO-SPCEs generate a higher background current than bare SPCEs. In order to achieve the highest signal-to-noise ratio for heavy metal detection, the percentages of GO to carbon ink with 0%, 1%, 2%, 3%, and 4% (w/w) were investigated by SIA-SWASV. Square wave anodic stripping voltammograms of 100 µg L^{-1} Cd(II), Pb(II), Cu(II), and Hg(II) on SbF-GO-SPCEs at various percentages of GO are illustrated in Fig. 4.5. It can be seen that peak current of all heavy metals decreased with increasing percentage of GO from 1% to 4%. Owing to increase the electrode surface area of GO could be the result of the larger background current [17, 38-40], as shown in Fig. 4.6. Therefore, 1% (w/w) was selected as optimum amount of GO.
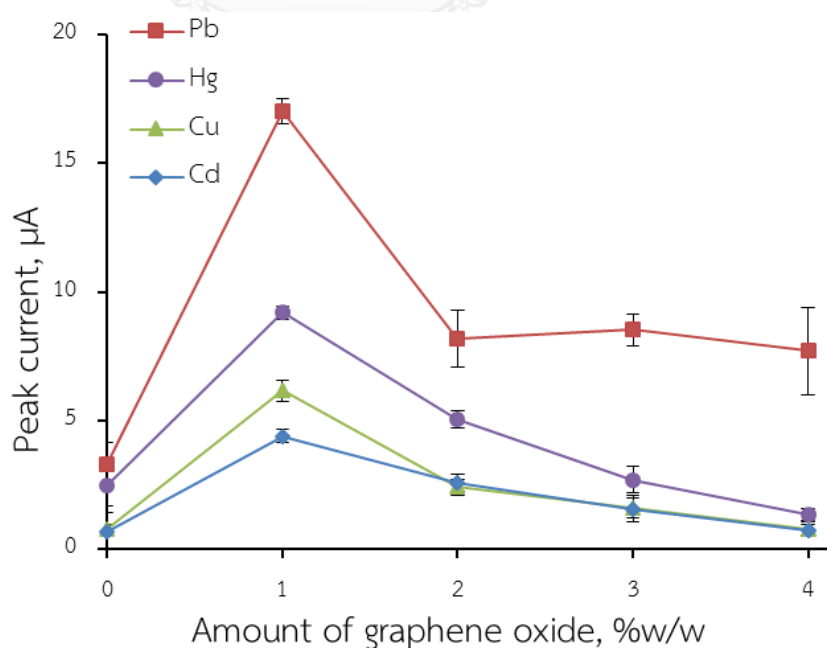
Figure 4.5   Effect of the amount of GO in SbF-GO-SPCE upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II).

Figure 4.6 SIA-SWASV signals of the SbF/GO/SPCE for 100 μg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different amount of graphene oxide (0 – 4%(w/w))

4.3.2 Effect of antimony(III) concentration

Antimony film was selected to modify on GO-SPCE by on-line in situ electrodepositing between each run. Sb(III) solution was introduced into the flow system followed by aspiration of sample solution into the holding coil. Then, all solutions were dispensed to detection zone in the flow cell in order that the Sb was electrodeposited first, followed by the heavy metals in the sample solution. After finished each detection, the Sb film was cleaned by flowing the supporting electrolyte without applied any potential for 17 s. The concentration of Sb(III) was optimized by varying concentration from 0 to 1000 μg L^{-1} while the volume of Sb(III) solution remained constant. The peak current increased when increased Sb(III) concentration and then reached the highest value at 500 μg L^{-1} of Sb(III) solution with small standard deviation for all heavy metal ions. After that, the signals remained rather constant as shown in Fig. 4.7. Therefore, 500 μg L^{-1} of Sb(III) was selected as optimum concentration to minimize the amount of Sb(III).
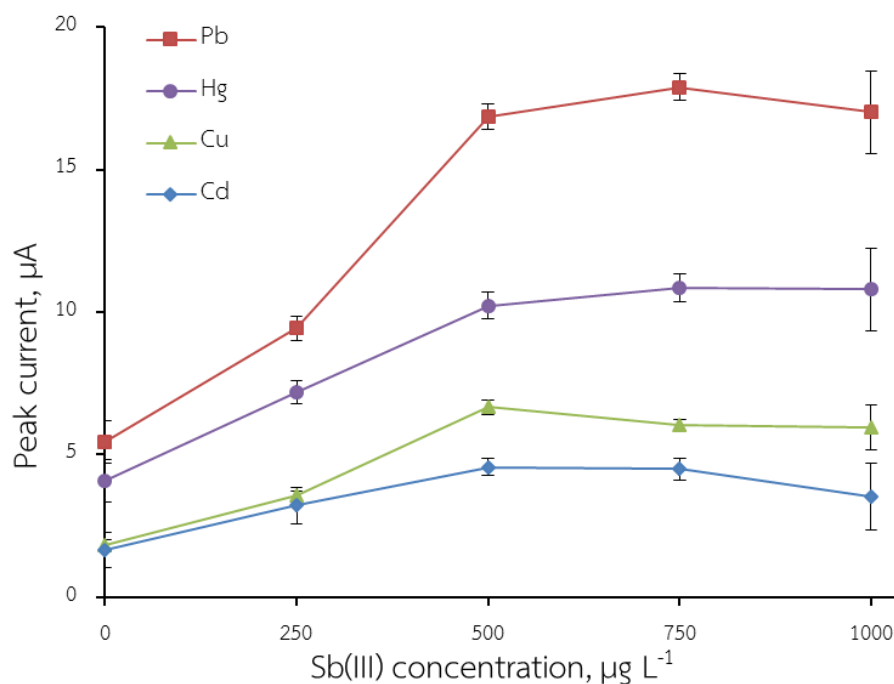
Figure 4.7 Effect of the concentration of Sb(III) upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.

Figure 4.8 SIA-SWASV signals of the SbF/GO/SPCE for 100 µg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different concentration of Sb(III) (0 – 1000 µg L^{-1})

4.3.3 Effect of concentration of supporting electrolyte

There has been widely reported that HCl is a good supporting electrolyte for heavy metal analysis [29-31]. The optimization of concentration of HCl as supporting electrolyte was carried out at different concentration from 0.01 to 1.00 M. The results showed that signal of all analytes increased with increasing HCl concentration and slightly decreased after 0.50 M of HCl due to hydrogen evolution at highly negative potential (Fig. 4.9). The standard deviations for all heavy metal ions at 0.50 M HCl were small. Moreover, at this concentration, there is enough amount of HCl to be a supporting electrolyte and the electrode can be cleaned, therefore 0.50 M of HCl was selected as optimum concentration of supporting electrolyte solution.
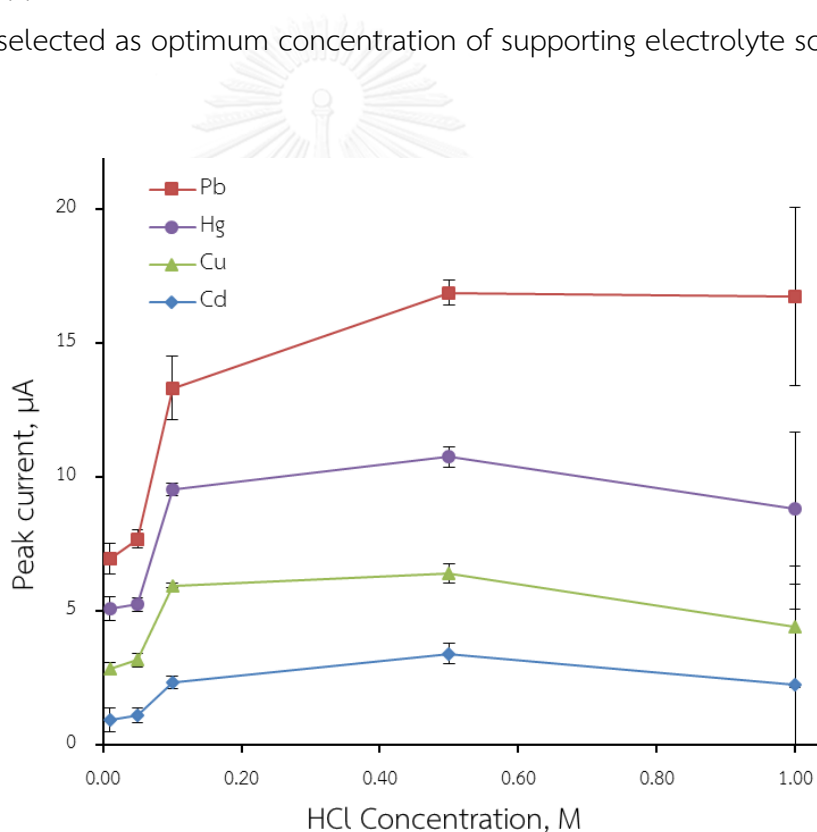
Figure 4.9 Effect of the concentration of HCl (supporting electrolyte) upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.
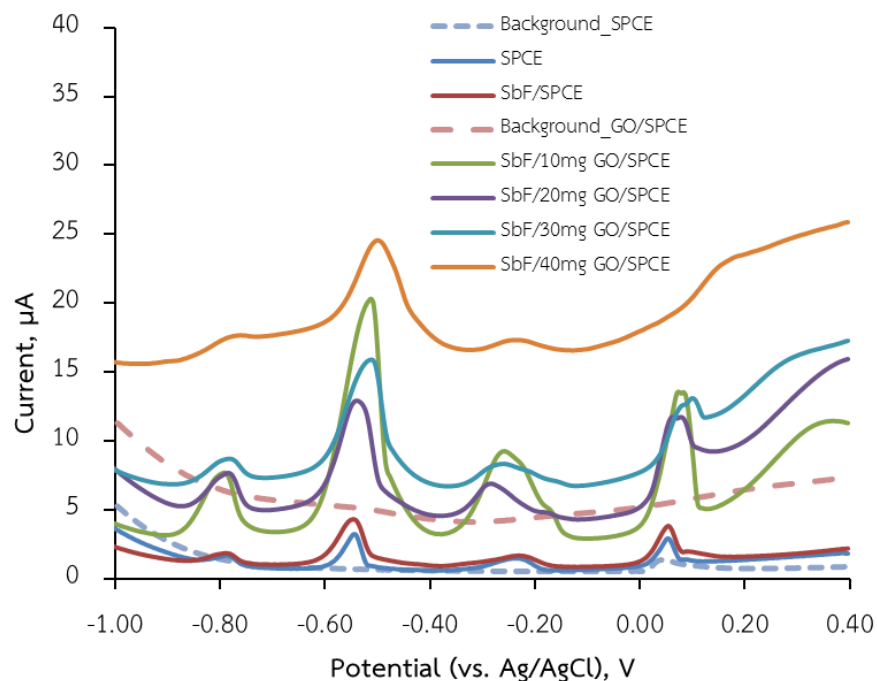
Figure 4.10 SIA-SWASV signals of the SbF/GO/SPCE for 100 μg L^{-1} of Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different type of supporting electrolyte.

4.4 Optimization of electrochemical parameters

In order to obtain the best detection and good reproducibility, SWASV parameters were studied including deposition potential, SW frequency, amplitude and increment potential. Thus, all conditions were investigated under previous optimized SbF-GO-SPCE and 0.50 M HCl as supporting electrolyte. To begin with, the effect of deposition potential was evaluated from -1.6 to -1.0 V with frequency of 50 Hz, amplitude of 40 mV and increment potential of 6 mV (Fig. 4.11). For all of heavy metal ions determination, the maximum anodic peak current was achieved with a deposition potential of -1.4 V vs. Ag/AgCl. For other deposition potential, the peak current were lower and poorer reproducibility (bigger standard deviation). Thus, -1.4 V vs. Ag/AgCl was selected as optimum deposition potential.

Figure 4.11   Effect of the deposition potential upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.

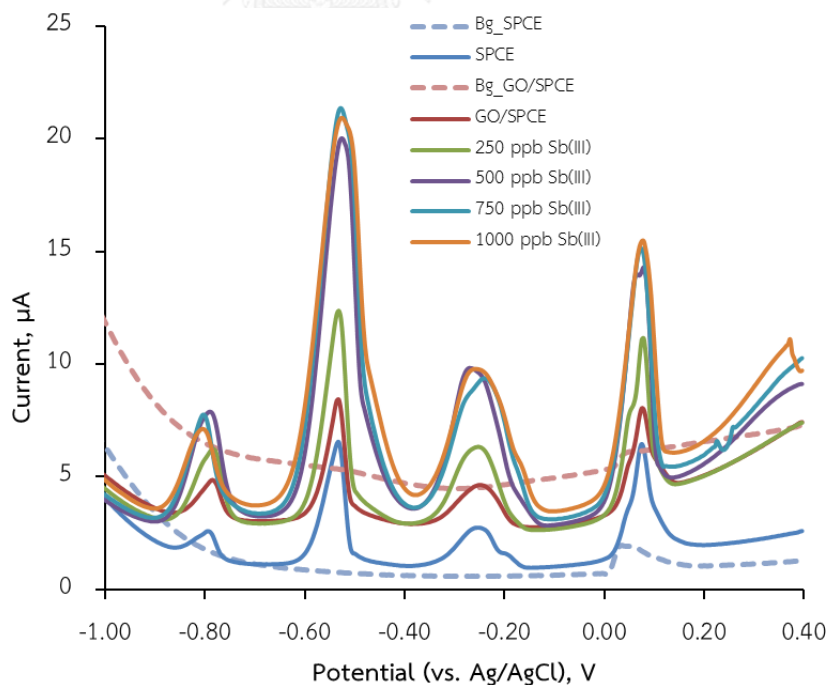**Figure 4.12**   SIA-SWASV signals of the SbF/GO/SPCE for 100 µg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different deposition potential (-1.0 to -1.6 V)

The frequency affected to the scan rate of determination system and was evaluated from 10 to 90 Hz at -1.4 V vs. Ag/AgCl as deposition potential, amplitude of 40 mV and increment potential of 4 mV (Fig. 4.13). The results showed that at 50 Hz high peak current with small standard deviation, well-separated peak shape for all heavy metal ions and fast determination were achieved. Therefore, optimum frequency of 50 Hz was selected.
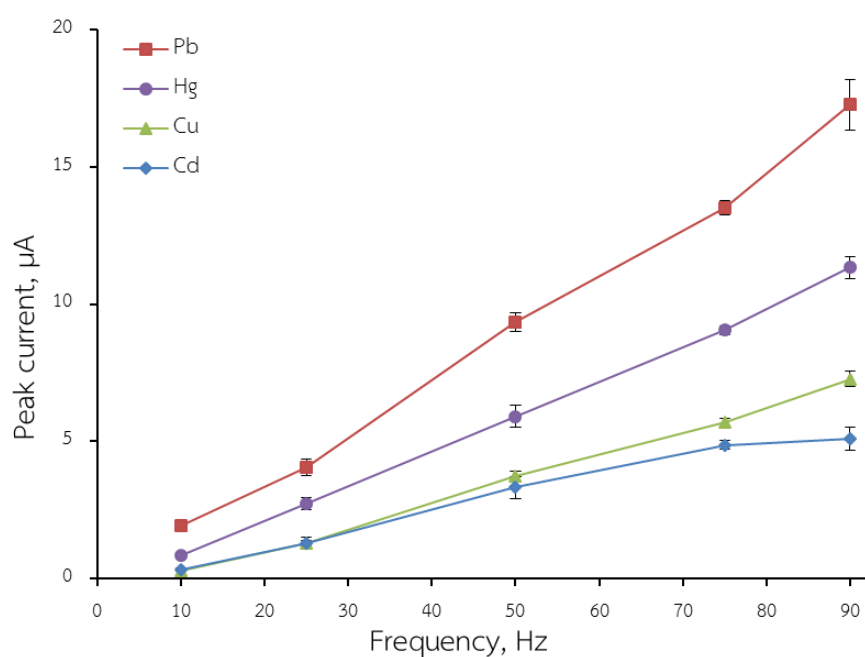
Figure 4.13 Effect of the SW frequency upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.
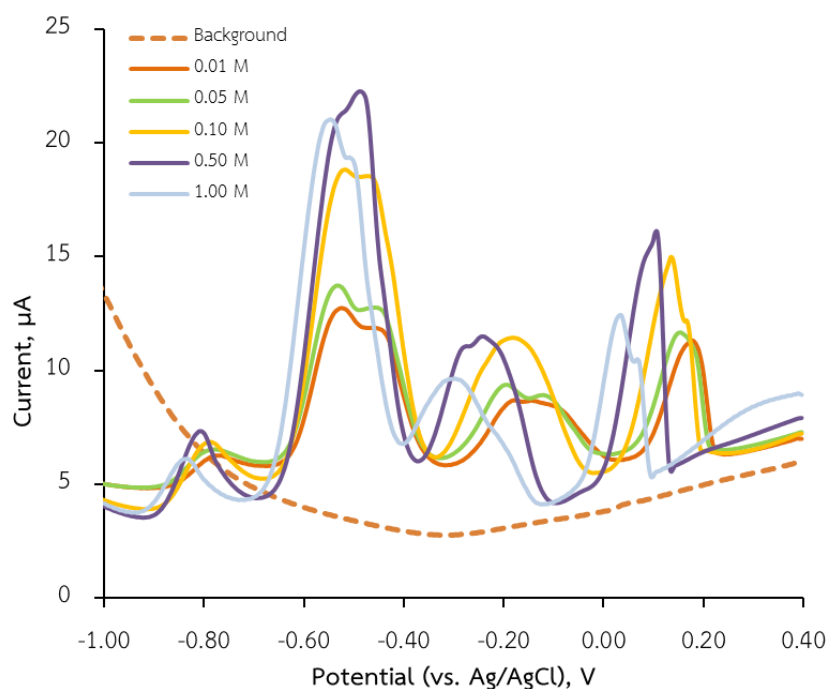
Figure 4.14   SIA-SWASV signals of the SbF/GO/SPCE for 100 μg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different frequency (10 – 90 Hz)

Next, SW amplitude was studied from 20 to 100 mV, with -1.4 V vs. Ag/AgCl as deposition potential, frequency of 50 Hz and increment potential of 4 mV (Fig. 4.15). The signals of all heavy metals were steadily increased from 20 to 80 mV and after 80 mV, signals were started to decrease. Increasing of amplitude also increased all signals for both background and analytes therefore the shape of peaks should be considered. For this reason, amplitude of 40 mV was selected.
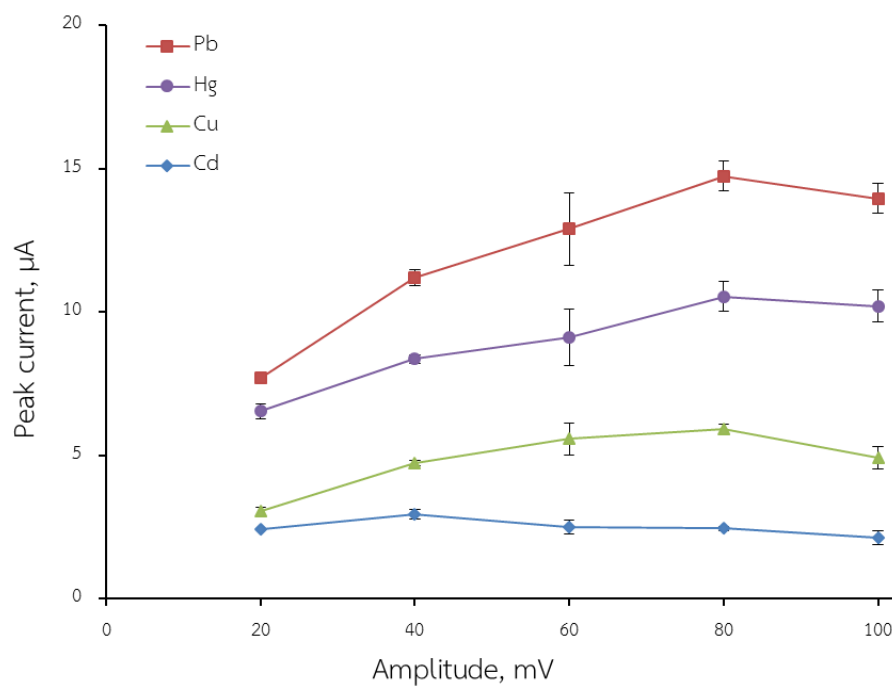
Figure 4.15   Effect of the amplitude upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.

Figure 4.16   SIA-SWASV signals of the SbF/GO/SPCE for 100 µg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different amplitude (20 – 100 mV)

Finally, the influence of increment potential was investigated from 2 to 10 mV, with deposition potential of -1.4 V vs. Ag/AgCl, frequency of 50 Hz and amplitude of 40 mV (Fig. 4.17). The increasing of increment potential would lower the number of points of measurement graph (lower precision of determined peak current position). However, at high increment potential, the signal obtained was high thus the peak current and number of data point must be considered. As a result, increment potential of 6 mV shown enough point for forming the graph and small standard deviation of peak for all heavy metal ions was selected.
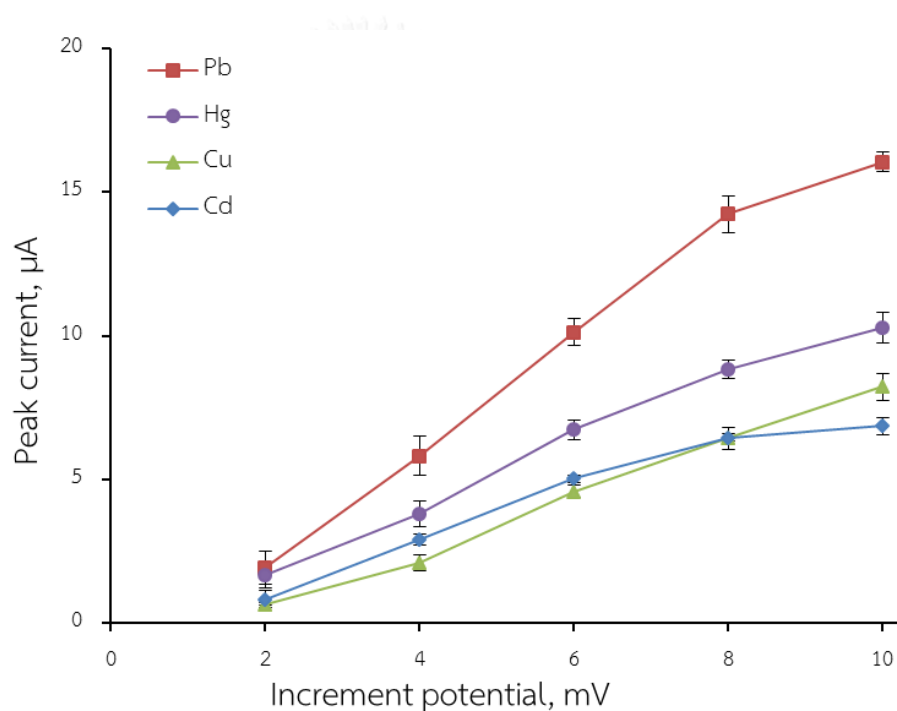
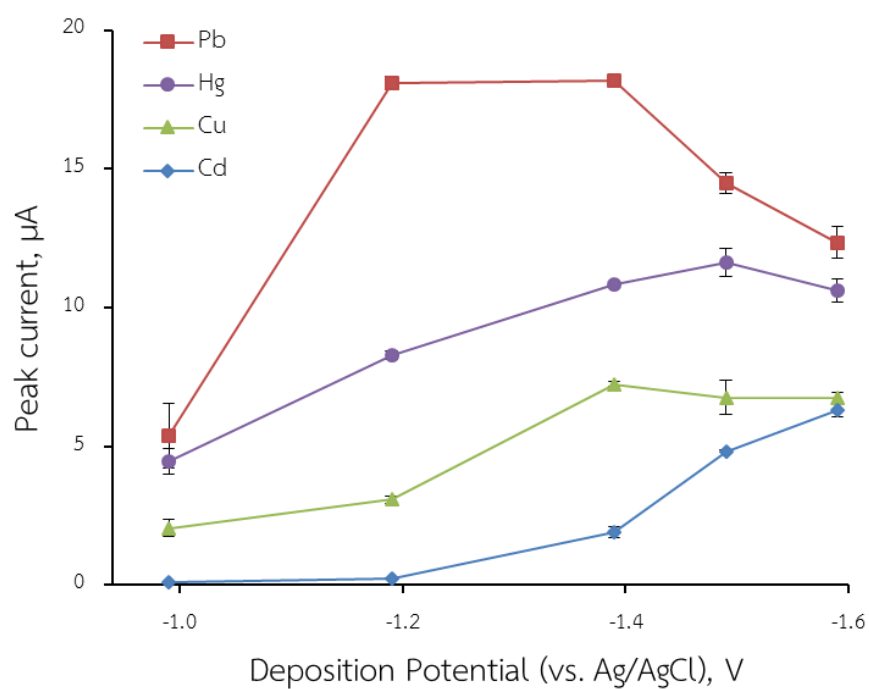**Figure 4.17**   Effect of the increment potential upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.
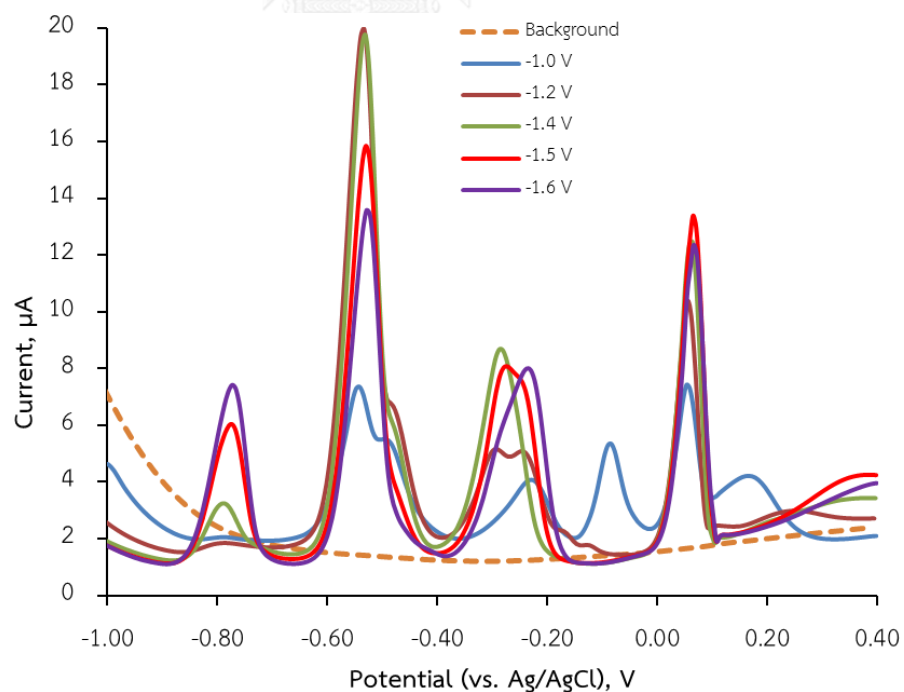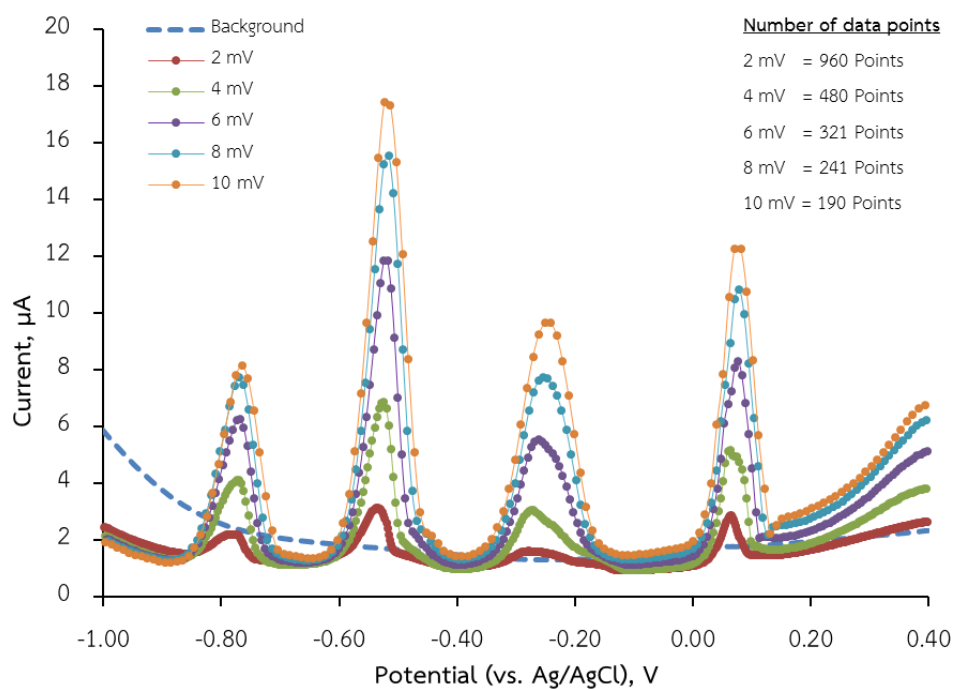
Figure 4.18   SIA-SWASV signals of the SbF/GO/SPCE for 100 µg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different increment potential (2 – 10 mV)

4.5 Optimization of SIA operation parameters

The other factors that influence the sensitivity and analysis time of each SWASV detection in flow-based analysis are sample volume and flow rate of the solution in the electrodeposition step. These parameters are related to deposition time, changing the sample volume and/or the flow rate will affect to the deposition time. Hence, the sample volume was set at 1.0 mL. The effect of flow rate was studied in the range of 5 to 40 µL s^{-1} and at different deposition times (Fig 4.19). It can be seen that the peak current decreased when the flow rate was increased. The highest signal was obtained at 5 µL s^{-1} however at that speed the analysis was time-consuming and had poor reproducibility. Thus, the flow rate of 10 µL s^{-1} preformed high current, smaller standard deviation for all heavy metal ions and shorter analysis time was selected.
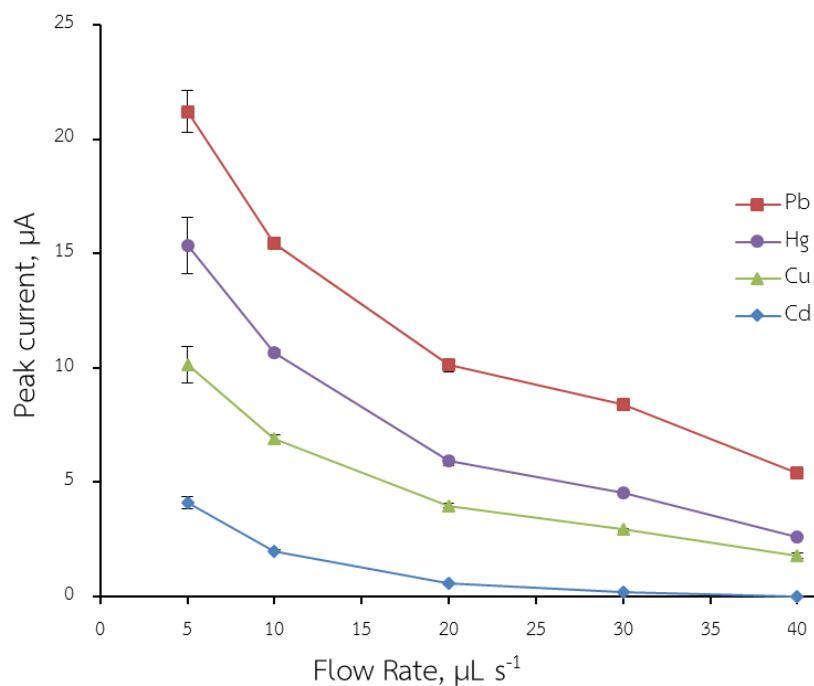
Figure 4.19 Effect of the flow rate during deposition upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.
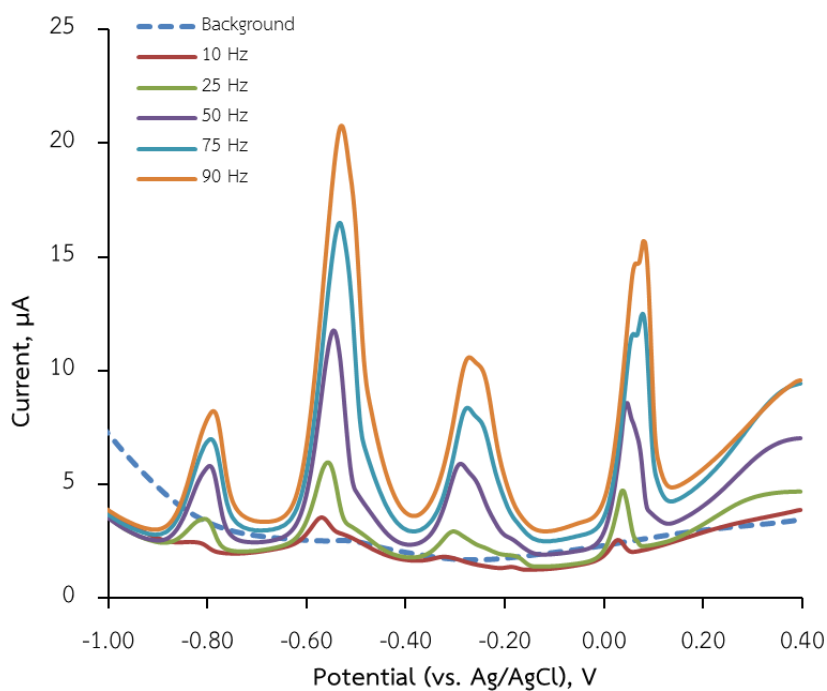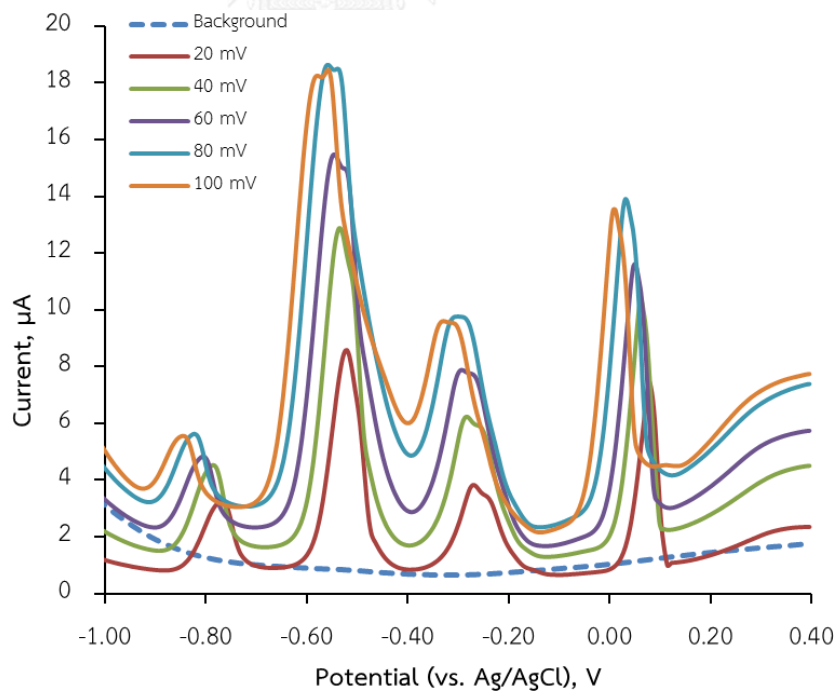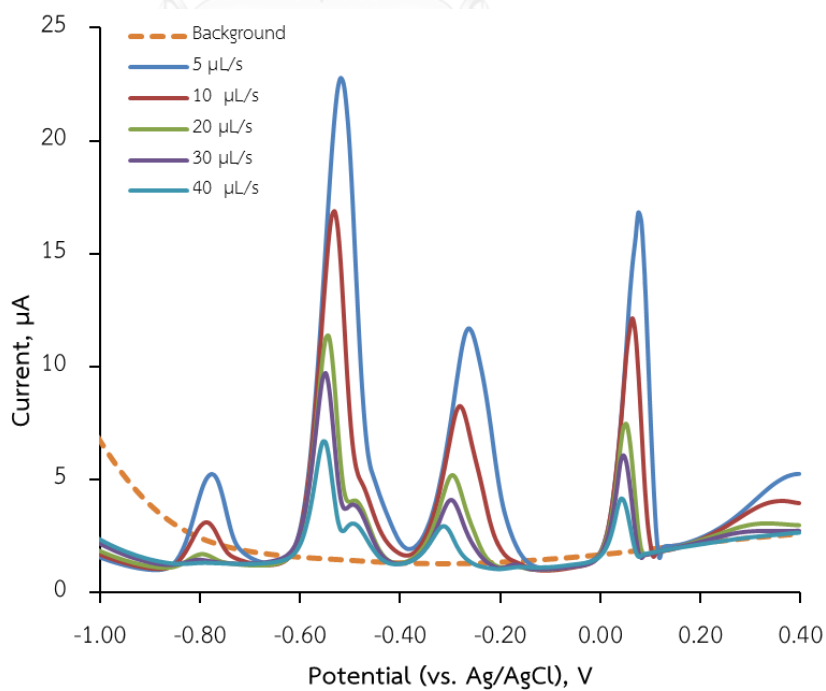

Figure 4.20 SIA-SWASV signals of the SbF/GO/SPCE for 100 µg L^{-1} Cd(II), Pb(II), Cu(II) and Hg(II) on 0.5M HCl at different flow rate during deposition (5 - 40 µL s^{-1})

After finish each analysis, the Sb film and all deposited metal was cleaned by flowing 0.5 M HCl which was the supporting electrolyte. To reduce waste, cleaning solution volume was investigated. The results showed that at 300 µL of 0.5 M HCl a small peak at around +0.05 V was still observed, whereas 500 µL of 0.5 M HCl can clean the electrode surface for all heavy metal ions (Fig. 4.21). Therefore, 500 µL of 0.5 M HCl was selected as optimum volume of cleaning solution.
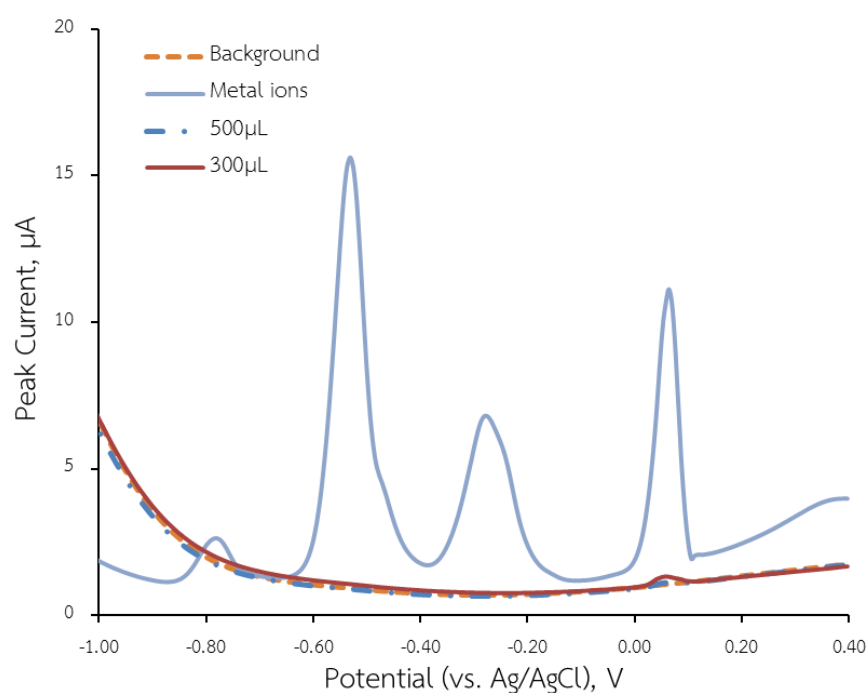
Figure 4.21   Effect of the 0.5 M HCl at 500 µL (dash dot line) and 300 µL (solid line) of cleaning solution upon the stripping voltammetric response at 100 µg L^{-1} of Pb(II), Cd(II), Cu(II), and Hg(II) using SbF/GO/SPCE.

4.6 Analytical performance

Under the optimized conditions, the calibration data for the simultaneous detection of the heavy metal ions was studied in the concentration range of 0.1 to 1.5 µM (Fig. 4.22). The peak currents were found to be directly proportional to the concentration of all analytes, with good linear regression correlation coefficients (R^2) of 0.996. The limit of detection (LOD) and limit of quantification (LOQ) were achieved

from 3σ/S and 10σ/S, where **σ** is the standard deviation of the blank measurement (n=10) and S is the slope of calibration curve. Summary of analytical data are presented in Table 4.1.

Figure 4.22   SIA-SWASV signals on the SbF/GO/SPCE of different concentrations of Cd(II), Pb(II), Cu(II) and Hg(II) in 0.5 M HCl, simultaneously. Inset are the calibration plots of signal and the heavy metal concentration.

Table 4.1   The analytical performance of simultaneous determination of heavy metal ions by SIA-SWASV using SbF-GO-SPCE under optimized conditions.

Metal ion	Linear dynamic range (µM)	R^2	LOD (µM)	LOQ (µM)
Cd(II)	0.3-1.5	0.9974	0.054	0.178
Pb(II)	0.1-1.3	0.9971	0.026	0.087
Cu(II)	0.3-1.5	0.9969	0.060	0.202
Hg(II)	0.1-1.3	0.9983	0.066	0.222

Moreover, under the same optimal conditions, each of heavy metal was analyzed separately. The relationship of anodic peak currents and the concentration was obtained (Fig. 4.23). Analytical characteristics for individual determination of heavy metal ions are summarized in Table 4.2. Under the same condition, it can be seen that LOD of Cd(II), Pb(II) and Cu(II) for individual detection were better when compared to simultaneous detection, however, the LOD of Hg(II) under simultaneous detection was better than that of individual detection. This phenomena could be explained by the intermetallic compounds [41-45] formed among the four target heavy metal ions and the competition for the limited area of active site on the modified electrode, although, the exactly reason for how these ions influence on each other is remain vague at present. Moreover, the activity of each metal is different from each other, thus single determination of Hg(II) was poorer performance because mercury can formed intermetallic compound with the other heavy metal ions and commonly used to improve the sensitivity in heavy metal ions detection. Absent of other metal ions caused the lower sensitivity of Hg(II) as well. Nevertheless, the separation between the anodic stripping peaks is big enough, hence the simultaneous or selective detection using SbF-GO-SPCE is practicable.
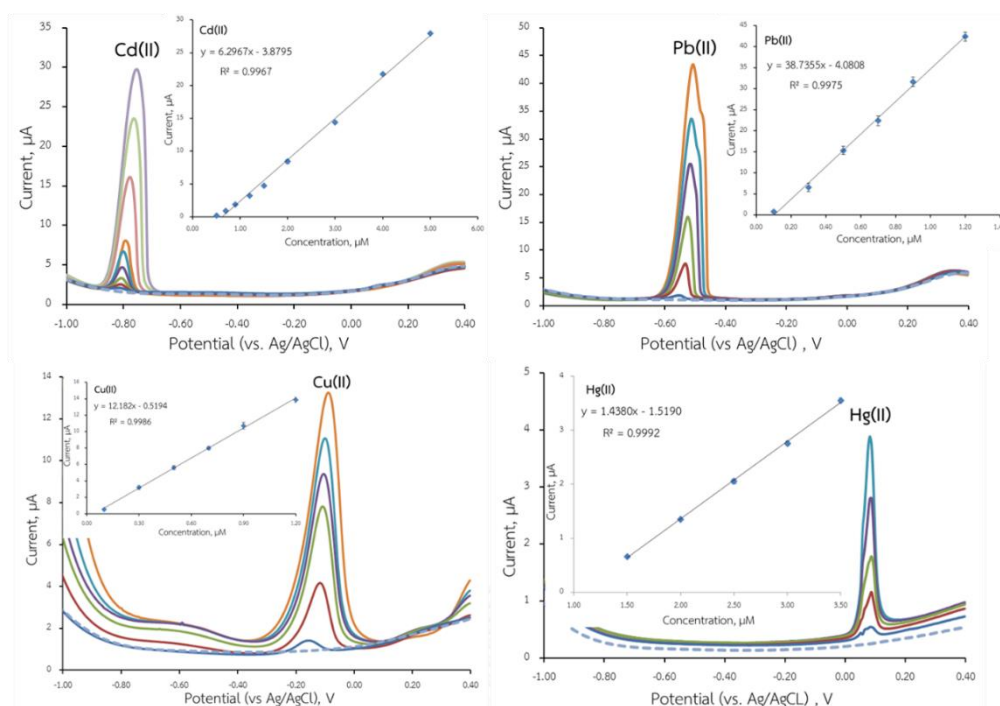
Figure 4.23   SIA-SWASV signals on the SbF/GO/SPCE of different concentrations of Cd(II), Pb(II), Cu(II) and Hg(II) in 0.5 M HCl, individually. Inset is the calibration plot of signal and the heavy metal concentration.
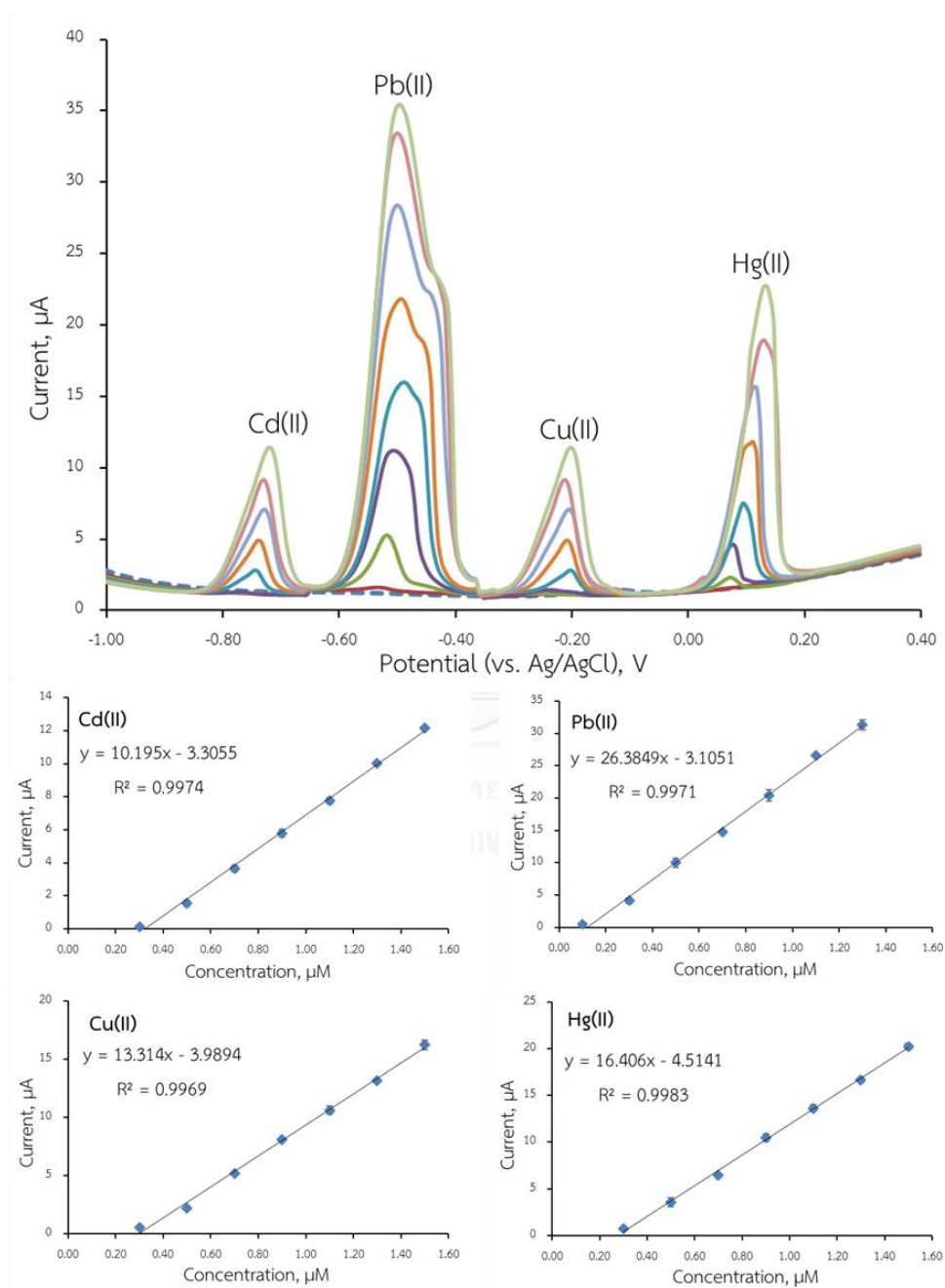
Table 4.2   The analytical performance of individual determination of heavy metal ions by SIA-SWASV using SbF-GO-SPCE under optimized conditions.

Metal ion	Linear dynamic range (µM)	R^2	LOD (µM)	LOQ (µM)
Cd(II)	0.5-5.0	0.9967	0.044	0.146
Pb(II)	0.1-1.2	0.9975	0.003	0.012
Cu(II)	0.1-1.2	0.9986	0.029	0.097
Hg(II)	1.5-3.5	0.9992	0.952	3.174

Even though the sensitivity of this modified electrode was not the best when compared with some work reported before, SbF-GO-SPCE can be easily fabricated and provided simultaneous analysis of four analytes. Besides, SbF-GO-SPCE could use repeatedly with renewable of electrode surface between successive detection and can also be used for a long total experimental time.

4.7   Interference study

To analyze the target heavy metal ions in real water sample, various ions in such water can be found. Hence, the interference of many cations (Na^+, K^+, Mg^{2+}, Ca^{2+} and Al^{3+}) and some of anions (F^-, Br^-, NO_3^-, HCO_3^-, CO_3^{2-} and SO_4^{2-}) that might be found in sewage and sea water were investigated. Their concentrations were 100-fold higher than analyte concentrations. The recovery of all target metal ions was in the range of 95.0 – 105.0% which was acceptable according to Guidelines for Standard Method Performance Requirements, AOAC official methods of analysis [46]. The results showed that none of the selected ions were considered interfered with the voltammograms of the solution of all heavy metal ions.

4.8   Analytical application

To evaluate the proposed method, this system was applied under optimized conditions for the simultaneous quantification of all target ions in sewage, fertilizer waste and sea water. All sample solutions were prepared by mixed 50 µL of each filtered water sample with 0.5 M HCl which was a supporting electrolyte to final volume of 10 mL. Prepared samples were introduced to determination system without any further pretreatment.

For sewage and fertilizer waste from a metal-plating factory in Thailand, preliminary testing found that only Cu(II) was detected. Thus, standard addition method was used to quantify concentration of copper solely. The average concentration of Cu(II) in sewage and fertilizer were found to be 38.02 µM and 68.70 µM, respectively (Fig. 4.24). In addition, the calculated standard deviations were relatively small, which suggested an acceptable level of reproducibility. To validate the proposed method, the results were compared with standard method obtained by AAS. A paired t-test with degrees of freedom of 4 was achieved on the data obtained. The calculated t-values between the two pairs of methods were 0.8369 for sewage and 0.2466 for fertilizer waste. Statistical analysis shown that the t-value for 4 degree of freedom at 95% confidence interval (2.7764) was greatly larger than the above-

mentioned experimental t-values. The results indicated that there were no significant difference between two methodologies for the heavy metals determination. It can be concluded that the purposed method is successful and suggesting that these results are reliable and acceptable.
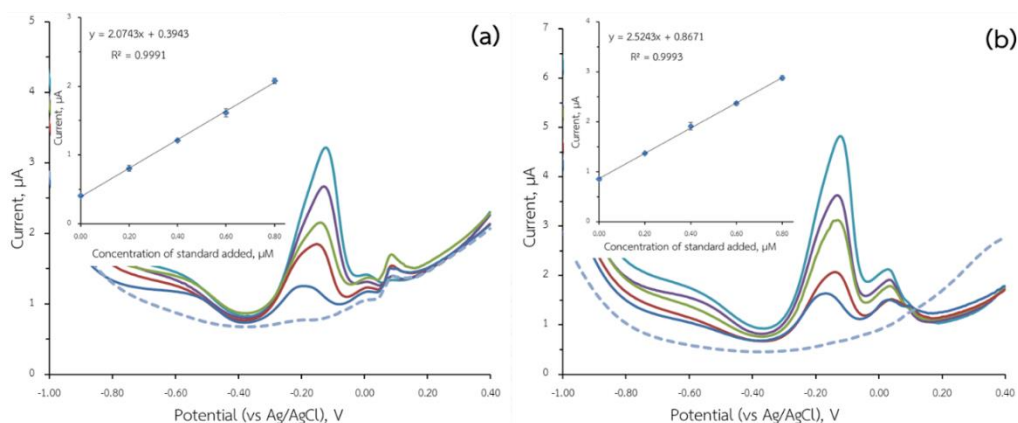
Figure 4.24   SIA-SWASV signals on the SbF/GO/SPCE at different concentrations of Cu(II) standard added sewage sample (a) and fertilizer waste sample (b) in 0.5 M HCl. Inset is the standard addition calibration plot of signal and the heavy metal concentration.

For sea water, the concentration of target ions was studied by calibration method without pretreatment. No anodic peak current of heavy metal ions was obtained in the unspiked sea water sample. The average concentrations of analytes in spiked samples were detected to be slightly higher than expected amount, with a 94.3 – 113 % recovery when spiked at 0.30, 0.50, 1.00 µM (Fig. 4.25). However, the reproducibility represented by standard deviations was reliable, which suggested by relatively low standard deviation.
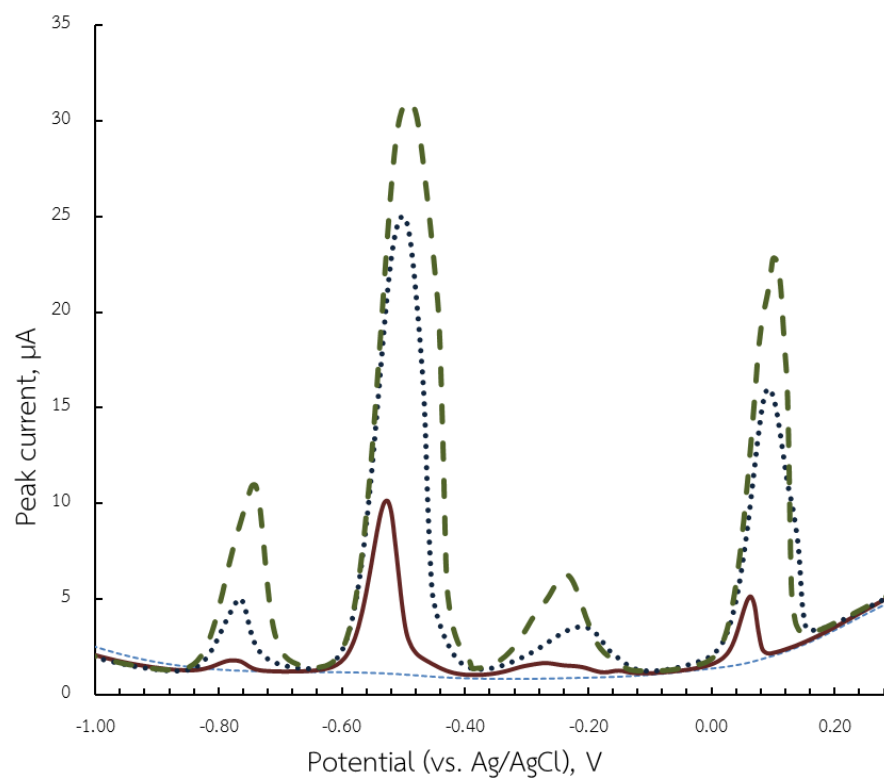
Figure 4.25   SIA-SWASV signals on the SbF/GO/SPCE of spiked sea water samples at 0.30 µM (solid line), 0.50 µM (dot line), 1.00 µM (dash line) of Cd(II), Pb(II), Cu(II) and Hg(II) in 0.5 M HCl.

CHAPTER V

CONCLUSIONS AND FUTURE WORK

5.1   Conclusions

In this study, we successfully developed a new electrochemical platform using in-situ antimony film and graphene oxide modified screen-printed electrode coupled with automated SIA-SWASV. The purposed system provides relatively low-cost, rapid, selective, and sensitive quantification method for Cd(II), Pb(II), Cu(II) and Hg(II) individually and simultaneously. This method can analyze 18 samples h^{-1} with high precision and small sample consumption over batch analysis system. Finally, it could be shown that applications of real water samples monitoring for heavy metal ion detection was achieved.

5.2   Future works

The SIA-SWASV system used in this research is highly interesting for on-site analysis since it is a compact size sensor platform comprising of both a sample preconcentration part and three electrode system for electrochemical detection in one set. Moreover, the screen-printed electrode also provided feasibility to fabricate in a mass production with relatively low cost and simplicity. From the advantages mentioned above, the electrochemical flow cell should be designed for highly sensitive detection of heavy metal ions by SWASV couple with an automated flow-based system. Sensitivity and selectivity of the SbF-GO-SPCE is another interesting issue. The further study about intermetallic compound should be done to confirm the effect of metallic bond to each other. Finally, all of the platform should be designed to generate as a device for on-site analysis. For example, combining SIA and electrochemical platform to each other and setting in a same place will increase portability performance for unskilled user.

REFERENCES

[1] S. Khazaeli, N. Nezamabadi, M. Rabani, H.A. Panahi, A new functionalized resin and its application in flame atomic absorption spectrophotometric determination of trace amounts of heavy metal ions after solid phase extraction in water samples, Microchemical Journal, 106 (2013) 147-153.

[2] M.A. Álvarez, G. Carrillo, Simultaneous determination of arsenic, cadmium, copper, chromium, nickel, lead and thallium in total digested sediment samples and available fractions by electrothermal atomization atomic absorption spectroscopy (ET AAS), Talanta, 97 (2012) 505-512.

[3] M.T. Naseri, M.R. Hosseini, Y. Assadi, A. Kiani, Rapid determination of lead in water samples by dispersive liquid-liquid microextraction coupled with electrothermal atomic absorption spectrometry, Talanta, 75 (2008) 56-62.

[4] C. Cui, M. He, B. Chen, B. Hu, Restricted accessed material-copper(II) ion imprinted polymer solid phase extraction combined with inductively coupled plasma-optical emission spectrometry for the determination of free Cu(II) in urine and serum samples, Talanta, 116 (2013) 1040-1046.

[5] A. Beiraghi, S. Babaee, M. Roshdi, Simultaneous preconcentration of cadmium, cobalt and nickel in water samples by cationic micellar precipitation and their determination by inductively coupled plasma-optical emission spectrometry, Microchemical Journal, 100 (2012) 66-71.

[6] L. Zhao, S. Zhong, K. Fang, Z. Qian, J. Chen, Determination of cadmium(II), cobalt(II), nickel(II), lead(II), zinc(II), and copper(II) in water samples using dual-cloud point extraction and inductively coupled plasma emission spectrometry, J Hazard Mater, 239-240 (2012) 206-212.

[7] C.Y. Tai, S.J. Jiang, A.C. Sahayam, Determination of As, Hg and Pb in herbs using slurry sampling flow injection chemical vapor generation inductively coupled plasma mass spectrometry, Food Chem, 192 (2016) 274-279.

[8] R.P. Lamsal, D. Beauchemin, Estimation of the bio-accessible fraction of Cr, As, Cd and Pb in locally available bread using on-line continuous leaching method

coupled to inductively coupled plasma mass spectrometry, Analytica Chimica Acta, 867 (2015) 9-17.

[9] Y. Wei, C. Gao, F.L. Meng, H.H. Li, L. Wang, J.H. Liu, X.J. Huang, SnO$_2$/Reduced Graphene Oxide Nanocomposite for the Simultaneous Electrochemical Detection of Cadmium(II), Lead(II), Copper(II), and Mercury(II): An Interesting Favorable Mutual Interference, The Journal of Physical Chemistry C, 116 (2012) 1034-1041.

[10] C. Gao, X.-Y. Yu, R.-X. Xu, J.-H. Liu, X.-J. Huang, AlOOH-Reduced Graphene Oxide Nanocomposites: One-Pot Hydrothermal Synthesis and Their Enhanced Electrochemical Activity for Heavy Metal Ions, ACS Applied Materials & Interfaces, 4 (2012) 4672-4682.

[11] U. Injang, P. Noyrod, W. Siangproh, W. Dungchai, S. Motomizu, O. Chailapakul, Determination of trace heavy metals in herbs by sequential injection analysis-anodic stripping voltammetry using screen-printed carbon nanotubes electrodes, Anal Chim Acta, 668 (2010) 54-60.

[12] N. Soltani, H. Haddadi, M. Asgari, N. Rajabzadeh, Adsorptive stripping voltammetric detection of thorium on the multi-walled carbon nanotube modified screen printed electrode, Sensors and Actuators B: Chemical, 220 (2015) 1212-1216.

[13] V. Sosa, C. Barcelo, N. Serrano, C. Arino, J.M. Diaz-Cruz, M. Esteban, Antimony film screen-printed carbon electrode for stripping analysis of Cd(II), Pb(II), and Cu(II) in natural samples, Anal Chim Acta, 855 (2015) 34-40.

[14] D. Riman, D. Jirovsky, J. Hrbac, M.I. Prodromidis, Green and facile electrode modification by spark discharge: Bismuth oxide-screen printed electrodes for the screening of ultra-trace Cd(II) and Pb(II), Electrochemistry Communications, 50 (2015) 20-23.

[15] M.Á.G. Rico, M. Olivares-Marín, E.P. Gil, Modification of carbon screen-printed electrodes by adsorption of chemically synthesized Bi nanoparticles for the voltammetric stripping detection of Zn(II), Cd(II) and Pb(II), Talanta, 80 (2009) 631-635.

[16] V. Sosa, N. Serrano, C. Ariño, J.M. Díaz-Cruz, M. Esteban, Sputtered bismuth screen-printed electrode: A promising alternative to other bismuth modifications

in the voltammetric determination of Cd(II) and Pb(II) ions in groundwater, Talanta, 119 (2014) 348-352.

[17] A.K. Geim, K.S. Novoselov, The rise of graphene, Nat Mater, 6 (2007) 183-191.

[18] V.K. Gupta, M.L. Yola, N. Atar, Z. Ustundağ, A.O. Solak, A novel sensitive Cu(II) and Cd(II) nanosensor platform: Graphene oxide terminated p-aminophenyl modified glassy carbon surface, Electrochimica Acta, 112 (2013) 541-548.

[19] W.K. Chee, H.N. Lim, I. Harrison, K.F. Chong, Z. Zainal, C.H. Ng, N.M. Huang, Performance of Flexible and Binderless Polypyrrole/Graphene Oxide/Zinc Oxide Supercapacitor Electrode in a Symmetrical Two-Electrode Configuration, Electrochimica Acta, 157 (2015) 88-94.

[20] M.R. Pourjavid, A.A. Sehat, M. Arabieh, S.R. Yousefi, M.H. Hosseini, M. Rezaee, Column solid phase extraction and flame atomic absorption spectrometric determination of manganese(II) and iron(III) ions in water, food and biological samples using 3-(1-methyl-1H-pyrrol-2-yl)-1H-pyrazole-5-carboxylic acid on synthesized graphene oxide, Mater Sci Eng C Mater Biol Appl, 35 (2014) 370-378.

[21] A.U. Chaudhry, V. Mittal, B. Mishra, Effect of graphene oxide nanoplatelets on electrochemical properties of steel substrate in saline media, Materials Chemistry and Physics, 163 (2015) 130-137.

[22] G.H. Hwang, W.K. Han, J.S. Park, S.G. Kang, Determination of trace metals by anodic stripping voltammetry using a bismuth-modified carbon nanotube electrode, Talanta, 76 (2008) 301-308.

[23] V. Arancibia, E. Nagles, C. Rojas, M. Gómez, Ex situ prepared nafion-coated antimony film electrode for adsorptive stripping voltammetry of model metal ions in the presence of pyrogallol red, Sensors and Actuators B: Chemical, 182 (2013) 368-373.

[24] H. Chang, L. Tang, Y. Wang, J. Jiang, J. Li, Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection, Anal Chem, 82 (2010) 2341-2346.

[25] M. Nasrollahzadeh, F. Babaei, P. Fakhri, B. Jaleh, Synthesis, characterization, structural, optical properties and catalytic activity of reduced graphene oxide/copper nanocomposites, RSC Advances, 5 (2015) 10782-10789.

[26] E. Svobodova-Tesarova, L. Baldrianova, M. Stoces, I. Svancara, K. Vytras, S.B. Hocevar, B. Ogorevc, Antimony powder-modified carbon paste electrodes for electrochemical stripping determination of trace heavy metals, Electrochimica Acta, 56 (2011) 6673-6677.

[27] A.M. Ashrafi, S. Cerovac, S. Mudrić, V. Guzsvány, L. Husáková, I. Urbanová, K. Vytřas, Antimony nanoparticle-multiwalled carbon nanotubes composite immobilized at carbon paste electrode for determination of trace heavy metals, Sensors and Actuators B: Chemical, 191 (2014) 320-325.

[28] E. Tesarova, L. Baldrianova, S.B. Hocevar, I. Svancara, K. Vytras, B. Ogorevc, Anodic stripping voltammetric measurement of trace heavy metals at antimony film carbon paste electrode, Electrochimica Acta, 54 (2009) 1506-1510.

[29] V. Guzsvany, H. Nakajima, N. Soh, K. Nakano, T. Imato, Antimony-film electrode for the determination of trace metals by sequential-injection analysis/anodic stripping voltammetry, Anal Chim Acta, 658 (2010) 12-17.

[30] W. Wonsawat, S. Chuanuwatanakul, W. Dungchai, E. Punrat, S. Motomizu, O. Chailapakul, Graphene-carbon paste electrode for cadmium and lead ion monitoring in a flow-based system, Talanta, 100 (2012) 282-289.

[31] E. Punrat, S. Chuanuwatanakul, T. Kaneta, S. Motomizu, O. Chailapakul, Method development for the determination of arsenic by sequential injection/anodic stripping voltammetry using long-lasting gold-modified screen-printed carbon electrode, Talanta, 116 (2013) 1018-1025.

[32] W.S. Hummers, R.E. Offeman, Preparation of Graphitic Oxide, Journal of the American Chemical Society, 80 (1958) 1339-1339.

[33] K. Hamsawahini, P. Sathishkumar, R. Ahamad, A.R. Yusoff, A sensitive, selective and rapid determination of lead(II) ions in real-life samples using an electrochemically reduced graphene oxide-graphite reinforced carbon electrode, Talanta, 144 (2015) 969-976.

[34] K.Z. Setshedi, M. Bhaumik, M.S. Onyango, A. Maity, High-performance towards Cr(VI) removal using multi-active sites of polypyrrole–graphene oxide nanocomposites: Batch and column studies, Chemical Engineering Journal, 262 (2015) 921-931.

[35] S. Su, B. Chen, M. He, B. Hu, Graphene oxide-silica composite coating hollow fiber solid phase microextraction online coupled with inductively coupled plasma mass spectrometry for the determination of trace heavy metals in environmental water samples, Talanta, 123 (2014) 1-9.

[36] Rattana, S. Chaiyakun, N. Witit-anun, N. Nuntawong, P. Chindaudom, S. Oaew, C. Kedkeaw, P. Limsuwan, Preparation and characterization of graphene oxide nanosheets, Procedia Engineering, 32 (2012) 759-764.

[37] F.T. Thema, M.J. Moloto, E.D. Dikio, N.N. Nyangiwe, L. Kotsedi, M. Maaza, M. Khenfouch, Synthesis and Characterization of Graphene Thin Films by Chemical Reduction of Exfoliated and Intercalated Graphite Oxide, Journal of Chemistry, 2013 (2013) 1-6.

[38] S. Park, R.S. Ruoff, Chemical methods for the production of graphenes, Nat Nanotechnol, 4 (2009) 217-224.

[39] A.A. Balandin, S. Ghosh, W. Bao, I. Calizo, D. Teweldebrhan, F. Miao, C.N. Lau, Superior thermal conductivity of single-layer graphene, Nano Lett, 8 (2008) 902-907.

[40] R.F. Service, Materials science. Carbon sheets an atom thick give rise to graphene dreams, Science, 324 (2009) 875-877.

[41] J. Pei, M.L. Tercier-Waeber, J. Buffle, Simultaneous determination and speciation of zinc, cadmium, lead, and copper in natural water with minimum handling and artifacts, by voltammetry on a gel-integrated microelectrode array, Anal Chem, 72 (2000) 161-171.

[42] A.H.I. Ben-Bassat, A. Azrad, Intermetallic compounds formed in mixed (complex) amalgams—I. The systems: copper—mercury, zinc—mercury and copper—zinc—mercury, Electrochimica Acta, 23 (1978) 63-69.

[43] R.J. Grim, Catalytic Activity of an Intermetallic Compound of Cadmium and Copper in the Vapor-phase Reduction of Nitrobenzene, The Journal of Physical Chemistry, 46 (1942) 464-469.

[44] D.F. Tibbetts, J. Davis, R.G. Compton, Sonoelectroanalytical detection of lead at a bare copper electrode, Fresenius J Anal Chem, 368 (2000) 412-414.

[45] J. Schiewe, K.B. Oldham, J.C. Myland, A.M. Bond, V.A. Vicente-Beckett, S. Fletcher, Linear-scan anodic stripping voltammetry with thin-film electrodes: theory of the stripping stage and experimental tests, Analytical Chemistry, 69 (1997) 2673-2681.

[46] AOAC, AOAC official methods of analysis, Appendix F: Guidelines for Standard Method Performance Requirements, 2012.

APPENDIX

Table A1 Two-tailed Student t-values

Degrees of Freedom	Confidence Interval			
	90% $\alpha = 0.10$	95% $\alpha = 0.05$	99% $\alpha = 0.01$	99.9% $\alpha = 0.001$
1	6.31	12.7	63.7	637
2	2.92	4.30	9.92	31.6
3	2.35	3.18	5.84	12.9
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96
7	1.89	2.36	3.50	5.41
8	1.86	2.31	3.36	5.04
9	1.83	2.26	3.25	4.78
10	1.81	2.23	3.17	4.59
11	1.80	2.20	3.11	4.44
12	1.78	2.18	3.05	4.32
14	1.76	2.14	2.98	4.14
16	1.75	2.12	2.92	4.01
18	1.73	2.10	2.88	3.92
20	1.72	2.09	2.85	3.85
30	1.70	2.04	2.75	3.65
50	1.68	2.01	2.68	3.50
∞	1.64	1.96	2.58	3.29

Hibbert, D. Brynn., Gooding, J. Justin. <u>Data analysis for chemistry: an introductory guide for students and laboratory scientists</u>. Oxford University Press, Inc., 2006

VITA

Miss Prasongporn Ruengpirasiri was born on December 11, 1990 in Bangkok, Thailand. She received her Bachelor's degree of Science (Chemistry) with first class honor from Chulalongkorn University, Bangkok, Thailand in 2012. Next, she accordingly becomes a Master's degree of Science (Analytical Chemistry) of academic year 2015 from Chulalongkorn University.

Proceeding:

Prasongporn Ruengpirasiri, Orawon Chailapakul, Suchada Chuanuwatanakul, "Method development for simultaneous determination of trace heavy metals by sequential injection-anodic stripping voltammetry using antimony-graphene oxide modified electrode", Proceeding of the 5th Asian Symposium on Advanced Materials: Chemistry, Physics & Biomedicine of Functional and Novel Materials (ASAM-5), Pusan National University, Busan, South Korea, November 1-4, 2015, pp 190-194.

Scholarship:

She has got a scholarship from Science Achievement Scholarship of Thailand in 2009 – 2014. In addition, she was received Japan Student Services Organization Student Exchange Support Program (JASSO) for short time research at Japan Advanced Institute of Science and Technology (JAIST) in December 2014 to February 2015. Moreover, the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) was granted for research fund in 2014 – 2015.

Award:

2nd place poster presentation "Method development for simultaneous determination of trace heavy metals by sequential injection-anodic stripping voltammetry using antimony-graphene oxide modified electrode" – ASAM-5: the 5th Asian Symposium on Advanced Materials: Chemistry, Physics & Biomedicine of Functional and Novel Materials (ASAM-5), Pusan National University, Busan, South Korea, November 1-4, 2015