Determination of Lead (II) and Cadmium (II) in Water Lily Stems by Anodic Stripping Voltammetry using a Bismuth Film Electrode Modified with Mesoporous Silica Nanoparticles and Chitosan

Monthira Somkid

A Thesis Submitted in Partial Fulfillment of the Requirements for
the Degree of Master of Science in Chemistry (Analytical Chemistry)
Prince of Songkla University
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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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Candidate

I hereby certify that this work has not been accepted in substance for any other degree, and is not being currently submitted in candidature for any degree.

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(Miss Monthira Somkid)
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ชื่อวิทยานิพนธ์	การวิเคราะห์หาปริมาณตะกั่วและแคดเมียมในสายบัว ด้วยวิธีแอโนดิกสทริปปิงโวลแทมเมตรี โดยใช้ขั้วไฟฟ้าฟิล์มบิสมัทปรับแต่งด้วยอนุภาคนาโนซิลิกาและไคโตซาน
ผู้เขียน	นางสาวมลธิรา สมคิด
สาขาวิชา	เคมีวิเคราะห์
ปีการศึกษา	2560

บทคัดย่อ

ตะกั่วและแคดเมียมในสิ่งแวดล้อม มีความเป็นพิษต่อสิ่งมีชีวิตแม้จะได้รับเพียงปริมาณเล็กน้อย ดังนั้น การวิเคราะห์หาปริมาณตะกั่วและแคดเมียมที่มีอยู่ในธรรมชาติ จึงเป็นสิ่งสำคัญที่จะนำไปสู่การประเมินคุณภาพด้านสิ่งแวดล้อมและด้านสุขภาพ

ในงานวิจัยนี้ ได้พัฒนาวิธีการเตรียมขั้วไฟฟ้าบิสมัทแบบ ex-situ บนขั้วไฟฟ้ากลาสสิคาร์บอนปรับแต่งด้วยนาโนซิลิกา รูพรุนขนาดเมโซ (2- 50 นาโนเมตร) ที่ถูกตรึงด้วยไคโตซาน โดยใช้เป็นขั้วไฟฟ้าทำงานในการวิเคราะห์หาปริมาณของตะกั่วและแคดเมียมในตัวอย่างสายบัว ด้วยวิธีแอโนดิกสทริปปิงโวลแทมเมตรี ขั้วไฟฟ้านี้มีความเป็นมิตรต่อสิ่งแวดล้อมมากกว่าขั้วปรอทที่นิยมใช้กัน นอกจากนี้การเลือกใช้ไคโตซานเป็นสารตรึงนาโนซิลิกาบนผิวขั้วไฟฟ้านั้นมีราคาถูกกว่าแนฟฟิออน ทำการตรวจหาปริมาณของโลหะด้วยเทคนิคดิฟเฟอร์เรนท์เชียลพัลส์โวลแทมเมตรี ภายใต้สภาวะที่เหมาะสม ประกอบด้วย ศักย์ไฟฟ้าและเวลาที่ก่อให้เกิดการพอกพูนด้วยไฟฟ้าของฟิล์มบิสมัท ความเข้มข้นของไคโตซาน ความเป็นกรด-เบสของ 0.1 โมลาร์ กรดไฮโดรคลอริก/โพแทสเซียมคลอไรด์ ศักย์ไฟฟ้าและเวลาที่ก่อให้เกิดการพอกพูนด้วยไฟฟ้าของตะกั่วและแคดเมียม เวลาในการปรับสมดุล พัลส์แอมปลิจูด และอัตราการสแกน

ภายใต้สภาวะที่เหมาะสมจากการทดลองได้กราฟเป็นเส้นตรงในช่วง 2 – 40 ไมโครกรัมต่อลิตร ด้วยค่าสหสัมพันธ์ของเส้นตรงของตะกั่วและแคดเมียม (R^2) เท่ากับ 0.998 เมื่อใช้เวลาที่ก่อให้เกิดการพอกพูนด้วยไฟฟ้านาน 240 วินาที ขีดจำกัดต่ำสุดของการตรวจวัดและขีดจำกัดต่ำสุดของการหาปริมาณของตะกั่วและแคดเมียม เท่ากับ 0.3 และ 1.1 ไมโครกรัมต่อลิตร และ 0.1 และ 0.4 ไมโครกรัมต่อลิตร ตามลำดับ ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ สำหรับ 20 ไมโครกรัมต่อลิตรของตะกั่วและแคดเมียม เท่ากับ 2.26 and 1.71 เปอร์เซ็นต์ ตามลำดับ วิธีนี้ให้ค่าร้อยละการได้กลับคืนของการวิเคราะห์โลหะที่ช่วง 93.3 –101.9 สำหรับตะกั่ว และ 99.3 – 103.7 สำหรับแคดเมียม ศึกษาการรบกวนของไอออนบวกที่ส่งผลต่อการตรวจวัดตะกั่วและ

แคดเมียม ได้แก่ แคลเซียม ทองแดง แมกนีเซียม สังกะสี นิกเกิล ปรอท และเหล็ก ผลการศึกษา พบว่าทองแดงทำให้สัญญาณการตรวจวัดตะกั่วและแคดเมียมลดลง ซึ่งสามารถแก้ปัญหาดังกล่าวด้วยการเติม 0.01 มิลลิโมลาร์ เฟอโรไซยาไนด์ลงในสารละลายที่ทำการวิเคราะห์

เมื่อนำวิธีที่ศึกษานี้ไปวิเคราะห์หาปริมาณของตะกั่วและแคดเมียมในตัวอย่างสายบัว บริเวณพื้นที่ทะเลน้อย พบว่าปริมาณตะกั่วและแคดเมียมที่ตรวจพบ อยู่ที่ 0.00-0.29 มิลลิกรัมต่อกิโลกรัม และ 0.00-0.10 มิลลิกรัมต่อกิโลกรัม ตามลำดับ ซึ่งปริมาณดังกล่าวต่ำกว่าค่ามาตรฐานที่กำหนดไว้โดยองค์การอนามัยโลก ที่กำหนดให้ปริมาณของตะกั่วและแคดเมียมที่พบในส่วนที่รับประทานได้ของพืช ไม่เกิน 0.5 มิลลิกรัมต่อกิโลกรัม สำหรับตะกั่ว และ 0.2 มิลลิกรัมต่อกิโลกรัม สำหรับแคดเมียม และเมื่อเปรียบเทียบผลการวิเคราะห์จากวิธีที่ศึกษากับเทคนิคอินดักทีพลีคัพเพิลพลาสมา ออฟติคอลอีมิสชันสเปกโทรเมทรี ด้วยวิธีการทดสอบความแม่น T-test พบว่าทั้งสองวิธีได้ผลการวิเคราะห์ไม่แตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่น 95% ได้ผลการวิเคราะห์ที่คล้ายคลึงกัน

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Major Program	Analytical Chemistry
Academic Year	2017

ABSTRACT

Lead and cadmium in the environment can be toxic to human beings, even at very low concentration. Therefore, quantitative determination of Pb(II) and Cd(II) is the great importance for quality of environmental and also health assessment.

In this work, the preparation of ex-situ bismuth film on glassy carbon electrode modified with mesoporous silica nanoparticles (2 - 50 nm) and chitosan used as a working electrode was developed and evaluated for reliable quantification of Pb(II) and Cd(II) by anodic stripping voltammetry in water lily stem samples. This electrode is more environmentally friendly than the commonly used mercury working electrode. Moreover, the selected chitosan as strong adhesion reagent for silica nanoparticles coating is cheaper than Nafion. The resulting accumulated metals were stripped off by differential pulse anodic stripping voltammetry (DPASV) with optimized conditions of plating potential and plating time of bismuth film, concentration of chitosan, pH of 0.1 M HCl/KCl, deposition potential and deposition time of Pb(II) and Cd(II) detection, equilibration time, pulse amplitude and step potential.

Under the optimum condition, the linear ranges were 2 – 40 μg/L for with the linear correlation coefficient (R^2) of 0.998 for both Pb(II) and Cd(II) for 240 s deposition time. The limit of detection (LOD) and limits of quantification (LOQ) were obtained of 0.3 μg/L and 1.1 μg/L for Pb(II) and 0.1 μg/L and 0.4 μg/L for Cd(II), respectively. Relative standard deviations (%RSD) of 20 μg/L Pb(II) and Cd(II) determinations obtained from this method were 2.26 and 1.71 %, respectively.

The method has percentage recoveries for the determination of those metals in range of 93.3 –101.9 for Pb(II) and 99.3 –103.7 for Cd(II). Effect of interferences caused by cations commonly found in water lily, especially, Ca(II), Cu(II), Mg(II), Zn(II), Ni(II), Hg(II) and Fe(III) levels were investigated. Cu(II) interference in this method, which could improve the stripping peak signal of Pb(II) and Cd(II) and eliminate Cu(II), 0.01 mM of ferrocyanide added to the solution.

This studied method was applied to determine Pb(II) and Cd(II) in water lily stem samples in Thale Noi were found in range of 0.00-0.29 mg/kg and 0.00-0.10 mg/kg, respectively, which lower than the guideline values of edible portions of plants (0.5 mg/kg for Pb(II) and 0.2 mg/kg for Cd(II)) given by the World Health Organization (WHO). This method was validated to the determine the Pb(II) and Cd(II) with ICP-OES and the results were tested by accuracy (t-Test). They were not significant difference at the confidence level of 95%.

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Monthira Somkid

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS = Atomic absorption spectroscopy
ASV = Anodic stripping voltammetry
BiFE = Bismuth film electrode (BiFE)
BiONPS = Bismuth oxide nanoparticles
CMGCE = Chitosan modified glassy carbon electrode
Ct-MGC = Chitosan-modified glassy carbon
CTS = Chitosan, poly-[1→4]-ß-D-glucosamine
CV = Cyclic voltammetry
DPASV = Differential pulse anodic stripping voltammetry
DPV = Differential pulse voltammetry
EDX = Energy dispersive X-ray
EMIM TCB = 1-ethyl-3-methylimida-zolium tetracyanoborate
g = Gramme
GCE = Glassy carbon electrode
ICP-MS = inductively coupled plasma-mass spectrometry
ICP-OES = Inductively coupled plasma-optical emission spectrometry
IL = Ionic liquid
i_p = Peak current
LOD = Limit of detection
LOQ = Limit of quantification
LSV = Linear sweep voltammetry
M = Molar (Mole per liter)
MFE = Mercury-film electrode
mg/kg = Milligram per kilogram
µg/L = Microgram per liter
mg/L = Milligram per liter
ml = Milliliter
mM = Millimole per liter (Millimolar)
mV = Millivolts

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

Nafion/Bi/NMC/GCE = Nafion-bismuth/nitrogen doped microporous carbon composite modified glassy carbon electrode
NanoSiO$_2$/BiFE = Mesoporous silica nanoparticles modified bismuth film electrode
ND = Non detectable
NMC = Nitrogen doped microporous carbon
RSD = Relative standard deviation
s = Second (time)
SD = Standard deviation
SEM = Scanning electron microscopy
S/N = Signal to noise ratio
SWASV = Square wave anodic stripping voltammetry
SWV = Square wave voltammetry
V = Volts
v/v = Volume by volume
WHO = World Health Organization
ZIF-8 = Zeolitic imidazolate framework-8
σ = The population standard deviation

CHAPTER 1
INTRODUCTION

1.1 Overview of the research

The Thale Noi is a freshwater lake in the uppermost of Songkhla Lake, between 7°45'55″ N and 100°05'15″ E, in the Southern part of Thailand. Its area is about 27 square kilometers with average 1.5 meters deep (Segers and Pholpunthin, 1997). In the past fishing is the main occupation of people around Thale Noi but currently they make a living on crafts especially handicrafts made from Krajood (*Lepironia articalata*). To make the products colorful, the plant needs to be dyed with substance containing heavy metals, particularly, lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu) and nickel (Ni) (Vipul *et al*., 2014). The resulting wastewater contaminated with heavy metals has been released to certain water resources of Thale Noi.

Phytoremediation is the use of plants to clean up a contamination from water, soils and sediment. This technology is cost effective and environmental friendly (Bieby *et al*., 2011). In addition, the aquatic plants, such as water lily (macrophyt). Their roots have an ability to absorb and accumulate heavy metal from polluted water resources (Mohamed and Serag, 2003).

Nymphaea lotus (Water lily) is a herbaccous aquatic plant, which is widely distributed in Thale Noi. Its root is restricted to rhizomotous form and the leaves known as lily pads, lay on the surface of the water. The leaves shapes are particularly round and the colour is from primary medium to deep olive green. The leaves are like another terrestrial plant leaves which capture sunlight for photosynthesis and gas exchange. Water lily stems are projected into the sediment and connected with the root that important to transportation process of mineral such as nitrogen (Rahim, 2014). Their flowers are complete with sepals, petals, stamens and carpel (Usman and Abdus-Salam, 2011). Furthermore, tropical water lily owns flowers with the special colors of blue, violet and bluish purple which hardy water lily lacks of. For this reason, the former is more favorable by people (Zhu *et al*., 2012). When focusing on the fact that water lily stems is edible portions. Its grown in soils contaminated with heavy metals possess a possible risk to human health.

Many researches presented the determination of heavy metals in Thale Noi such as the determination of lead and chromium concentration in water (Arnop, 1999) and the determination of arsenic and lead concentration in water, sediment and aquatic plants (e.g. esthwaite waterweed (*Hydrilla verticillata*), coontail hornwort (*Ceratophyllum demersum*), blue water lily (*Nymphaea stellata*) and water lily (*Nymphaea lotus*) (Phojanaluk *et al*., 2015).

According to the study on heavy metals contents in water lily plants, they have higher ability for heavy metals uptake including lead (Pb), cadmium (Cd), iron (Fe), arsenic (As), copper (Cu), nickel (Ni) and mercury (Hg) (Rahim, 2014). There are a number of possibilities for metal exposure or intoxication. Lead and cadmium exposure is the most common.

Many methods have been employed for heavy metal determination such as inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-optical emission spectrometry (ICP-OES), atomic absorption spectrometry (AAS) and electroanalytical method. Spectroscopic methods are high cost and need extraction and preconcentration which are time consuming with contamination and danger of losses (Somer *et al*., 2015). Electroanalytical methods, especially anodic stripping voltammetry (ASV) is considered as the most powerful technique due to its high sensitivity, high speed, low detection limit, simplicity, capacity to multielement determination and relatively inexpensive (Kannika *et al*., 2013).

The selection of a proper electrode material is very important in voltammetry. For the past six decades mercury has been the most commonly used electrode material for electrochemical (stripping) measurement in various electrode configurations. Due to its well-known toxicity, there have been numerous attempts to replace mercury with some other non-toxic or less-toxic electrode material (Borgo *et al*., 2015), nowadays, numerous new electrode materials and methods are desired to replace it. A variety of studies have demonstrated the applicability of bismuth film electrode (BiFE) as a possible alternative for electrochemical stripping analysis of trace heavy metals. The bismuth-film electrode (BiFE)–consisting of a thin film of bismuth deposited on a carbon substrate – has been proposed as an alternative to mercury electrodes.

Previously bismuth thin was deposited onto a glassy carbon electrode (GCE) (Lemma *et al.*, 2015). However, to improve the electrochemical properties of this electrode, various modifiers were used on the electrode surface. The incorporation of nanoparticles (e.g. nano-silica) onto electrode surface was also known to enhance electroanalytical properties of the electrode, primarily due to their increased surface area coupled with the enhanced mass transfer effect (Yang *et al.*, 2014). In addition, Nafion is one of the most extensively employed strong adhesion reagents for silica nanoparticles coating but it is expensive. Therefore, Chitosan is an attractive polymer for use in this thesis, because it is an inexpensive biopolymer.

The principle of the method is in accordance with that proposed by Khaled, *et al.*, 2010 who mentioned that accumulation of MCl_4^{2-} at the electrode surface where the metal ions are then reduced to the corresponding metals by application of sufficiently negative potential. In the next step, the metals are reoxidized back into the solution and the resulting stripping peak constitutes the analytical signal. Selective enhancement could be explained by the electrostatic attraction between the negatively charged metal chlorocomplexes and the protonated amino groups ($–NH_3^+$) of chitosan molecule, the electrode process can be expressed as follows:

Enriching process:

$C_6H_9O_4NH_2|_{ed} + H^+ \rightarrow C_6H_9O_4\overset{+}{N}H_3|_{ed}$

$C_6H_9O_4\overset{+}{N}H_3|_{ed} + PbCl_4^{2-} \rightarrow C_6H_9O_4\overset{+}{N}H_3 + PbCl_4^{2-}|_{ed}$

Deposition process:

$C_6H_9O_4\overset{+}{N}H_3 - PbCl_4^{2-}|_{ed} + 2e^- \rightarrow C_6H_9O_4\overset{+}{N}H_3 - PbCl_2^{2-}|_{ed} + 2Cl^-$

Stripping process:

$C_6H_9O_4\overset{+}{N}H_3 - PbCl_2^{2-}|_{ed} + 2Cl^- - 2e^- \rightarrow C_6H_9O_4\overset{+}{N}H_3|_{ed} + PbCl_4^{2-}$

The electrochemical reaction process of cadmium is like that of lead.

The main objectives are to improve the analytical performance of ASV for determination of lead and cadmium (such as high reproducibility and sensitivity, more convenient, rapid, inexpensive and environmental friendly operation) and to apply the proposed method to the analysis of real samples.

1.2 Introduction to lead and cadmium

1.2.1 Sources and effect of lead and cadmium in the environment

Lead and cadmium are heavy metals, which are natural constituents of the earth crust and present in the environment as a result of weathering and erosion of parent rocks (Samiey *et al*., 2014). In addition to natural sources, they are introduced in ecosystems through wastewaters originating from anthropogenic sources such as batteries, metallurgy and dye industries (Rajkumar *et al*., 2015). A large amount of lead and cadmium is released every year into the environment, which will affect the soil and water and hence affect animals and plants.

Soil and water exposed to heavy metals results in both animal and plant metal poisoning. Humans at the end of the food chain are exposed on any long- or short-term influence of heavy metal poisoning (Nelkel *et al*., 2015). Lead and cadmium exposure are the most common. Those can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations (Bieby *et al*., 2011)

1.2.2 Physical and chemical properties of lead and cadmium

Lead is a heavy metal which classified to be in group IVA of the periodic table. It is a soft, bright silvery metal. It is easy to beat, press or straighten and soluble in nitric acid. A summary of the physical properties of lead is given in Table 1-1.

Table 1-1 Physical and chemical properties of lead

Physical properties	Lead properties
Element symbol	Pb
Atomic mass	207.19
Atomic number	82
Oxidation state	0, 2, 4
Atomic radius	180 pm
Melting point (STP)	$327.46^{o}C$
Boiling point (STP)	$1749\ ^{o}C$
Density ($20^{o}C$)	$11.34\ g\ cm^{-3}$
Heat of fusion	$4.77\ kJ\cdot mol^{-1}$
Heat of vaporization	$179.5\ kJ\cdot mol^{-1}$
Heat capacity (25 °C)	$26.650\ J\cdot mol^{-1}\cdot K^{-1}$
Electron configuration	$[\,Xe]\ 4f^{14}\ 5d^{10}\ 6s^{2}\ 6p^{2}$
Young's modulus	16 GPa

Source: http://en.wikipedia.org/wiki/Lead (03/03/2016).

Cadmium is a heavy metal which classified in group IIB of the periodic table. It is a soft, bluish white metal. It is insoluble in water and is not flammable. A summary of the physical properties of cadmium is given in Table 1-2.

Table 1-2 Physical and chemical properties of cadmium

Physical properties	Cadmium properties
Element symbol	Cd
Atomic mass	112.41
Atomic number	48
Oxidation state	0, 2
Atomic radius	151 pm
Melting point (STP)	320.90 ^{o}C
Boiling point (STP)	765 ^{o}C
Density (20^{o}C)	8.65 g cm^{-3}
Heat of fusion	4.77 kJ·mol^{-1}
Heat of vaporization	179.5 kJ·mol^{-1}
Heat capacity (25 °C)	26.650 J·mol^{-1}·K^{-1}
Electron configuration	[Kr] 4d^{10} 5s^{2}
Young's modulus	16 GPa

Source: http://en.wikipedia.org/wiki/ Cadmium (03/03/2016).

1.2.3 Toxicology of lead and cadmium

Lead and cadmium are highly toxic and have an adverse health effects to the living organism and environment. Lead gives rise to adverse effects in several organs and systems in all known animal species, such as blood, central nervous system, kidneys, reproductive and immune systems (Chen *et al*., 2014). The permissible limit in edible portions of plants recommended by WHO is 0.5 mg/kg. Cadmium is the third of the "big three" of heavy metals with the greatest potential hazard in addition to lead and mercury. A disease known as “Itai Itai” in Japan is associated with cadmium poisoning, which results in multiple fractures in the body (Shetty, 2006). Cadmium accumulates in the human body affecting negatively several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system (Vipul *et al.*, 2014). The permissible limit in edible portions of plants recommended by WHO is 0.2 mg/kg.

1.3 Anodic Stripping Voltammetry

In anodic stripping voltammetry is a two-step technique. It involves a pre-concentration step and a stripping step (Figure 1-1). The first step included the electrolytic deposition of the metal ions onto a working electrode surface at a suitable potential. The second step included the application of a voltage scan to the working electrode. At a specific potential, it causes the stripping of the metal ions accumulated onto the surface of working electrode as thin film or amalgam into the solution.

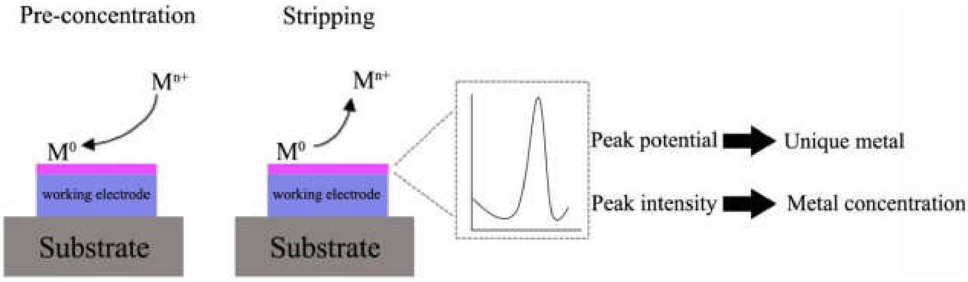


Figure 1-1 Anodic stripping voltammetry (ASV) principle according to literature (Gregory *et al.*, 2015).

Voltammetry involves monitoring the current generated upon application of a potential sweep. There are several types of voltammetric techniques, such as linear sweep voltammetry (LSV), cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV), depending on the potential-time waveforms of the applied potential function. As they offer better signal-to-noise ratios, DPV and SWV can offer better sensitivity than LSV and CV, so DPV and SWV are the most commonly used methods (Gao and Huang, 2013).

The stripping step is performed using any voltammetric technique. When the wave form is differential pulse, the technique is known as differential pulse anodic stripping voltammetry (DPASV). The high sensitivity of the DPASV can be attributed to the high ratio of Faradaic to Non-Faradaic currents generated in the electrochemical cell (Shirley, 1999).

1.4 Working electrodes

The working electrode is an important component of the ASV. The analyte is reduced or oxidized here. Glassy carbon electrode is often used for the analysis of heavy metals. Nowadays, several types of materials are used to modify the surface of glassy carbon electrode (GCE) including films, polymers and nanomaterial**.** There is a need to develop alternate electrodes that possess the same attractive properties as Hg because of its toxicity, stability and volatility issues (Prerna *et al.*, 2004).

1.4.1 Glassy carbon electrode (GCE)

(Silwana, 2012) The structure of glassy carbon consists of graphite planes randomly organised in a complex topology. Glassy carbon electrodes is special preparation through a carefully controlled heating program of pre-modelled phenolic resin form in an inert atmosphere. Unlike many non-graphitising carbons, it is highly resistant to acid attack and impermeable to gases. Glassy carbon is an isotropic material, it does not require a particular orientation in the electrode device. The performance of the electrode depends on the polishing materials and the procedures. Surface treatment is usually employed to create active and reproducible surface of the glassy carbon electrode and to enhance its analytical performance. Some additional activation steps have also been used to enhance the performance such as electro-chemical, chemical, vacuum heat, or laser treatment. The properties of carbonaceous materials significantly depend on the manufacturing process.

Carbon electrodes exhibit an environmentally friendly and a useful alternative to substitute mercury electrodes, which have noble metal surfaces, or a narrow cathodic range, which have the formation of oxide layers during voltammetric procedures, relatively inexpensive and limitations in terms of reproducibility (Thalita *et al.*, 2014).

1.4.2 Modified glassy carbon electrode (GCE) surfaces

The characteristics and properties of certain materials to modify the surface of glassy carbon electrode (GCE) are addressed here.

1.4.2.1 Bismuth film modified glassy carbon electrode (BiFE)

In 2000, a new type of electrode, the bismuth film electrode (BiFE) consisting of a thin film of bismuth deposited on a carbon substrate has been proposed as an alternative to mercury electrode in ASV (Christos *et al.*, 2008). A comparison of the electrochemical properties between bismuth film electrode and mercury film electrode (MFE) that are bismuth is more environmentally friendly with negligible toxic, high sensitivity, simplicity of preparation, insensitivity to dissolved oxygen (which is an essential property for on-site monitoring) and well defined and highly reproducible stripping signal. Moreover, the bismuth-based electrodes exhibit stripping due to its ability to form “fused” alloy with other metals similar to mercury (Yang *et al.*, 2014).

There are three methods to generate a bismuth film. The first method, by preplating it from an acidic media which is called the ex-situ preparation. The second method, by codeposition with the analyte which is known as the in-situ set up, and The third method by electrode fabricated by a film, such as BiF_3 or Bi_2O_3, to generate the Bi(s) coating (Karim and Fariba, 2014). Ex-situ plating is adaptable as electroplating conditions are independent of analysis conditions but more time consuming and complex than in situ plating. Moreover, BiFEs prepared with the ex-situ method can be reused and the surface of the electrode is reactivated after each analysis by polarization at an adequate potential which is more negative than the oxidation of bismuth but more positive than the oxidation stripping potential of other metal ions. In practice, a short cleaning step of 10–30 s at −0.35 V to –0.40 V in stirred solution is sufficient. In the case of method, the bismuth film is stripped after each analysis at a potential more positive than the oxidation of bismuth, typically in the range of 0.0 V to + 0.3 V for a few tenth of seconds. After that a new bismuth film is re-plated (Gregory *et al.*, 2015).

1.4.2.2 Chitosan modified glassy carbon electrode

Chitosan, poly-[1→4]-ß-D-glucosamine (CTS), is one of the most abundant natural polymers. Its pKa value of ~6.5, so at lower pH solutions (<pKa), its primary amines are protonated, making chitosan a cationic polyelectrolyte that is soluble in aqueous solution. At higher pH solutions (>pKa), these amines are deprotonated making chitosan neutral and insoluble (Figure 1.2).

a pKa value of ~6.5

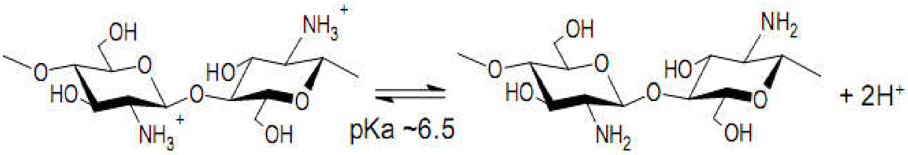


Figure 1-2 Chitosan’s amines confer pH-responsiveness (Eunkyoung*et al.*, 2015).

Chitosan finds several applications in pharmacology, environmental studies and food industries. The interest in this biopolymer for electrochemical applications is due to the combination of film-forming ability, good adhesion to electrode surfaces, high water permeability, biocompatibility, non-toxicity, high mechanical strength and susceptibility to chemical modifications (Carlos *et al.*, 2009).

1.4.2.3 Nanomaterial-modified electrodes

Mesoporous nanomaterials are described as nanomaterials whose pore diameters lie in the range between 2 and 50 nm (Liangming *et al.*, 2010). Mesoporous materials attracted much attention for their large surface area, fast adsorption kinetics, controllable pore size and pore arrangement. Due to the abundant silanol groups located at the internal surfaces, the mesoporous materials could be functionalized with alkoxy silanes containing different organic groups. These functionalized silica behaved as effective sorbents in the removal of heavy metals (Penghui *et al.*, 2010).

1.5 Review of literatures

Nowadays, a number of modifiers were used to improve the electrochemical properties of the glassy carbon electrode (GCE). The modified electrode was applied to the determination of heavy metals by electrochemical techniques. Various different applications of the modified electrodes are reviewed here.

Xianzeng *et al.*, (1998) studied the possible mechanism of electrochemical reaction of the surface of glassy carbon electrode modified with chitosan for the determination of precious metal elements: Au, Ag, Pt and Pd in low pH medium. The complex anionic form of these elements combines with the protonated group ($–NH_3^+$) in the chitosan molecule through an ion-association complex. The metal ions can be enriched selectively to produce sensitive anodic stripping peak current. The results of determination are consistent with standards. The possible mechanism of electrochemical reaction has been investigated by CV, UV and FT-Raman spectrometry, revealing that the metal ions combine with chitosan in different ways. The ion-association complex of Pt(II) and Pd(II) with chitosan was found to be tighter than that of Au(III) and Ag(I).

Guanghan *et al.*, (2001) studied the preconcentration and voltammetric method of chitosan modified glassy carbon electrode (CMGCE) for the determination of total Fe by cathodic stripping voltammetry. The CMGCE can be used to preconcentrate the complex of Fe and $C_2O_4^{2-}$ from aqueous solutions containing 3×10^{-3} mol/L KCl (pH 3.0). Its sensitivity, reproducibility and stability were found to be satisfactory. Factors influencing the cathodic stripping performance including deposition potential, deposition time, pH of solution, the concentration of modifier, scan rate and UV absorption curves were investigated to obtain optimum conditions. Under optimized conditions, the linear response was obtained in the concentration range of 2×10^{-8} to 3×10^{-7} g/ml Fe with a detection limit of 1×10^{-8} g/ml for a 2 min deposition time. The CMGCE exhibited the relative standard deviation of 3.67% for six repetitive measurements of a solution containing 2×10^{-7} g/ml Fe^{3+}. Furthermore, the developed electrode was successfully applied to the determination of total iron in tap water and mineral water directly.

Zanta and Martínez (2007) studied the preparations and voltammetric characteristics of chitosan-modified glassy carbon (Ct-MGC) and platinum electrodes. The Ct-MGC electrode used for pre-concentration and quantification of trace amounts of platinum in solution, which the reaction mechanism can explain that at low pH medium, the complex of Pt with protonated group $–NH_3^+$ in the chitosan molecule has been confirmed by FT–IR spectra studies of Ct film before and after adsorption for Pt. Moreover, cyclic voltammetric studies showed that chitosan has a wider range of potential window in positive potential with complexity capacity groups. On the other hand, during quantification of Pt in solution by differential pulse technique using Ct-MGC electrodes, a linear range and limit of detection, 2.26×10^{-6} to 7.18×10^{-6} mol/L and 3.5×10^{-8} mol/L , can be determined respectively. On the basis of voltammetric studies during ethanol electro-oxidation at CG/Ct/Pt electrodes, it can be concluded that chitosan modification with Pt may be used as proton exchange membrane for micro fuel cells in the field of energy conversion.

Martínez *et al.*, (2010) presented the surface of glassy carbon electrode modified with chitosan for the determination trace metals (Cu, Pb, Cd, Co, As and Pt). The modified surface exhibited an affinity to chelating metal ions in solution, forming complexes. The measurements were carried out by differential pulse stripping voltammetry. The response of proposed method is linear in the concentration ranges 3.99×10^{-6} to 3.91×10^{-5} mol/L for Cu(II), 1.99×10^{-6} to 1.58×10^{-5}mol/L for Pb(II), 1.59×10^{-5} to 6.23×10^{-5}mol/L for Cd(II), 7.99×10^{-6} to 5.04×10^{-5} mol/L for As(IV), 8.19×10^{-6} to 3.59×10^{-5} mol/L for Pt(IV) and 6.11×10^{-4} to 2.78×10^{-3} mol/L for Co(II). Cyclic voltammetry experiments were performed to study the behavior of polymeric surface in the presence of differential metals. Moreover, chitosan modified electrodes are found to cheaper than the spectroscopy adsorption analysis and non toxicity.

Hong *et al.*, (2010) presented the bismuth film glassy carbon electrode for the determination of lead by the differential pulse voltammetry method. This method shows a good linear relationship in the measuring range of 10 to 110 ug/L, which is $I = 0.1002C + 0.0539$ and is characterized by simple, rapid and sensitive. The experimental analysis shows that the concentration of lead in tea samples is 1.667 μg/g and the recovery rate is 91.2% ~ 101.8%. Compared to mercury-based electrode, bismuth-film carbon is better than common electrode, as it has excellent dissolution characteristics of volt-ampere. The characteristics of bismuth electrodes are close to mercury. Bismuth film electrode potentiometric stripping analysis was found to have high sensitivity and easy to update the electrode surface. Additionally bismuth is not toxic so it could replace mercury, resulting in better acceptance for application.

Lemma *et al.*, (2011) studied the application of bismuth film modified glassy carbon electrode for determination of lead. Ex-situ preparation of the bismuth film deposited on the glassy carbon surface from 2 mmol/L $Bi(NO_3)_3$ in 1 mol/L HCl as a plating solution, at –1.1 V (vs. Ag/AgCl) for 300 s. The effect of several experimental parameters including deposition time, pH of the solution, Bi(III) concentration, potential, pulse amplitude and pulse width were optimized. The measurements were carried out by differential pulse anodic stripping voltammetric in acetate buffer (pH 5.0) in the concentration range of 7.5 nmol/L to 12.5 μmol/L. Three linear calibration plots in the range 7.5 nmol/L to 0.1 μmol/L, 0.25 to 1 μmol/L, 2.5 to 12.5 μmol/L with regression coefficients of 0.991, 0.986 and 0.978 respectively were obtained. The theoretical detection limit for lead was calculated to be 5.25 nmol/L for 5 min deposition time and sensitivity 83.97 A L/mol from equation $Y = 83.967X + 0.371$. This method presented good sensitivity and relatively free from coexisting ions in excess amounts. But there were interferences like Cu(II), Hg(II) and Cr(III) which can inhibit to lower the detection limit. Acceptable agreement was found between the results obtained and the values of certified reference material.

Shijie *et al.*, (2012) reported the method for the synthesis of BiNPs on the electrode surface, which was carried out by ion-exchange in Nafion film coated on the electrode surface and subsequent electrochemical reduction of Bi(III) ions to BiNPs on site. The BiNPs modified electrode used to determination of Pb(II) by anodic stripping voltammetry with a linear range of 1.0–90 nM. The detection limit is 0.3 nM with 5 min accumulation. The BiNPs modified electrode has been successfully used for determination of Pb(II) by anodic stripping voltammetry with a linear range of 1.0–90 nM. The detection limit is 0.3 nM with 5 min accumulation. In addition, the properties of the resulting BiNPs modified electrode were investigated by scanning electron microscopy, an energy dispersive X-ray microanalyser and electrochemistry. The properties of the resulting BiNPs modified electrode were investigated by scanning electron microscopy, an energy dispersive X-ray microanalyser and electrochemistry. Almost 10 nm BiNPs were decorated in the matrix of Nafion film and showed excellent electrochemical activity. Under the optimum conditions, the BiNPs/NA/GCE exhibited high sensitivity, good linearity, a low detection limit and wide linear range for determination of Pb(II) in the lake water and tap water samples, with good agreement with results obtained by the standard ICP-MS method.

Qi *et al*., (2013) reported the application of bismuth film electrode (BiFE) for simultaneous determination of Pb(II), Cd(II) and Zn(II) using differential pulse stripping voltammetry. The BiFE could be prepared in situ when the sample solution contained a suitable amount of $Bi(NO)_3$, and its analytical performance was evaluated for the simultaneous determination of Pb(II), Cd(II) and Zn(II) in solutions. The limits of detection were found to be 0.19 ug/L for Zn(II) and 0.28 ug/L both Pb(II) and Cd(II), with a preconcentration time of 300 s. Under the optimum conditions, the BiFE presented good accuracy and reliability and was successfully applied to measure Pb(II), Cd(II) and Zn(II) in tea infusion and leaf samples, with good agreement with results obtained by the standard AAS method. The advantageous characteristics of bismuth electrodes is low toxicity of Bi, inexpensive instrumentation, simple operation and pretreatment, The proposed method is also green and will be great potential for on-site monitoring of heavy metals in environmental, food and medical samples, while avoiding the toxicity associated with mercury-based electrodes.

Die *et al*., (2013) studied the interfering effect of Cu(II) on the anodic stripping voltammetry of Pb(II) and Cd(II) at bismuth film electrodes. The presence of a 10-fold molar excess of Cu(II) could decrease the stripping signals of 70% for Pb(II) and 90% for Cd(II). The decrease in sensitivity was ascribed to the competition between copper and bismuth or the metal ions for surface active sites. Energy dispersive X-ray (EDX) and scanning electron microscopy (SEM) analysis suggested a large decrease in the amount of bismuth nanoparticles formed on the electrode surface in the presence of Cu(II) occurred, validating the competition between copper and bismuth ions for surface active sites. To improve the stripping peak signals of Pb(II) and Cd(II) and eliminate Cu(II) interference in ASV by adding ferrocyanide ion to the solution. Furthermore, the study method was successfully applied to measure

Pb(II) and Cd(II) in water samples and the method was validated by ICP-MS technique.

Lili *et al.*, (2014) presented the Nafion-bismuth/nitrogen doped microporous carbon (NMC) composite modified glassy carbon electrode (Nafion/Bi/ NMC/GCE). The NMC was derived from a zeolitic imidazolate framework-8 (ZIF-8), which good dispersibility, large specific surface area (941 m^2/g), high nitrogen content (25.0%) and good electrical conductivity of the NMC materials, as well as the synergistic effects of Nafion and bismuth film contributed to the enhanced signals of the electrode during the differential pulse anodic stripping voltammetry (DPASV) measurements for simultaneous detection of trace Cd(II) and Pb(II) in aqueous solution. The response of proposed method is linear in the concentration ranges 2-10 ug/L and 10-100 ug/L for Cd(II) and 0.5-10 ug/L and 10-100 ug/L for Pb(II). The detection limits were estimated to be 1.5 ug/L for Cd(II) and 0.05 ug/L for Pb(II). Under the optimum conditions, the Nafion/Bi/NMC/GCE presented the good selectivity, stability and reproducibility with the RSD of the stripping signals less than 5% for both Cd(II) and Pb(II) and was successfully applied to measure Cd(II) and Pb(II) in tap water samples.

Yang *et al.*, (2014) developed and evaluated a mesoporous silica nanoparticles modified bismuth film electrode (NanoSiO_2/BiFE) was applied with square wave anodic stripping voltammetry (SWASV) for the simultaneous determination of trace Pb(II) and Cd(II) in water samples. Parameters involved to the voltammetric analysis and the developed electrode were optimized. The calibration curve was obtained in the concentration range of 2-150 μg/L with the detection limit of 0.2 μg/L for Pb(II) and 0.6 μg/L for Cd(II) for 120s deposition time. In addition, the surface morphology of fabricated NanoSiO_2 were characterized by scanning electron microscopy (SEM). It has been shown that bismuth nanoparticles improve voltammetric performance due to the enhanced surface area. Under the optimum conditions, the NanoSiO_2/BiFE presented good accuracy and reliability and was successfully applied to measure Pb(II) and Cd(II) in real water samples, with good agreement with results obtained by the standard ICP-MS method.

Die *et al.*, (2014) presented bismuth film electrode coated with [1-ethyl-3-methylimida-zoliumtetracyanoborate (EMIMTCB)] as an ionic liquid and

Nafion was used to simultaneously determine lead and cadmium by anodic stripping voltammetry. With the mechanical stability of Nafion, the high conductivity of IL and the unique features of the bismuth film, the modified electrode exhibited very attractive stripping performance. Moreover, the surface characteristics of the modified BiFE were investigated by scanning electron microscopy (SEM), the results showed that fibril-like bismuth nanostructures were formed on the porous Nafion polymer matrix. Under optimized conditions, the calibration curve was obtained in the concentration range of 10–120 mg/L with the detection limit of 0.2 mg/L for Pb(II) and 0.5 mg/L for Cd(II) + for 120s deposition. The NC/IL-BiFE were exhibited the relative standard deviation (1.9 and 2.5% for Pb(II) and Cd(II), respectively) of nine repetitive measurements of a solution containing 20 mg/L Pb(II) and Cd(II). Furthermore, the developed electrode was successfully applied to the determination of Pb(II) and Cd(II) from water samples and the results were in satisfactory agreement with ICP-MS determination.

Borgo *et al*., (2015) presented the bismuth film electrode (BiFE) under more acidic medium was applied for determination of Zn(II) in tap water sample, with anodic stripping voltammetric detection. *In-situ* simultaneous deposition of zinc and bismuth at -1.2 V for 120 s was carried out in a supporting electrolyte containing 0.01 mol/L hydrochloric acid (pH 2.0) and 0.5 mol/L potassium sodium tartrate tetrahydrate as the complexing agent. The addition of potassium sodium tartrate tetrahydrate into the measurement solution improved the electroanalytical performance of BiFE via inhibition of hydrogen evolution. A single well-defined anodic stripping peak was observed at -1.1 V for the oxidation of Zn to Zn(II), which was used as the analytical signal. The calibration curve was in the concentration range of 10 to 100 μg/L with the detection limit being 1.3 μg/L (r = 0.998) associated with 300 s accumulation time. Repeatability expressed as relative standard deviation was 4.8%, for a 30 μg/L Zn(II) solution (n = 10). The BiFE method was validated by comparing the results with those obtained by the ICP-OES standard method.

Chen *et al*., (2016) presented the bismuth oxide nanoparticles (BiONPs) and the chitosan (CS) modified glassy carbon electrode was applied for simultaneously determination of lead and cadmium with differential pulse anodic stripping voltammetry. The modified electrode exhibited the superior electrochemical property of bismuth oxide nanoparticles and the good absorption capacity of chitosan, the self-made electrode demonstrated excellent analytical performance. Scanning electron microscopy image shows that the mean diameter of BiONPs is about 235 nm. In addition, the sensitivity of the BiONPs-CS-GCE is much better than that of the bare GCE and the CS-GCE. Under the optimized conditions, the BiONPs-CS-GCE applied to the analysis of Pb(II) and Cd(II). The linear range of the electrode was 0.4–2.8 μM for Pb(II) and 0.8–5.6 μM for Cd(II), with a limit of detection of 0.15 μM for Pb(II) and 0.05 μM for Cd(II), respectively. The fabricated electrode was successfully applied for the detection of Pb(II) and Cd(II) in spiked tap water was applied and the experimental analysis shows that gave the recovery rate from 99 to 122 % for Pb(II), and from 96 to 120 % for Cd(II).

From all literature reviews mentioned above, bismuth modified glassy carbon electrode (BiFE) have been employed in different combinations, e.g. in-situ and ex-situ BiFE prepared, bismuth nanoparticles, polymer coated BiFE, etc. for electrochemical techniques for determination of heavy metals. However, a few reports about the BiFE modified additionally with mesoporous silica nanoparticles. Normally, Nafion is one of the most extensively employed as a strong adhesion reagent for silica nanoparticles coating but it is expensive. Therefore, in this investigation the feasibility of using chitosan as a strong adhesion reagent due to the fact that it is cheaper than Nafion. Additionally, a number of articles reports its use for modification of the glassy carbon electrode surface for the determination of heavy metals. Here fabricated electrode of NanoSiO$_2$–CTS/BiFE was applied to simultaneous determination of Pb(II) and Cd(II) in water lily stems samples.

1.6 Objectives

1.6.1 To optimize conditions of the method for determination of Pb(II) and Cd(II) by anodic stripping voltammetry with NanoSiO$_2$–CTS/BiFE.

1.6.2 To apply the method to determine Pb(II) and Cd(II) in water lily stems in Thale Noi, Phatthalung Province, South of Thailand.

1.6.3 To risk assess of the concentration of Pb(II) and Cd(II) in edible portions of plants (water lily stems) with the permissible limit recommended by WHO.

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and materials

2.1.1 Standard chemicals

- Stock solution (1000 mg/L) of lead, cadmium, copper, mercury, nickel and zing (Fluka, Switzerland)

2.1.2 General chemicals and solvents

- Mesoporous silica nanoparticles size 200 nm pore size 4 nm, (Sigma-Aldrich, Sudan)
- Low molecular weight chitosan, (Sigma-Aldrich, Sudan)
- Bismuth(III) nitrate pentahydrate (Assay 98.0% min), AR grade (Fluka, Switzerland)
- Hydrochloric acid (Assay 37.0% min), AR grade (Lab scan, Thailand)
- Potassium chloride (Assay 99.8% min), AR grade (RFCL, India)
- Acetic acid (Assay 99.7% min), AR grade (Lab scan, Thailand)
- Nitric acid (Assay 69% min), AR grade (J.T.Baker, China)
- Hydrogen peroxide (Assay 30.0% min), (Merck, Germany)
- Calcium nitrate (Assay 99.0% min), AR grade (Ajax Finechem, Australia)
- Magnesium chloride (Assay 99.0% min), AR grade (Carlo Erba, Italy)
- Ultra pure water resistivity 18 MΩ obtained by passing deionized water through a ELGA water purification system (ELGA, England)

2.1.3 Samples

Water lily stems samples from Thale Noi in Phatthalung Province, South of Thailand. The samples were collected from five regions at Thale Noi. The sampling point show on page 32.

2.2 Instruments and apparatus

2.2.1 Potentiostat

- Powerlab 2/20 Potentiostat (ADInstrument, Australia) with Echem software version 2.1.16 as shown in Figure 2-1.
- Computer system
- IME663 interface (Metrohm, Switzerland)
- Nitrogen gas, High purity 99.99%, (TIG, Thailand)

2.2.2 Inductively coupled plasma optical emission spectrometer

- Inductively couple plasma optical emission spectrometer, Optima 8000 (Perkin-Elmer, USA) from the Scientific Equipment Center.

2.2.3 Electrochemical cell and electrodes (Figure 2-2)

- Ag/AgCl (3 M KCl) served as the reference electrodes (Metrohm, Switzerland)
- Glassy carbon electrode (3 mm diameter) as a working electrode (Windsor Scientific Ltd., UK)
- Platinum wire as an auxiliary electrodes (Number 6.1204.120, Metrohm, Switzerland)
- 50 ml of Electrochemical cell (Metrohm, Switzerland)

2.2.4 Apparatus

- Oven (Memmert, Germany)
- Water bath (Memmert, Germany)
- pH meter Model pH 510 (EUTECH Instrument, Netherlands)
- Balance 4 digits (Mettler Toledo, Switzerland)
- Microlitre pipette model: SL200 (0.5-10 µL) (BRAND, USA)
- Microlitre pipette model: SL1000 (20-200 µL) (BRAND, USA)

2.2.5 Materials and glassware

- Alumina powder 0.05 micron diameter (Buehler, USA)
- Whatman filter paper No.1
- Polyethylene bags
- Polypropylene bottles

All glassware such as volumetric flasks, beakers, cylinder, test tube and pipette are Pyrex. These glassware were soaked in 10% (v/v) nitric acid for overnight and then rinsed at least three times with ultra pure water.

Figure 2-1 Powerlab 2/20 Potentiostat (ADInstrument, Australia)

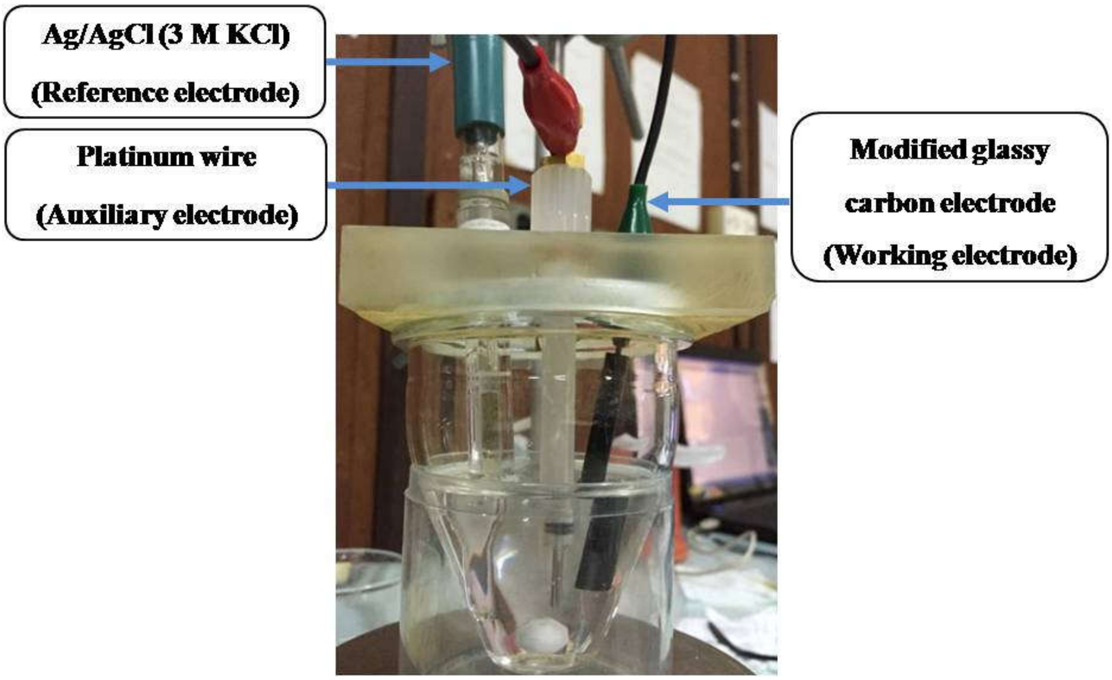


Figure 2-2 Electrochemical cell and electrodes

2.3 Methodology

2.3.1 Preparation of reagents

All chemicals used are analytical reagent grade and all standard and reagent solutions were prepared with ultrapure water (Milli Q water, resistivity of 18.2 MΩ cm^{-1}) obtained from a Millipore water purification system (Millipore, Sweden).

2.3.1.1 Preparation of stock standard solutions

Stock standard solutions of 100 mg/L Pb(II) and Cd(II) were prepared by using 1,000 mg/L Pb(II) and Cd(II) standard solutions and diluted for the corresponding stock solutions.

2.3.1.2 Hydrochloric acid solution

Concentrated hydrochloric acid solution 82.8 ml was added into ultrapure water and the solution was made up to 1000 ml to obtain 1 mol/L hydrochloric acid solution.

1 mol/L hydrochloric acid solution 100 ml was added into ultrapure water and the solution was made up to 1000 ml to obtain 0.1 mol/L hydrochloric acid solution.

2.3.1.3 Bi (III) solution

Bi (III) solution 0.01 mol/L was prepared by dissolving 4.8500 g of bismuth(III) nitrate pentahydrate $(BiNO_3)_3{\cdot}5H_2O$ in 1 mol/L hydrochloric acid solution and the volume was made up to 1000 ml in a volumetric flask.

0.01 mol/L Bi(III) solution 200 ml was added into 1 mol/L hydrochloric acid solution and the solution was made up to 1000 ml to obtain 2 mmol/L Bi (III) solution.

2.3.1.4 Potassium chloride solution

Potassium chloride solution 0.1 mol/L was prepared by dissolving 7.4560 g of potassium chloride in ultrapure water and the volume was made up to 1000 ml in a volumetric flask.

2.3.2 Preparation of supporting electrolyte

A supporting electrolyte: 0.1 mol/L HCl/KCl was prepared by mixing 0.1 mol/L HCl and 0.1 mol/L KCl and the pH of the solution was adjusted to 2.2 controlled by pH meter.

2.3.3 Preparation of working electrode

2.3.3.1 Ex-situ preparation of bismuth film modified glassy carbon electrode (BiFE)

Glassy carbon electrode (GCE) was polished with water slurry of 0.05 µm Al_2O_3 powder (Buehler, USA) on a polishing kit to a visually shiny surface. Then it is sonicated in distilled water in order to remove alumina and any other contaminants from electrode's surface. The bismuth film is deposited on the glassy carbon electrode without removing oxygen, immersed in 1 mol/L HCl solution containing 2 mmol/L Bi(III) ion (Lemma et al., 2013) by electrolysis at - 0.80 V vs.

Ag/AgCl for 150 s while stirring the solution. After the electrode was modified with bismuth, it was rinsed with distilled water and dried.

2.3.3.2 Preparation of NanoSiO$_2$–chitosan/bismuth film modified glassy carbon electrode (NanoSiO$_2$–CTS/BiFE)

To prepare the NanoSiO$_2$–chitosan as a modifying solutions, 10 ml chitosan solution [0.2% w/v in acetic acid solution (2 mol/L)] was prepared and the pH was adjusted to 6.0 with 1 mol/L NaOH, then 2 mg NanoSiO$_2$ was added with mechanical stirring at room temperature. A highly viscous solution of NanoSiO$_2$–chitosan was obtained. Then a 2 µl drop of NanoSiO$_2$–chitosan was placed onto the glassy carbon electrode modified with bismuth film with a micropipette (Brand, USA) and allowed to dry in the air.

2.3.4 Reference electrode

The porous position should be immersed in a 3M aqueous potassium chloride solution. The reference electrode should also be removed from the electrochemical cell and stored in this solution between experiments (this is particularly important when using non-aqueous solvent systems). Occasionally, air bubbles will form in the solution next to the porous point; these should be removed by gently flicking the end of the electrode (BAS Epsilon, 2000).

2.3.5 Optimization of operating conditions

The optimization procedures for all parameters were carried out by using 20 µg/L Pb(II) and Cd(II) standard solution. Each parameter was optimized by varying it while keeping other parameters constant. The optimum value was then used for all experiments. The following parameters for Powerlab 2/20 Potentiostat (ADInstrument, Australia) with Echem software version 2.1.16.were summarized in Table 2-1.

Table 2-1 Optimized operating conditions for Powerlab 2/20 Potentiostat

Parameter	Optimum value	Studied Range
Plating potential for ex-situ BiFE formation	-0.8 V	-0.6-(-1.0 V)
Plating time for ex-situ BiFE formation	150 s	60-300 s
Deposition potential for Pb(II) and Cd(II) detection	-1.1 V	-0.8-(-1.3 V)
Deposition time for Pb(II) and Cd(II)detection	240 s	60-420 s
Effect of pH 0.1 mol/L HCl/KCl	pH 2.2	pH 1.7-2.4
Chitosan concentration	0.20 %w/v	0.10 – 0.30 %w/v
Preconcentration time	10 s	0-40 s
Step potential	5 mV	1-20 mV
Pulse amplitude	50 mV	50-150 mV

2.3.5.1 Signal

A comparison of the sensitivities for 20 μg/L Pb(II) and Cd(II) analysis between square wave and differential pulse mode with the parameters are shown in Table 2-2.

Table 2-2 The comparison between square wave and differential pulse mode

Parameters	SWASV	DPASV
Accumulation step		
Deposition potential	-1000 mV	-1000 mV
Deposition time	180 s	180 s
Equilibration time	10 s	10 s

Parameters	SWASV	DPASV
Measuring step		
Potential window	-1000 to -300 mV	-1000 to -300 mV
Frequency	25 Hz	-
Step potential	5 mV	5 mV
Amplitude	25 mV	50 mV

2.3.5.2 Effect of pH 0.1 mol/L HCl/KCl

The influence of the pH of 0.1 mol/L HCl/KCl solution was examined by varying the pH from 1.7 to 2.4 (1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3 and 2.4). The optimal pH was chosen by considering on metal stripping signals of 20 μg/L Pb(II) and Cd(II) with the same conditions and same parameters in section 2.3.5.

2.3.5.3 Effect of chitosan concentration

Chitosan concentration was optimized between 0.10 to 0.35 % wt (0.10, 0.15, 0.20, 0.25, 0.30 and 0.35 % wt). The optimal chitosan concentration was chosen by considering on metal stripping signals of 20 μg/L Pb(II) and Cd(II) with the same conditions and same parameters in section 2.3.5.

2.3.5.4 Deposition potential of pre-plated bismuth film modified electrode

The formation of the Bi film on the GCE was optimized using a 2 mmol/L Bi(III) solution in 1 mol/L HCl used as plating solution. The effects of deposition potential was varied from -0.6 to -1.0 V (-0.6, -0.7, -0.8, -0.9 and -1.0 V) versus silver/silver chloride reference electrode in stirred solution using a deposition time of 120 s. The optimal deposition potential was chosen by considering on metal stripping signals of 20 μg/L Pb(II) and Cd(II) with the same conditions and same parameters in section 2.3.5.

2.3.5.5 Deposition time of pre-plated bismuth film modified electrode

The bismuth film plating time was used to control the thickness of the bismuth film. The effect of the bismuth plating time was studied in the range 60–300 s (60, 120, 150, 180, 240 and 300 s) were tried with the deposition potential of -0.8 V in stirred solution versus silver/silver chloride reference electrode. The stripping voltammogram was recorded in the differential pulse mode with the same conditions and same parameters in section 2.3.5.

2.3.5.6 Deposition potential for Pb(II) and Cd(II) detection

To achieve maximum sensitivity in the voltammetric response, first of all instrumental parameters for the deposition potential were examined with standard solution containing 20 μg/L Pb(II) and Cd(II). Then the deposition potential was varied from -0.8 to -1.3 V (-0.8, -0.9, -1.0, -1.1, -1.2 and -1.3 V) versus silver/silver chloride reference electrode in stirred solution using a deposition time of 180 s. The stripping voltammogram was recorded in the differential pulse mode with the same conditions and same parameters in section 2.3.5. Three replicates were performed for each potential.

2.3.5.7 Deposition time for Pb(II) and Cd(II) detection

To increase peak current and to improve the sensitivity of the method, the influence of deposition time was investigated with standard solution containing 20 μg/L Pb(II) and Cd(II), deposition time periods between 60–360 s (60, 120, 180, 240, 300 and 360 s) were tried with the deposition potential of -1.1 V in stirred solution versus silver/silver chloride reference electrode. The stripping voltammogram was recorded in the differential pulse mode with the same conditions and same parameters in section 2.3.5 and three replicates were performed for each time period.

2.3.5.8 Equilibration time

The influence of equilibration time on peak current was carried out by varying equilibration time in a range of 0-40 s (0, 10, 20, 30 and 40 s). The dependence of deposition efficiency upon equilibration time was studied with for 20 μg/L Pb(II) and Cd(II) in 0.1 mol/L HCl/KCl (pH 2.2). The stripping peak was performed with the optimum conditions in section 2.3.5 and three replicates were performed for each.

2.3.5.9 Step potential

The effects of step potential on peak current were studied by varying the range of 1-20 mV (1, 2, 5, 10 and 20 mV). The concentration of Pb(II) and Cd(II) used was 20 µg/L in 0.1 mol/L HCl/KCl (pH 2.2). The stripping was performed with the optimum conditions as described in section 2.3.5 and three replicates were performed for each.

2.3.5.10 Pulse amplitude

The effect of amplitude on peak current was studied by varying amplitudes in the range of 25-150 mV with 25 mV increment (25, 50, 75, 100, 125, 150 mV). The concentration of Pb(II) and Cd(II) used was 20 µg/L in 0.1 mol/L HCl/KCl (pH 2.2). The stripping peak was performed with the optimum conditions in section 2.3.5 and three replicates were performed for each.

2.3.6 Analytical performances of ASV methods

2.3.6.1 Linear range

The stock standard solution of Pb(II) and Cd(II) were diluted with ultrapure water to obtain various concentrations with the range from 2 to 40 µg/L. The 50.0 ml of each concentration was analyzed by Powerlab 2/20 Potentiostatat the optimum conditions in section 2.3.5. The linear dynamic range was obtained by plotting the current versus the concentration of metal ions. The linearity of response was evaluated from the correlative coefficient of the linear curve.

2.3.6.2 Limit of detection (LOD)

The limit of detection (IUPAC definition) is expressed as the smallest concentration that can be detected with a certainty of more than 95%. Limit of detection for ASV techniques was determined based on $3\sigma/m$ (Coelho *et al.,* 2002) where;

σ = the standard deviation of 10 measurements of 2 µg/L Pb(II) and Cd(II) adding in blank solution, due to the fact that no peak appeared at blank solution.

m = the slope of the calibration graphs

2.3.6.3 Limit of quantification (LOQ)

The limit of quantification is expressed as the smallest concentration that can be quantified with suitable precision and accuracy. Usually the limit of quantification is evaluated as the signal to noise ratio (S/N) that is equivalent to 10 times of the standard deviation of the noise ($S/N=10\sigma$). However, the determination of the limit of quantification for ASV techniques was studied based on $10\sigma/m$ (Coelho *et al.,* 2002) where;

σ = the standard deviation of 10 measurements of 2 µg/L Pb(II) and Cd(II) adding in blank solution, due to the fact that no peak appeared at blank solution

m = the slope of the calibration graphs

2.3.6.4 Precision

Precision is the measure of the degree of repeatability of an analytical method under the same condition. Normally it is expressed as a percentage of the relative standard deviation (%RSD) for a statistically significant number of samples. The calculation of %RSD is given as:

$$\%\mathrm{RSD} = \frac{\mathrm{SD}}{\overline{\mathrm{X}}} \times 100 \quad ; \ \mathrm{SD} = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \overline{x})^2}{(n-1)}} \qquad \ldots\ldots\ldots\ldots\ldots(2.1)$$

Where;

SD = standard deviation

n = total number of values

x_i = each individual value to calculate the mean

$\overline{x}$ = the mean of *n* values

In this study, the precisions were investigated by measuring the degree of repeatability for analyses of Pb(II) and Cd(II) standard solutions in the concentration of 20 µg/L. The experiments for the Pb(II) and Cd(II) standard solutions were repeated 10 times.

2.3.6.5 Accuracy

The terms accuracy is expressed as the relative percent error term (%RE). The following calculation of relative percent error can be calculated from the equation below:

$$\%\mathrm{RE} = \frac{\text{Measured value - Real value}}{\text{Real value}} \times 100 \qquad \ldots\ldots(2.2)$$

In this study, the accuracy was obtained from water lily stems solution samples spiked with 20 µg/L of Pb(II) and Cd(II). The stripping peak was performed with the optimum conditions in section 2.3.5 and three replicates were performed for each.

2.3.7 General procedure for determination of lead and cadmium

The measurements were carried out in a conventional three-electrode cell, at the room temperature. All DPASV experiments were carried out with a Powerlab 2/20 Potentiostat (ADInstrument, Australia) with Echem software version 2.1.16. The electrochemical cell containing a glassy carbon electrode modified with ex-situ bismuth film combined with mesoporous silica nanoparticles and chitosan (NanoSiO$_2$CTS/BiFE) as working electrode, Ag/AgCl (3M KCl) and Platinum wire (Metrohm, Switzerland) as the reference and auxiliary electrodes, respectively were used containing 0.1 mol/L HCl/KCl (pH 2.2). Standard solution of Pb(II) and Cd(II) were added into the cell and the mixed solution was stirred at the potential of -1.1 V for 240 s. Following the preconcentration step, the stirring was stopped. After 10 s of quiet time, the differential pulse anodic stripping voltammetry (DPASV) was performed by potential scan from –1.1 to –0.4 V with pulse amplitude 50 mV, pulse duration 20 ms and potential step of 5 mV. The stripping peaks were observed at -0.460 V. All measurements were made at the room temperature (ca. 25-35 ^{o}C).

2.4 Effect of interferences

The effect of various common ions was evaluated with respect to their interferences with the stripping peak of Pb(II) and Cd(II) by adding foreign ions to 50 ml solution containing 20 µg/L of Pb(II) and Cd(II). The interference include Ca(II), Cu(II), Mg(II), Zn(II), Ni(II), Hg(II) and Fe(III). The stripping was performed with the optimum conditions in section 2.3.5 and three replicates were performed for each.

2.5 Application of this investigation method in water lily *(Nymphaea lotus*)

2.5.1 Sampling and sample storage

Water lily samples shown in Fig 2-3 were collected from five sites in Thala Noi is a freshwater lake in the uppermost of Songkhla Lake, Phatthalung Province, South of Thailand shown in Fig 2-4. The samples collection at sites is shown in Table 2-3 and Fig 2-5. The water lily samples were cut by using plastic-ceramic knife based on interest parts which was stems, were kept in polyethylene resealable zipper plastic bags and labeled and later transported to the laboratory by kept in the ice box. On arrival at the laboratory, the samples were washed with distilled water to remove any adhering soil/dust particles. The water lily stems samples were stored in polyethylene resealable zipper plastic bags and the frozen at -5 °C until further analysis.

Fig 2-3 Water lily samples in Thale Noi showing stems, flowers and leaves.

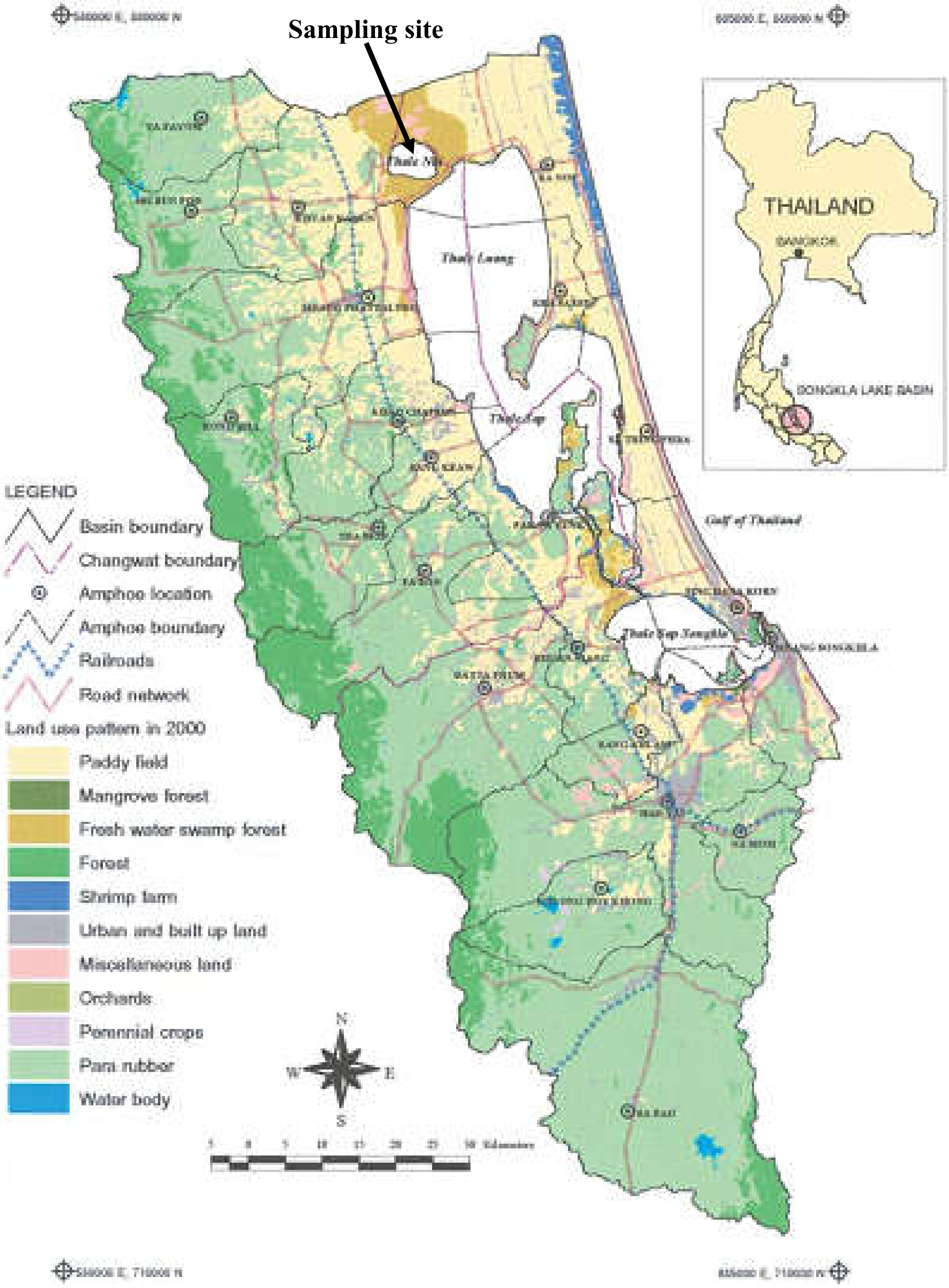


Fig 2-4 Location of Songkhla Lake Basin according to Charlchai *et al*., 2001.

Table 2-3 Sampling point identified by using the global positioning system (GPS)

Site	Sampling point	Longitude and latitude
1	Khlong Ban Khao	E 100° 7′ 40″ N 7° 47′ 21″
2	Khlong Ban Bon	E 100° 9′ 33″ N 7° 48′ 7″
3	Khlong Nang Riam	E 100° 11′ 6″ N 7° 47′ 5″
4	KhlongYuan	E 100° 7′ 58″ N 7° 46′ 14″
5	Ao Na Klang	E 100° 7′ 40″ N 7° 46′ 43″

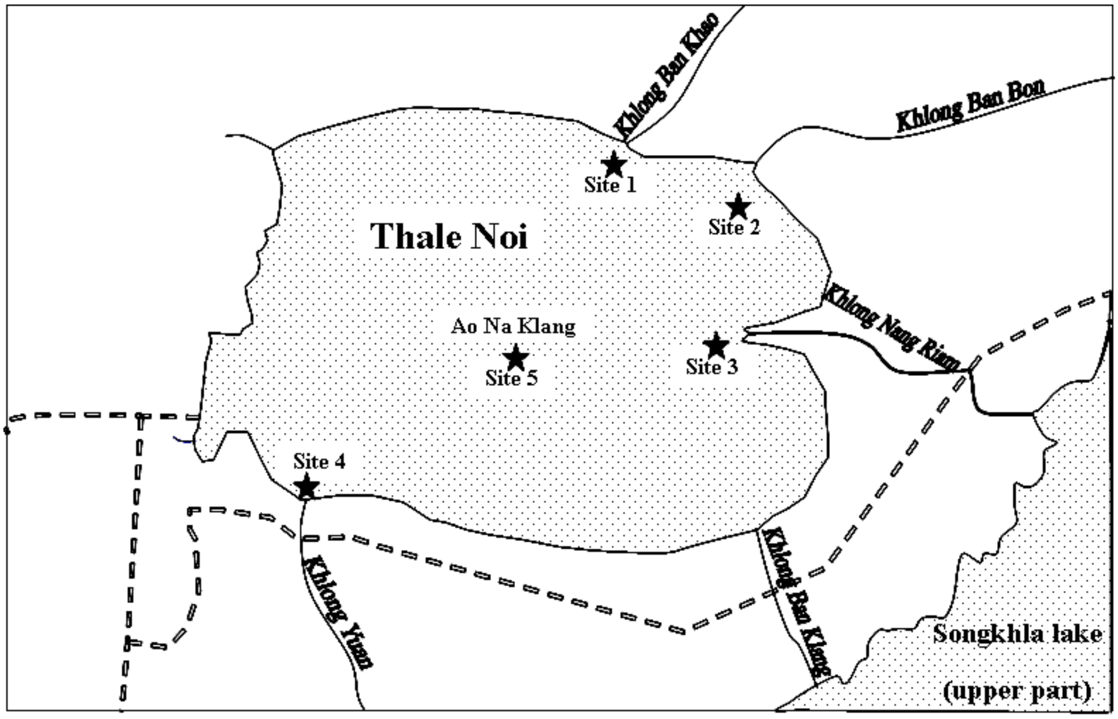


Fig 2-5 Location of sampling sites within Thale Noi.

2.5.2 Sample pretreatment

Water lily stems samples were defrosted and rinsed three times with distilled water. Then, the samples were cut by using plastic-ceramic knife and placed in different Petri dishes before being dried in oven at 105 °C until reaching constant weights. The dried samples were grinded to be powder with non-metal pestle and mortar and collected in polyethylene bags until analysis.

2.5.3 Sample digestion by water bath digestion method

Water lily stems samples were digested using water bath digestion method (Phojanaluk *et al.*, 2015). 0.5 g dry weight of sample was weighed. The samples then transferred into the test tube followed by addition 2.0 ml of 65% of nitric acid (HNO_3). The test tube was placed on the water bath and heated at 95ºC about 1.5 hour. Then, the sample was left to cool at room temperature and added 0.5 ml of 30% hydrogen peroxide (H_2O_2) as oxidizing agent. The test tube was placed on the water bath and heated at 95ºC about 30 min. After that the digested sample was left to cool at room temperature, filtered through Whatman No. 1 filter paper and diluted to 10 ml with ultrapure water for determined Pb(II) and Cd(II) using ICP-OES. In the case of DPASV the sample solution 1 ml was added in to electrochemical cell and diluted to 50 ml with 0.1 mol/L HCl/KCl (pH 2.2).

CHAPTER 3
RESULTS AND DISCUSSION

The results from the determination of Pb(II) and Cd(II) in water lily stem samples carried out by anodic stripping voltammetry with NanoSiO$_2$–chitosan/ bismuth film modified glassy carbon electrode (NanoSiO$_2$–CTS/BiFE) are as follows:

3.1 Optimization of ASV parameters

3.1.1 Operational principle of stripping voltammetric determination of lead and cadmium ions at the electrode

The principle of the method is in accordance with that proposed by Khaled, et al. who mentioned that accumulation of MCl_4^{2-} at the electrode surface where the metal ions are then reduced to the corresponding metals by application of sufficiently negative potential. In the next step, the metals are reoxidized back into the solution and the resulting stripping peak constitutes the analytical signal. Selective enhancement could be explained by the electrostatic attraction between the negatively charged metal chlorocomplexes and the protonated amino groups (–NH_3^+) of chitosan molecule, the electrode process can be expressed as follows:

Enriching process:

$C_6H_9O_4NH_2|_{ed} + H^+ \rightarrow C_6H_9O_4\overset{+}{N}H_3|_{ed}$

$C_6H_9O_4\overset{+}{N}H_3|_{ed} + PbCl_4^{2-} \rightarrow C_6H_9O_4\overset{+}{N}H_3 + PbCl_4^{2-}|_{ed}$

Deposition process:

$C_6H_9O_4\overset{+}{N}H_3 - PbCl_4^{2-}|_{ed} + 2e^- \rightarrow C_6H_9O_4\overset{+}{N}H_3 - PbCl_2^{2-}|_{ed} + 2Cl^-$

Stripping process:

$C_6H_9O_4\overset{+}{N}H_3 - PbCl_2^{2-}|_{ed} + 2Cl^- - 2e^- \rightarrow C_6H_9O_4\overset{+}{N}H_3|_{ed} + PbCl_4^{2-}$

3.1.2 Comparison of Pb(II) and Cd(II) stripping voltammogram between square wave and differential pulse mode

At the beginning, a comparison of Pb(II) and Cd(II) stripping voltammogram between square wave (SWASV) and differential pulse (DPASV) modes with the parameters shown in Table 3-1 was performed. The stripping peak for 20 µg/L Pb(II) and Cd(II) in different modes at NanoSiO$_2$-CTS/BiFE, the results exhibits the position of the peak potential of at -0.550 V for Pb(II) and -0.815 V for Cd(II). As shown in Figure 3-1, the current from DPASV is higher sensitivity than that of SWASV. Consequently, the differential pulse mode was chosen for all experiments.

Table 3-1 The comparison between square wave and differential pulse mode

Parameter	SWASV	DPASV
Accumulation step		
Deposition potential	-1000 mV	-1000 mV
Deposition time	180 s	180 s
Equilibration time	10 s	10 s
Measuring step		
Potential window	-1000 to -300 mV	-1000 to -300 mV
Frequency	25 Hz	-
Step potential	5 mV	5 mV
Amplitude	25 mV	50 mV

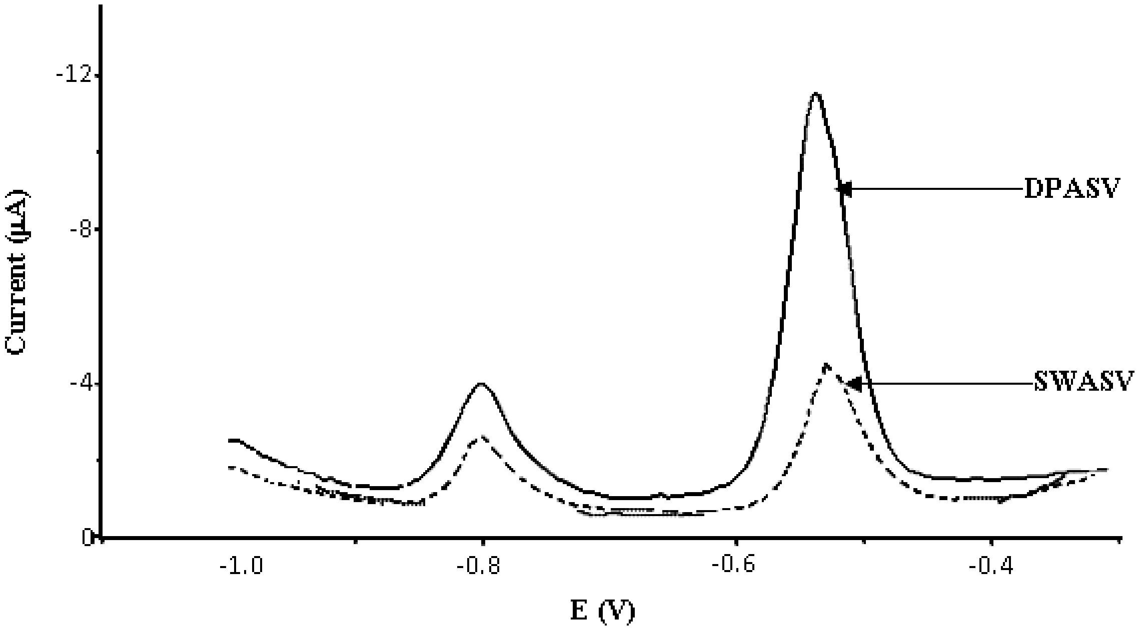


Fig 3-1 Stripping voltammogram of Pb(II) and Cd(II) in differential pulse mode and square wave mode of 20 µg/L Pb(II) and Cd(II) in 0.1 mol/L HCl/KCl (pH 2.2)

3.1.3 Optimization of pre-plated bismuth film modified electrode

3.1.3.1 Effects of plating potential of bismuth film formation

The formation of the Bi film on the GCE was optimized using a 2 mmol/L Bi(III) solution in 1 mol/L HCl used as plating solution. The effects of plating potential on the stripping peak currents of 20 µg/L Pb(II) and Cd(II) are shown in Appendix A-1 and Figure 3-2.

The plating potential was varied from -0.6 to -1.0 V with a constant plating time of 120 s. At the more negative deposition potentials, the peak current of Pb(II) and Cd(II) increase. But when the potential is more negative than -0.8 V, the electrode is not successfully modified. Therefore, a potential of -0.8 V vs Ag/AgCl selected as the optimum potential.

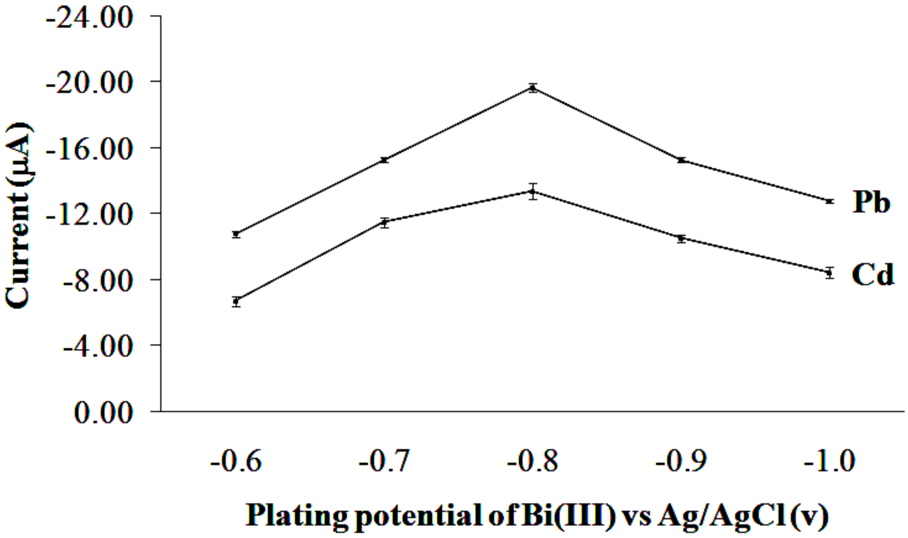


Fig 3-2 Effects of plating potential of bismuth film formation upon DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl (pH 2.2); deposition potential of -1.0 V for 180 s, equilibration time 10 s, pulse amplitude 50 mV and potential step of 5 mV

3.1.3.2 Effects of plating time of bismuth film formation

The bismuth film plating time was used to control the thickness of the bismuth film. The effect of the bismuth plating time on the stripping peak currents of 20 µg/L Pb(II) and Cd(II) are shown in Appendix A-2 and Figure 3-3. The plating time was studied in the range 60–300 s with a constant plating potential of -0.8 V vs Ag/AgCl. The Pb(II) and Cd(II) peak increased with increasing bismuth plating time up to 150 s and then both peaks decreased with increasing bismuth plating time due to film saturation.

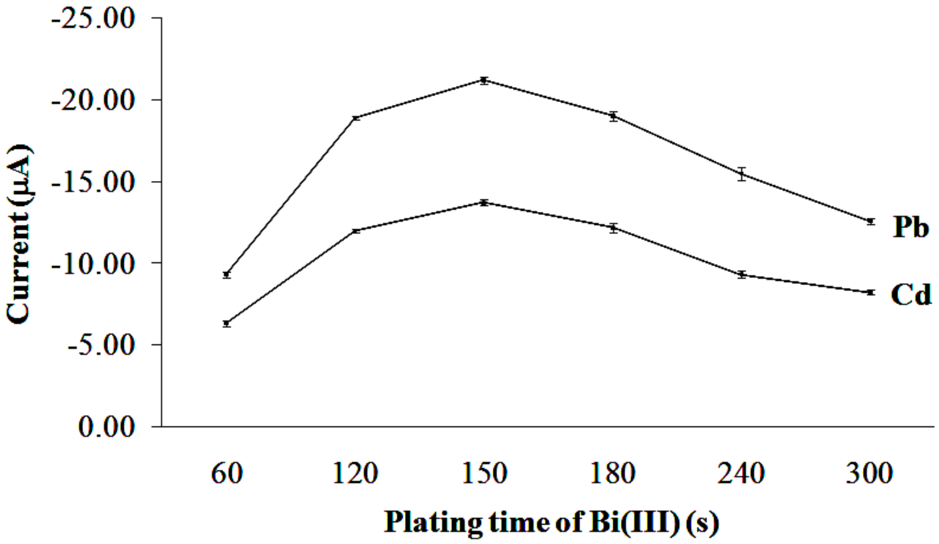


Fig 3-3 Effects of plating time of bismuth film formation upon DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl (pH 2.2); Other conditions are as in **Fig 3-2**

3.1.4 Effect of concentration of chitosan

The influence of the concentration of chitosan was investigated by varying the concentration of chitosan in the modifying solutions in the range of 0.10 to 0.35 %wt with the content of mesoporous silica nanoparticles (NanoSiO$_2$) fixed at 2 mg. In Appendix A-3 and Figure 3-4 shows that the peak height of Pb(II) and Cd(II) increased as the concentration of chitosan increased and reached a maximum at 0.20 %wt. Further increase of the concentration of chitosan in the modifying solutions decreased the peak height of Pb(II) and Cd(II), most probably due to the lower conductivity of chitosan. 0.20 %wt chitosan in the modifying solutions was chose for all subsequent work.

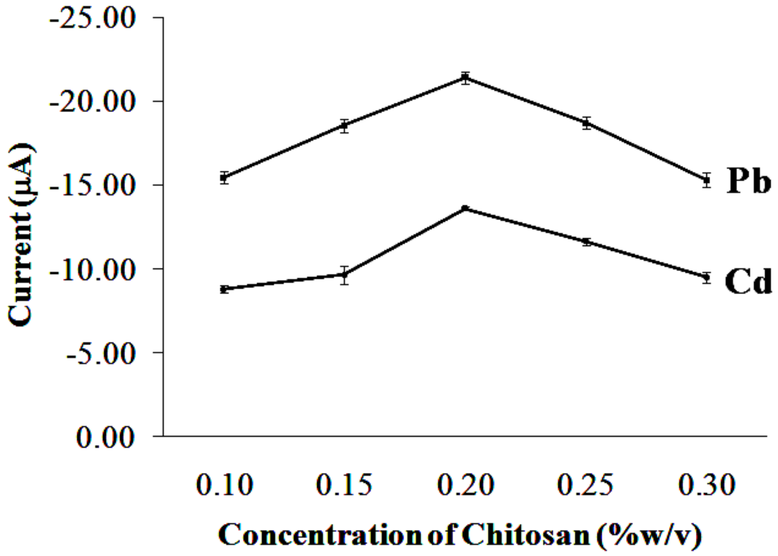


Fig 3-4 Effect of concentration of chitosan upon DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl (pH 2.2). Other conditions are as in **Fig 3-2**

3.1.5 Effect of pH 0.1 mol/L HCl/KCl

The pH of 0.1 mol/L HCl/KCl solution is an important parameter for the deposition of Pb(II) and Cd(II) on NanoSiO_2-CTS/BiFE and was examined by varying the pH from 1.7 to 2.4 with 20 µg/L Pb(II) and Cd(II) as displayed in Appendix A-4 and Figure 3-5. At pH 2.2 both Pb(II) and Cd(II) exits in the form of MCl_4^{2-} in 0.1 mol/L HCl/KCl medium, These species are anionic form which are suitable for sorption on protonated amino group (NH_3^+) in the chitosan molecule through anion exchange/electrostatic attraction. Thus, 0.1 mol/L HCl/KCl pH 2.2 was selected for the next experiments.

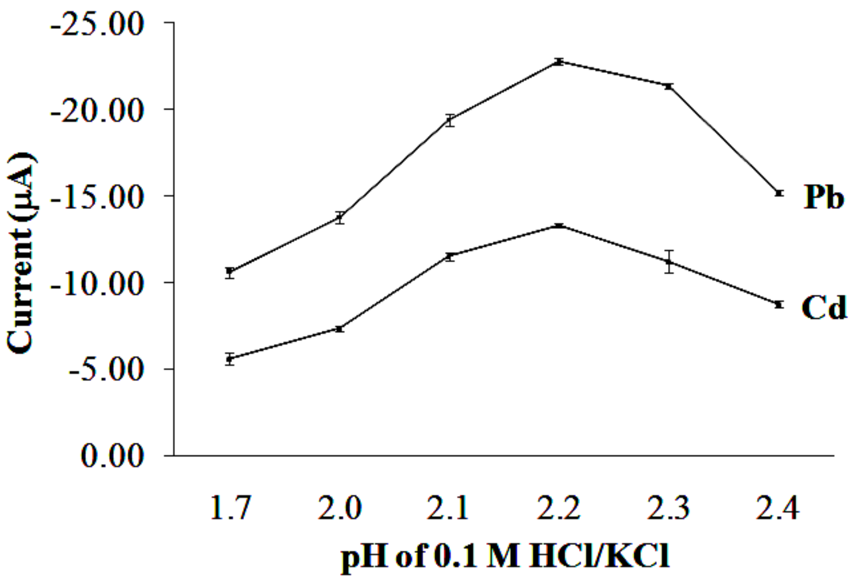


Fig 3-5 Effect of pH of 0.1 M HCl/KCl upon DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl. Other conditions are as in **Fig 3-2**

3.1.6 Optimization of the measurement parameters

3.1.6.1 Effect of deposition potential

Deposition potential is an important parameter for stripping techniques and it has substantial influence on the sensitivity of the determination. The effect of deposition potential was varied between -0.8 to -1.3 V using a deposition time of 180 s as shown in Appendix A-5 and Figure 3-6. The stripping currents of both analytes increased with the deposition potential shifting from -0.8 to -1.1 V and then decreased at more negative potentials. This decrease of the peak height may be due to the hydrogen evolution in the acid solution when the applied potential becomes more negative than -1.1 V. Therefore the deposition potential of -1.1 V was used for further experiments.

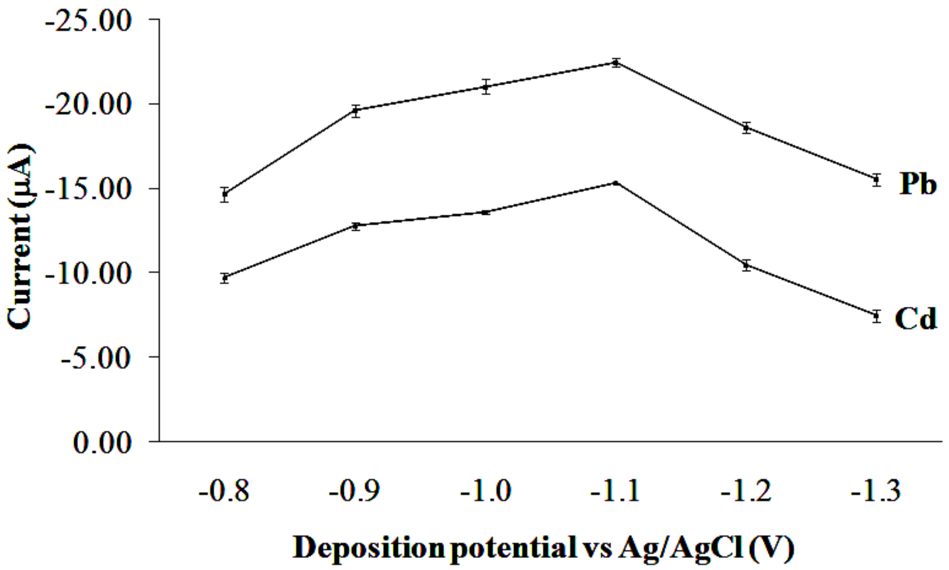


Fig 3-6 Effect of deposition potential up on DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl (pH 2.2). Other conditions are as in **Fig 3-2**

3.1.6.2 Effect of deposition time

The effect of deposition time for Pb(II) and Cd(II) detection were examined by varying deposition time within a range of 60-480 s at deposition potential of -1.1 V as shown in Appendix A-6 and Figure 3-7. It was found that at longer deposition time, higher sensitivity was obtained as expected. This is due to the fact that greater amount of analyte was deposited on NanoSiO$_2$-CTS/BiFE with increasing deposition time. However, the linear trends become not obvious due to the saturation loading of the electrode surface. In this study, a deposition time of 240 s was selected for further experiments.

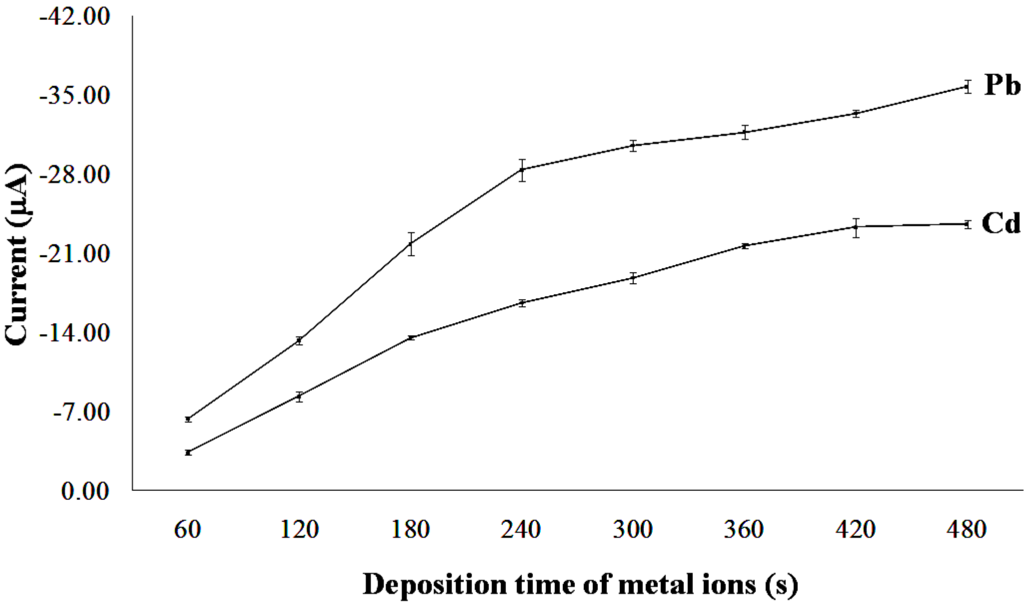


Fig 3-7 Effect of deposition time up on DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl (pH 2.2). Other conditions are as in **Fig 3-2**

3.1.6.3 Effect of equilibration time

The influence of equilibration time on peak current was carried out by varying equilibration time in a range of 0-40 s (0, 10, 20, 30 and 40 s) as shown in Appendix A-7 and Figure 3-8. In this study, equilibration time of 10 s was selected for further experiments.

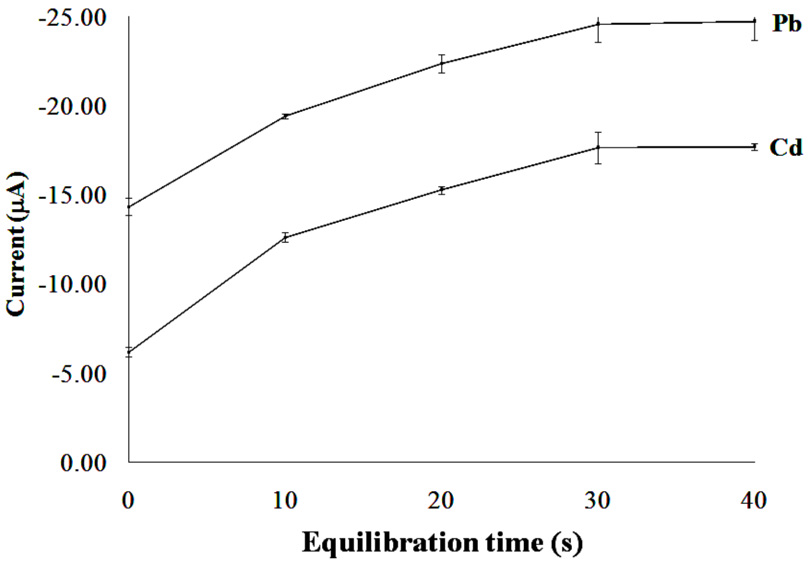


Fig 3-8 Effect of equilibration time up on DPASV signals of 20 µg/L Pb(II) and Cd(II) in 0.1 M HCl/KCl (pH 2.2). Other conditions are as in **Fig 3-2**

3.1.7 The optimum operation conditions

The parameter which affected analytical performance and the optimum conditions for determination of Pb(II) and Cd(II) using NanoSiO$_2$-CTS/BiFE as a working electrode are summarized in the Table 3-2.

Table 3-2 Optimized operating conditions for Powerlab 2/20 Potentiostat

Parameter	Optimum value	Studied Range
Plating potential for ex-situ BiFE formation	-0.8 V	-0.6–(-1.0 V)
Plating time for ex-situ BiFE formation	150 s	60–300 s
Deposition potential for Pb(II) and Cd(II) detection	-1.1 V	-0.8–(-1.3 V)
Deposition time for Pb(II) and Cd(II) detection	240 s	60–420 s
Effect of pH 0.1 mol/L HCl/KCl	pH 2.2	pH 1.7–2.4
Chitosan concentration	0.20 %w/v	0.10–0.30 %w/v
Equilibration time	10 s	0–40 s
Step potential	5 mV	1–20 mV
Pulse amplitude	50 mV	25–150 mV

3.2 Analytical performances of ASV method

3.2.1 Linear range

Under the optimal condition as shown in Table 3-2, the calibration graphs of Pb(II) and Cd(II) in the range of 2 to 40 µg/L of metal ions were constructed by plotting current (µA) versus concentration of metal ions (µg/L) the linear correlation coefficient. The calibration curves exhibited excellent linearity with a correlation coefficient (R^2) of 0.998 for both Pb(II) and Cd(II) after a 240 s deposition time as shown in Appendix B-1 and Figure 3.9. The stripping peak currents at peak potential of about -0.815 and -0.550 V versus Ag/AgCl were proportional to concentrations of Pb(II) and Cd(II), respectively. The voltammograms are shown in Figure 3.10.

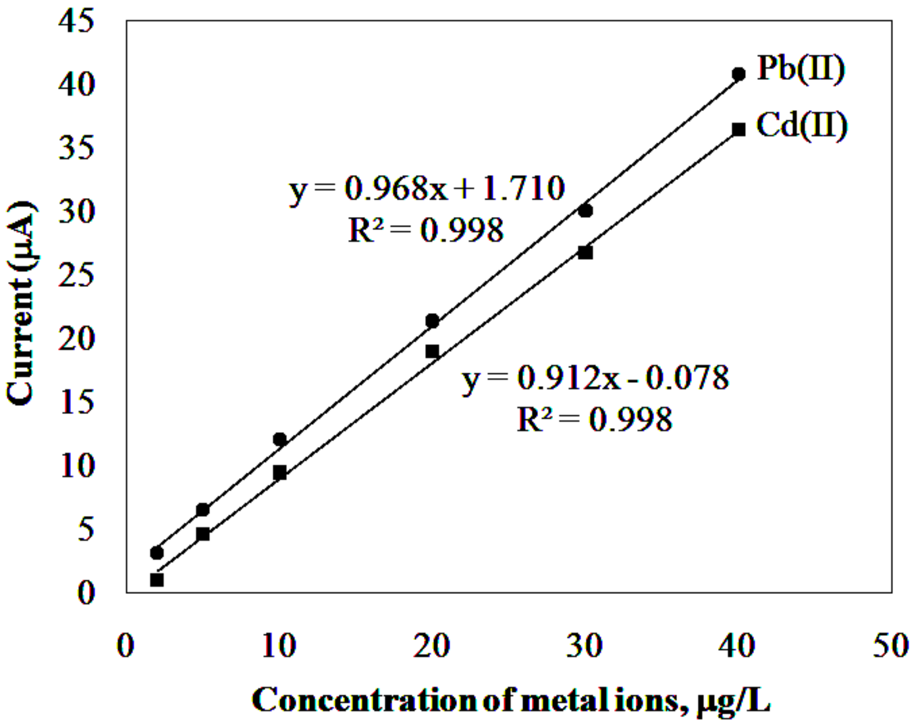


Figure 3-9 The calibration graphs of Pb(II) and Cd(II) in the range of 2 to 40 µg/L

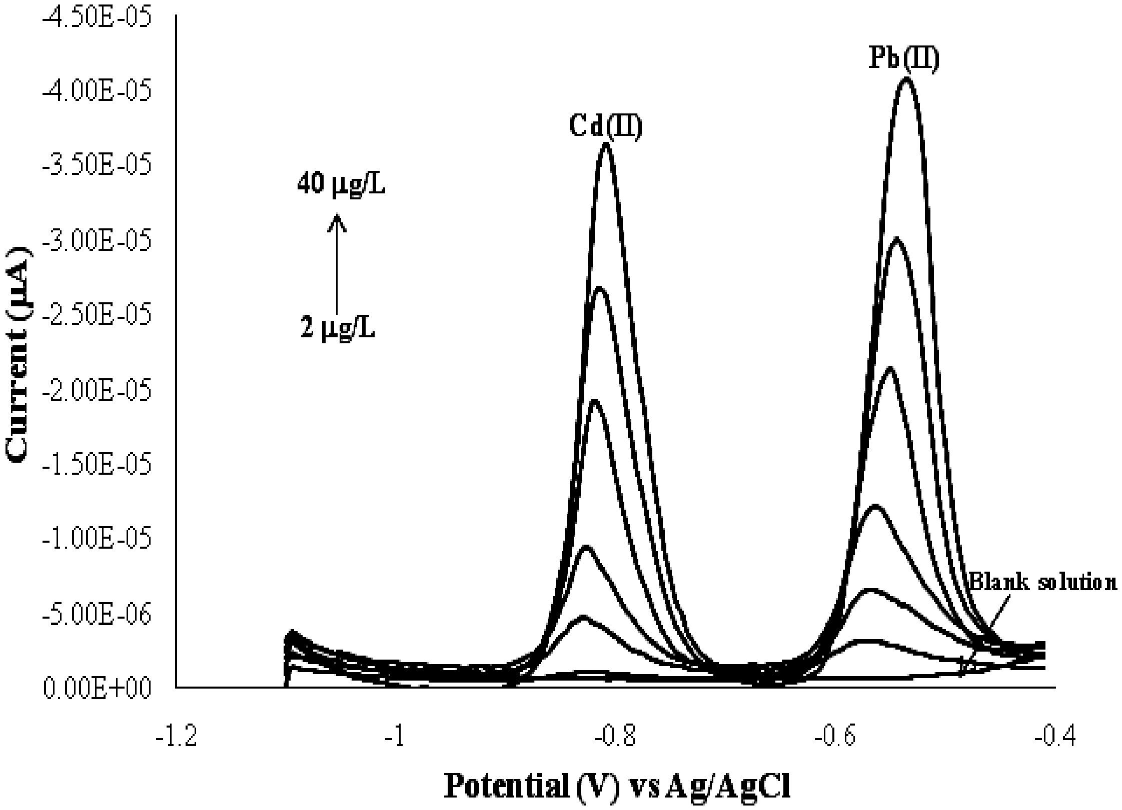


Figure 3-10 The voltammograms of Pb(II) and Cd(II) in the range of 2 to 40 μg/L

3.2.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of Pb(II) and Cd(II) were studied by measuring the current of ten replications of 2 μg/L Pb(II) and Cd(II). The limit of detection and limit of quantification were calculated following section 2.3.6.2 and 2.3.6.3, respectively (Coelho *et al.,* 2002).

The current of 2 μg/L Pb(II) and Cd(II) were carried out for evaluating limit of detection and limit of quantification, the results exhibits the limit of detection (LOD) and limit of quantification (LOQ) of 0.3 μg/L and 1.1 μg/L for Pb(II) and 0.1 μg/L and 0.4 μg/L for Cd(II), respectively. The results are shown in Table 3-3.

Table 3-3 Current from 10 replicates of 2 µg/L Pb(II) and Cd(II) (n = 10)

Replicate	**Current (µA)**		
	Pb(II)	**Cd(II)**	
1	3.183	1.053	
2	3.019	1.12	
3	3.015	1.013	
4	3.056	1.001	
5	3.163	1.022	
6	3.278	1.079	
7	3.19	1.056	
8	3.073	1.022	
9	2.944	1.097	
10	3.164	1.046	
Average	3.109	1.051	
SD (σ)	0.10	0.04	
%RSD	3.30	3.66	
Calibration Slope (m)	0.968	0.912	
LOD (3σ/m)	0.3	0.1	µg L^{-1}
LOQ (10σ/m)	1.1	0.4	µg L^{-1}

3.2.3 Accuracy and Precision

The accuracy of the system was studied by examining the relative percent error of Pb(II) and Cd(II) at spiked concentration of Pb(II) and Cd(II) standard solution of 2, 20 and 40 µg/L in water lily stem samples. The relative percent error for each sample was calculated from the calibration equation to obtain concentration of Pb(II) and Cd(II) and calculated from the equation (2.2). The obtained results are given in the Table 3.4, the relative percent error for Pb(II) and Cd(II) were in agreement with the value of spiked samples.

Table 3-4 Accuracy test for the studied method using water lily stem samples (n = 3) spiked with 20 µg/L of Pb(II) and Cd(II)

	Conc. Pb(II) (µg/L)	**%RE**	**Conc. Cd(II) (µg/L)**	**%RE**
Sample + 20 µg/L	20.1	100.5	18.6	93.0

In addition, the precision of the proposed method was also evaluated as %RSD of ten replication measurements. The %RSD of 20 µg/L Pb(II) and Cd(II) determinations obtained from this method were 2.26 and 1.71% , respectively. The results are shown in Table 3-5.

Table 3-5 The currents for evaluating the precision

Replicate	**Current (µA)**	
	Pb(II)	**Cd(II)**
1	21.008	19.083
2	20.300	18.807
3	21.235	18.944
4	20.308	19.553
5	20.432	19.005
6	20.147	19.398
7	20.089	19.156
8	20.248	18.540
9	21.061	19.532
10	21.219	18.887
Average	20.605	19.091
SD	0.47	0.33
%RSD	2.26	1.71

3.3 Interferences of some coexisting ions with the determination of Pb(II) and Cd(II)

The effect of the presence of other metal ions present in the solution on the anodic peak currents of Pb(II) and Cd(II) were evaluated. The interference study was performed by detecting with 20 μg/L Pb(II) and Cd(II) solutions with that in the copresence of 200 μg/L of one of the selected foreign ions. Using the criterion for interference of a relative error of more than ± 10% (Xiao *et al.*, 2014), no interference from the presence of Ca(II), Mg(II), Zn(II), Ni(II), Hg(II) and Fe(II). However, the interference caused by 10-fold Cu(II) is relatively bigger, with more than 30% change for both Pb(II) peak and Cd(II) peak

Table 3-6 Interferences of some metal ions (200 μg/L) on the stripping peak currents of 20 μg/L Pb(II) and Cd(II)

Interferences	**Contribution (%)** **(i_p.Pb(II) = 100%)**	**Contribution (%)** **(i_p.Cd(II) = 100%)**
Ca^{2+}	0	0
Mg^{2+}	0	0
Zn^{2+}	-7.08	-5.40
Ni^{2+}	-5.03	-7.27
Cu^{2+}	-31.00	-42.97
Hg^{2+}	-5.95	-6.02
Fe^{3+}	+2.84	+0.35

i_p : peak current

However, the interfering effect of Cu(II) can be eliminated by the addition of 0.01 mM of ferrocyanide added to the solution reagent (Yang *et al.*, 2014). This is because the interfering effect can be alleviated by masking the Cu(II) in the solution via selective complexation with ferrocyanide.

3.4 Electrochemical response of NanoSiO_2-CTS/BiFE

The differential pulse anodic stripping voltammograms of 20 μg/L Pb(II) and Cd(II) obtained with four different electrodes in the potential range of -1.1 to -0.4 V under the same conditions are shown in Figure 3-11. The BiFE provided higher peak currents than those obtained with the bare GCE, which is likely to be due to the contribution of the Bi film on the GCE surface. Bi can form “fused” alloys with lead and cadmium can also increase the stripping peak currents. Higher peaks still were found with the NanoSiO_2-CTS, likely to be due to the negatively charged metal chlorocomplexes (MCl_4^{2-}) and the protonated amino groups ($–NH_3^+$) of chitosan molecule. Moreover, the increased surface area of the silica nanoparticles provides increased number of the active surface sites for metal deposition. However, the highest peaks were obtained with the NanoSiO_2-CTS/BiFE because the bismuth-film used to improve electric conductivity of chitosan which increasing the stripping peak currents. These results indicated that the current responses for the detection of Pb(II) and Cd(II) can be increased by using a NanoSiO_2-CTS/BiFE which improve sensitivity about 1.5 time both peak when compared with BiFE and NanoSiO_2-CTS, about 2 time for Pb(II) and 3 time for Cd(II) when compared with bare glassy carbon electrode.

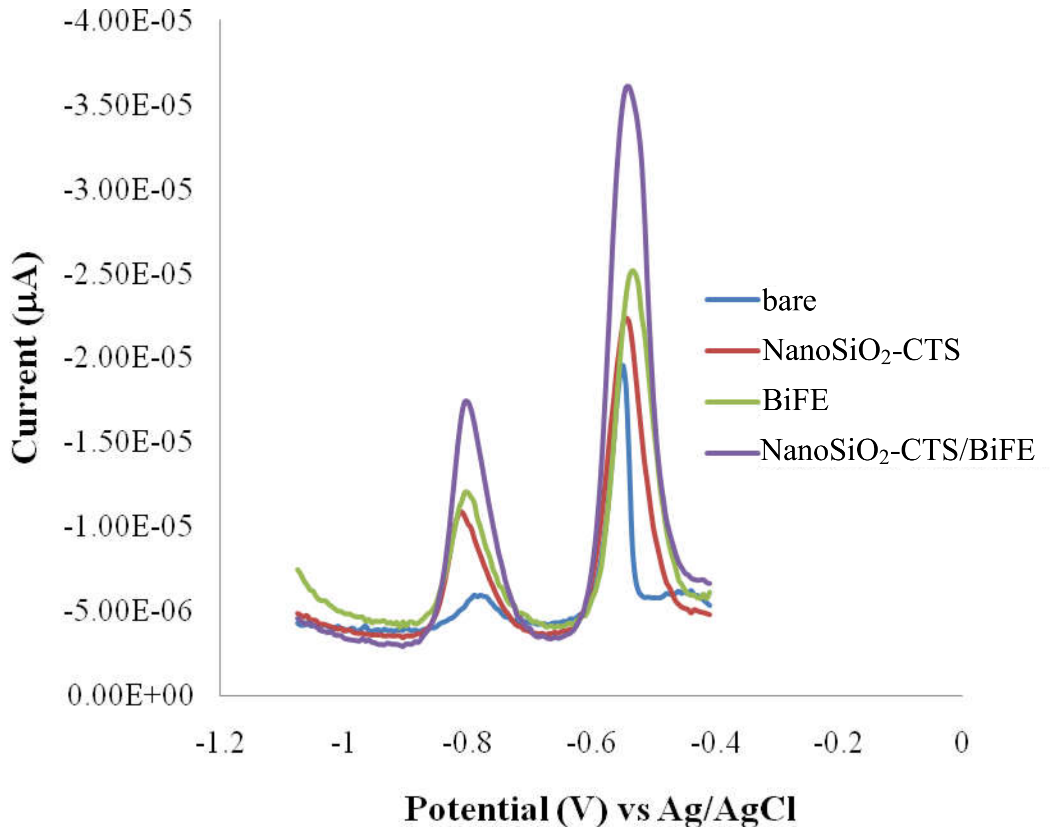


Figure 3-11 The differential pulse anodic stripping voltammograms of 20 µg/L Pb(II) and Cd(II) obtained with four different electrodes

3.5 Application of the studied method to water lily stem samples

3.5.1 Determination of Pb(II) and Cd(II) in water lily stem samples using the studied method (ASV)

The studied method was applied to the determination of Pb(II) and Cd(II) in water lily stem samples from five sites in Thale Noi, Phatthalung Province, South of Thailand.

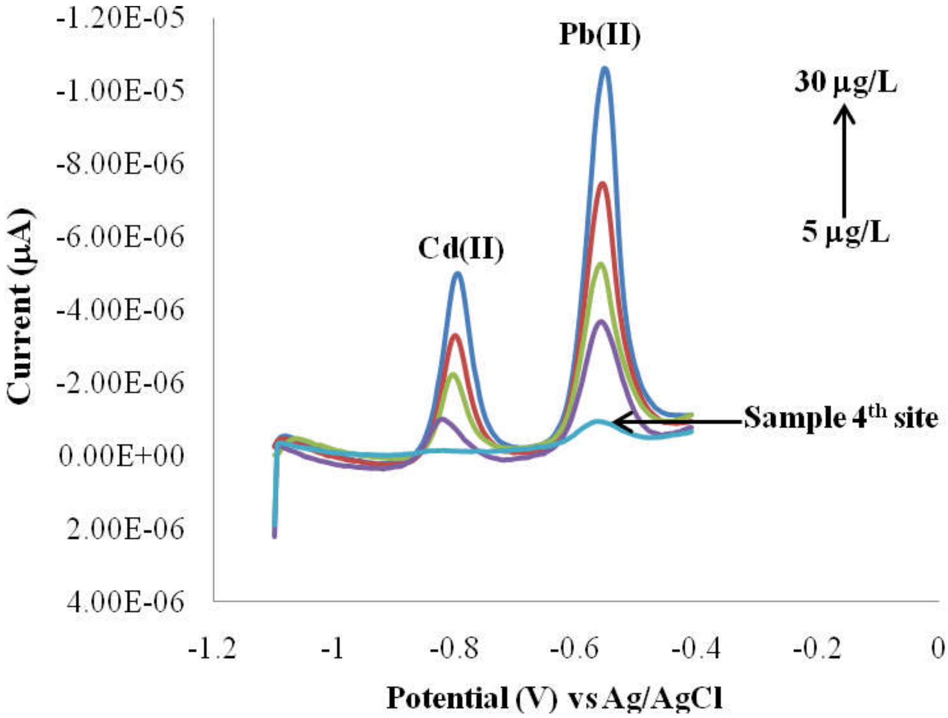
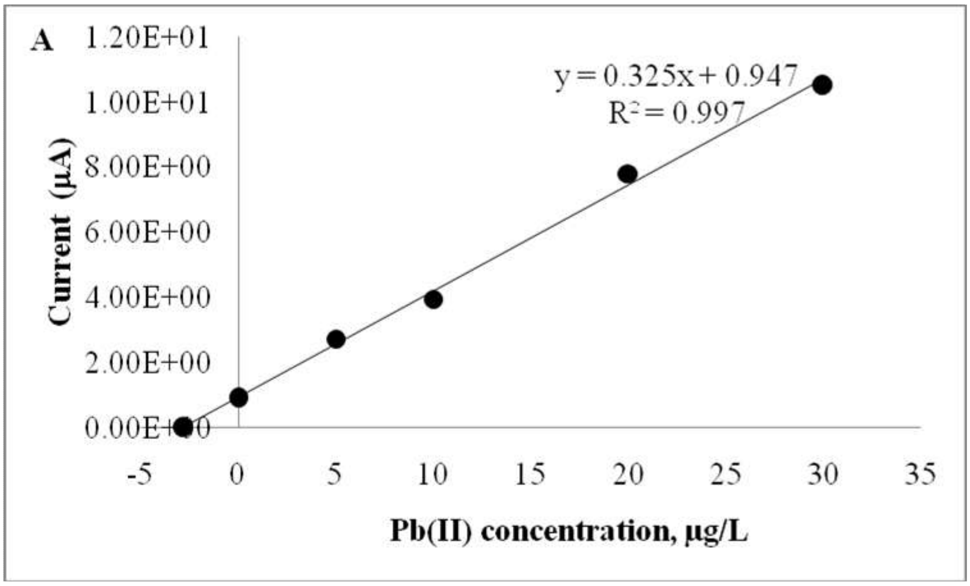


Figure 3-12 Differential pulse anodic stripping voltammograms of a water lily stem sample 4th site and standard solution of Pb(II) and Cd(II) ranging in concentration from 5 to 30 µg/L in 0.1 M HCl/KCl (pH 2.2). Other conditions are as in **Fig. 3-2**

The DPASV voltammogram of Pb(II) and Cd(II) determination in water lily stem sample from the 4th site at Thale Noi as shown in Figure 3-12 and the standard addition calibration curve of Pb(II) and Cd(II) in water lily stem sample are shown Table 3-15 and Figure 3-13.

Table 3-7 The results of standard addition calibration curve of Pb(II) and Cd(II) in water lily stem sample from the 4th site.

Pb(II) and Cd(II) conc. (µg/L)	**Current (µA)**	
	Pb(II)	**Cd(II)**
Sample 4th	0.902	0.141
	0.906	0.141
	1.000	0.130
Average	0.908	0.137
SD	0.01	0.01
%RSD	0.79	4.62
Sample 4th + 5 µg/L	2.751	0.912
	2.737	0.916
	2.669	0.926
Average	2.719	0.918
SD	0.04	0.01
%RSD	1.71	0.79
Sample 4th + 10 µg/L	3.904	1.882
	3.972	1.786
	3.916	2.000
Average	3.931	1.791
SD	0.04	0.09
%RSD	0.92	4.98
Sample 4th + 20 µg/L	7.832	2.929
	7.841	2.988
	7.747	2.949
Average	7.807	2.955
SD	0.05	0.03
%RSD	0.66	1.02
Sample 4th + 30 µg/L	10.470	4.794
	10.621	4.769
	10.562	4.791
Average	10.551	4.785
SD	0.08	0.01
%RSD	0.72	0.29



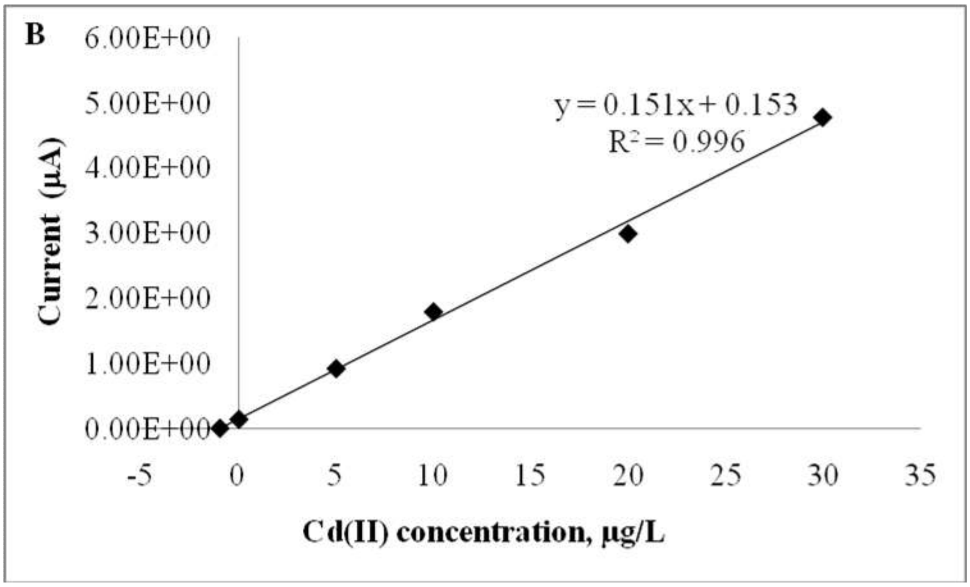


Figure 3-13 Standard addition calibration curve of (A) Pb(II) and (B) Cd(II) in water lily stem sample from the 4th region, Pb(II) and Cd(II) ranging in concentration from 5 to 30 µg/L

The results of Pb(II) and Cd(II) determination in water lily stem samples from five sites at Thale Noi are shown in Table 3-16 for Pb(II) and Table 3-17 for Cd(II). The results suggested that the concentrations of Pb(II) and Cd(II) in water lily stem were at the trace levels.

Table 3-8 The concentration of Pb(II) in water lily stem samples from Thale Noi.

Sites	**Pb(II) concentration (µg/L)**	**Pb(II) concentration (mg/kg)**
1	1.13	0.11
2	< LOQ	< LOQ
3	< LOQ	< LOQ
4	2.87	0.29
5	1.62	0.16

< LOQ: Lower than limit of quantitative.

Table 3-9 The concentration of Cd(II) in water lily stem samples from Thale Noi.

Sites	**Cd(II) concentration (µg/L)**	**Cd(II) concentration (mg/kg)**
1	< LOQ	< LOQ
2	< LOQ	< LOQ
3	< LOQ	< LOQ
4	0.98	0.10
5	0.65	0.07

< LOQ: Lower than limit of quantitative.

The concentrations of Pb(II) and Cd(II) in various water lily stem samples were found in the range of 0.00-0.29 mg/kg and 0.00-0.10 mg/kg, respectively, which are within the range of the permissible limit in edible portions of plants recommended by World Health Organization (WHO) (0.5 mg/kg for Pb(II) and 0.2 mg/kg for Cd(II)). Therefore, the Pb(II) and Cd(II) concentration in water lily stem samples under the investigation is safety for public health.

3.5.2 Comparison between the studied method and ICP-OES for Pb(II) and Cd(II) determination in water lily stem samples.

The results from determination of Pb(II) and Cd(II) using the studied method (DPASV) and ICP-OES as shown in Table 3-18 for Pb(II) and Cd(II). The means of Pb(II) and Cd(II) concentration in water lily stem samples determined by using the studied method (DPASV) and ICP-OES were compared.

From the statistical evaluation, it was found that the amount of Pb(II) by using the two methods was found to be in site at 1, 4 and 5. For the amount of Cd(II) was able to by using the studied method (DPASV). The results provided by the studied method (DPASV) and ICP-OES were in a good agreement [The limit of detection (LOD) of ICP-OES is 5.0 µg/L of Pb(II) and 1.0 µg/L of Cd(II)].

Table 3-10 The concentration of Pb(II) in water lily stem samples determined by the studied method under investigation and ICP-OES

Sites	**Concentration, (mg/kg)**			
	Pb(II)		**Cd(II)**	
	DPASV	**ICP-OES**	**DPASV**	**ICP-OES**
1	0.11	0.10	< LOQ	< LOQ
2	< LOQ	< LOQ	< LOQ	< LOQ
3	< LOQ	< LOQ	< LOQ	< LOQ
4	0.29	0.28	0.10	< LOQ
5	0.16	0.16	0.07	< LOQ
Mean	0.19	0.18	0.09	-
t calculate	2.0		-	
t Critical two-tail	4.3		-	

< LOQ: Lower than limit of quantitative.

It can be concluded that the proposed method is effective to be used for Pb(II) and Cd(II) determination in water lily stem samples. Moreover, the results from determination of Pb(II) by this method were compared to those obtained from the ICP-OES method. No significant difference between the two sets of results were found (paired t-test at the 95% confidence level where the calculated t value was smaller than critical t value (4.3)) as shown in Appendix C-1, implying that these results are acceptable.

CHAPTER 4
CONCLUSION

Lead and cadmium in the environment can be toxic to human beings, even at very low concentration. Thus, monitoring the level of heavy metal ions has become a serious environmental problem.

In this research, ex-situ bismuth film modified with mesoporous silica nanoparticles and chitosan (NanoSiO_2–CTS/BiFE) was used as a working electrode for simultaneously detection of Pb(II) and Cd(II) in water lily stem samples. The anion exchange/electrostatic attraction between the negatively charged metal chlorocomplexes (MCl_4^{2-}) and the protonated amino groups ($–NH_3^+$) of chitosan molecule. The resulting accumulated of MCl_4^{2-} on the surface of electrode were stripped off by differential pulse anodic stripping voltammetry (DPASV). The conditions for operating the system efficiently have been optimized and the optimum conditions were obtained as summarized in Table 3-9 (page 47).

Under optimized conditions, the linear correlation coefficient (R^2) of 0.998 for both Pb(II) and Cd(II) after a 240 s deposition time. The limits of detection (LOD) and limits of quantification (LOQ) were obtained of 0.3 μg/L and 1.1 μg/L for Pb(II) and 0.1 μg/L and 0.4 μg/L for Cd(II), respectively. Relative standard deviations (%RSD) of 20 μg/L Pb(II) and Cd(II) determinations obtained from this method were 2.26 and 1.71%, respectively. Effect of interferences caused by cations commonly found water lily, especially, Ca(II), Cu(II), Fe(III), Hg(II), Mg(II), Ni(II) and Zn(II), levels were investigated. Cu(II) interference in this method, which could improve the peak signal current of Pb(II) and Cd(II) and remove Cu(II), 0.01 mM of ferrocyanide added to the solution.

The modified electrode was applied to determination of Pb(II) and Cd(II) in water lily stem samples taken from five sites in Thale Noi, Phatthalung Province, South of Thailand. The developed method has accuracy for the determination of those metals in about 100.5 for Pb(II) and 93.0 for Cd(II). The concentration of Pb(II) and Cd(II) in various water lily stem samples were found in range of 0.00-0.29 mg/kg and 0.00-0.10 mg/kg, respectively, which is using a standard addition method. However, the concentrations of Pb(II) and Cd(II) in water lily stem samples were lower than the permissible limit in edible portions of plants (0.5 mg/kg for Pb(II) and 0.2 mg/kg for Cd(II)) issued by the World Health Organization (WHO). Therefore, the Pb(II) and Cd(II) concentration in water lily stem samples under the investigation is safety for public health.

The results were in satisfactory agreement with the reference values obtained by ICP-OES method, demonstrating the practical analytical utility of the method. Therefore, the bismuth film modified with mesoporous silica nanoparticles and chitosan (NanoSiO_2-CTS/BiFE) electrode was successfully applied to the simultaneous determination of Pb(II) and Cd(II) at trace levels in water lily stem samples.

For further study, various extraction methods for extraction and preconcentration of heavy metals from water lily stem samples in Thale Noi and detection of heavy metals by anodic stripping voltammetry using a bismuth film electrode modified with mesoporous silica nanoparticles and chitosan (NanoSiO_2-CTS/BiFE).

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APPENDICES

APPENDIX A

OPTIMIZATION OF THE MEASUREMENT PARAMETERS

Appendix A-1 Effect of plating potential on 20 µg/L Pb(II) and Cd(II) (n = 3)

Plating potential (V)	**Peak height (µA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
-0.6	-10.803	-6.737	1.66	4.38
-0.7	-15.303	-11.517	1.01	2.48
-0.8	-19.677	-13.387	1.29	3.52
-0.9	-15.293	-10.530	0.95	1.89
-1.0	-12.787	-8.473	1.02	4.13

Appendix A-2 Effect of plating time on 20 µg/L Pb(II) and Cd(II) (n = 3)

Plating time (s)	**Peak height (µA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
60	-9.297	-6.310	1.99	2.55
120	-18.877	-11.957	0.58	0.78
150	-21.180	-13.717	1.10	1.53
180	-18.990	-12.170	1.52	2.28
240	-15.460	-9.310	2.69	2.61
300	-12.567	-8.210	1.43	2.01

Appendix A-3 Effect of concentration of chitosan on 20 µg/L Pb(II) and Cd(II) (n = 3)

Concentration of chitosan (%wt)	**Peak height (µA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
0.10	-15.493	-8.797	2.28	2.45
0.15	-18.557	-9.640	2.21	5.35
0.20	-21.423	-13.620	1.68	0.75
0.25	-18.710	-11.643	1.95	1.90
0.30	-15.317	-9.500	2.68	3.14

Appendix A-4 Effect of pH 0.1 mol/L HCl/KCl on 20 µg/L Pb(II) and Cd(II) (n = 3)

pH 0.1 mol/L HCl/KCl	**Peak height (µA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
1.7	-10.623	-5.603	2.89	6.55
2.0	-13.770	-7.337	2.37	1.94
2.1	-19.430	-11.503	1.78	2.18
2.2	-22.797	-13.307	0.77	0.93
2.3	-21.387	-11.210	0.84	5.79
2.4	-15.220	-8.770	1.02	1.95

Appendix A-5 Effect of deposition potential on 20 μg/L Pb(II) and Cd(II) (n = 3)

Deposition potential (V)	**Peak height (μA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
-0.8	-14.673	-9.700	3.06	3.04
-0.9	-19.610	-12.773	1.99	1.94
-1.0	-21.023	-13.610	2.09	0.74
-1.1	-22.470	-15.320	1.12	0.17
-1.2	-18.610	-10.470	1.73	3.30
-1.3	-15.537	-7.480	2.43	4.83

Appendix A-6 Effect of deposition time on 20 μg/L Pb(II) and Cd(II) (n = 3)

Deposition time (s)	**Peak height (μA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
60	-6.337	-3.360	3.61	6.74
120	-13.277	-8.350	2.70	5.24
180	-21.823	-13.52	0.65	2.17
240	-28.400	-16.633	3.46	1.89
300	-30.563	-18.847	1.64	2.46
360	-31.733	-21.653	2.01	0.99
420	-33.387	-23.293	0.82	3.68
480	-35.767	-23.603	1.53	1.49

Appendix A-7 Effect of equilibration time on 20 μg/L Pb(II) and Cd(II) (n = 3)

Equilibration time (s)	**Peak height (μA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
0	-14.350	-6.213	3.31	4.33
10	-19.447	-12.633	0.65	2.17
20	-22.390	-15.283	2.22	1.52
30	-24.553	-17.650	3.86	5.04
40	-24.727	-17.720	4.08	1.08

APPENDIX B

Analytical performances STATISTICAL ANALYSIS

Appendix B-1 The current of Pb(II) and Cd(II) at the different concentrations (n = 3)

Concentration (µg/L)	**Current (µA)**		**%RSD**	
	Pb(II)	**Cd(II)**	**Pb(II)**	**Cd(II)**
2	-3.164	-1.046	0.17	0.06
5	-6.552	-4.611	0.15	3.60
10	-12.085	-9.459	0.50	1.06
20	-21.373	-18.949	0.08	0.38
30	-29.992	-26.715	0.39	0.37
40	-40.718	-36.379	0.33	0.63

APPENDIX C
STATISTICAL ANALYSIS

Appendix C-1 The comparison between the proposed method and the reference ICP–OES method was made by t-test

The results from determination of Pb(II) by this method were compared to those obtained from the ICP-OES method.

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.186666667	0.18
Variance	0.008633333	0.0084
Observations	3	3
Pearson Correlation	0.998136709	
Hypothesized Mean Difference	0	
df	2	
t Stat	**2**	
P(T<=t) one-tail	0.09175171	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.183503419	
t Critical two-tail	**4.30265273**	

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List of Publication and Proceeding

Monthira Somkid and Pipat Chooto. Optimization of differential pulse anodic stripping voltammetry for the simultaneous determination of lead (II) and cadmium (II) using a bismuth film electrode modified with mesoporous silica nanoparticles and chitosan. The 40th National Graduate Research Conference. October 20 – 21, 2016, Hat Yai, Songkhla, Thailand.