Flow Injection – Anodic Stripping Voltammetry for As(III) and As(V) Determination

Amornrat Suwan-in

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Analytical Chemistry
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ชื่อวิทยานิพนธ์	โฟลอินเจคชัน – อะโนดิกสทริปปิงโวลแทมเมตรีสำหรับการวิเคราะห์ As(III) และ As(V)
ผู้เขียน	นางสาวอมรรัตน์ สุวรรณอินทร์
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บทคัดย่อ

งานวิจัยนี้พัฒนาการหาปริมาณ As(III) และ As(V) ด้วยเทคนิคโฟลอินเจคชัน – อะโนดิกสทริปปิงโวลแทมเมตรี โดยใช้ขั้วฟิล์มทองเคลือบบนขั้วกลาสซีคาร์บอนเป็นขั้วไฟฟ้าทำงาน ขั้วซิลเวอร์/ซิลเวอร์คลอไรด์เป็นขั้วไฟฟ้าอ้างอิง และขั้วสแตนเลส บล็อค เป็นขั้วไฟฟ้าช่วย ได้ศึกษาสภาวะที่เหมาะสมต่อการเตรียมขั้วไฟฟ้าฟิล์มทอง ได้แก่ ศักย์ไฟฟ้าการเกาะติด -0.40 โวลต์ เวลาในการเกาะติด 90 วินาที ความเข้มข้นของสารละลายทอง 100 มิลลิกรัมต่อลิตร และอัตราการไหลของสารละลายทอง 1.0 มิลลิลิตรต่อนาที ขั้วไฟฟ้าฟิล์มทองที่เตรียมขึ้นสามารถนำมาใช้วิเคราะห์อย่างต่อเนื่องได้ 8 ครั้งโดยค่าความไวในการวิเคราะห์ไม่เปลี่ยนแปลง สำหรับการศึกษาสภาวะที่เหมาะสมต่อการวิเคราะห์ As(III) พบว่าความเข้มข้นที่เหมาะสมของสารละลายกรดไฮโดรคลอริกซึ่งใช้เป็นสารละลายเกื้อหนุนคือ 0.2 โมลต่อลิตร ศักย์ไฟฟ้าในการเกาะติด -0.30 โวลต์ อัตราการสแกน 90 มิลลิโวลต์ต่อวินาที อัตราการไหล 2.5 มิลลิลิตรต่อนาที และเวลาในการเกาะติด 240 วินาที

สำหรับการวิเคราะห์ As(V) ซึ่งไม่ตอบสนองทางไฟฟ้าเคมีที่สภาวะดังกล่าวจึงจำเป็นต้องรีดิวซ์เป็น As(III) ก่อนการวิเคราะห์ ได้ทำการศึกษาตัวรีดิวซ์ชนิดต่างๆ ได้แก่ โซเดียมไทโอซัลเฟต โซเดียมไดไทโอไนท์ แอล-ซิสทีอีน โพแทสเซียมไอโอไดด์ และสารละลายผสมระหว่างกรดแอสคอร์บิค และโพแทสเซียมไอโอไดด์ พบว่าโพแทสเซียมไอโอไดด์มีประสิทธิภาพดีที่สุด จากการศึกษาสภาวะที่เหมาะสมพบว่าความเข้มข้นของโพแทสเซียมไอโอไดด์ 6.5 มิลลิโมลาร์ และเวลาในการรีดิวซ์ 45 นาที ให้ประสิทธิภาพในการรีดิวซ์ As(V) เป็น As(III) ดีที่สุด

ประสิทธิภาพของวิธีวิเคราะห์ พบว่า ความเป็นเส้นตรงของ As(III) และ As(total) มีค่าอยู่ในช่วง 1.0 ถึง 30 ไมโครกรัมต่อลิตรและ 2.0 ถึง 100 ไมโครกรัมต่อลิตร ตามลำดับ ขีดจำกัดในการตรวจวัด As(III) มีค่า 0.42 ไมโครกรัมต่อลิตร และ As(total) มีค่า 1.47 ไมโครกรัมต่อลิตร ขีดจำกัดในการหาปริมาณ As(III) มีค่า 1.39 ไมโครกรัมต่อลิตร และ As(total) มีค่า 4.90

ไมโครกรัมต่อลิตร ส่วนเบี่ยงเบนมาตรฐานสัมพัทธ์ (n =11) ในการวิเคราะห์ As(III) น้อยกว่า 5 เปอร์เซ็นต์ (ที่ระดับความเข้มข้น 2 และ 20 ไมโครกรัมต่อลิตร) และในการวิเคราะห์ As(total) น้อยกว่า 8 เปอร์เซ็นต์ (ที่ระดับความเข้มข้น 10 และ 100 ไมโครกรัมต่อลิตร)

นอกจากนี้ยังทำการทดสอบความถูกต้องในการวิเคราะห์ โดยการวิเคราะห์สารอ้างอิงมาตรฐานของตัวอย่างน้ำธรรมชาติ (เอส อาร์ เอ็ม 1640) ผลการศึกษาพบว่ามีค่าความถูกต้องของการวิเคราะห์ดี สำหรับผลการศึกษาร้อยละการได้กลับคืนของวิเคราะห์ในตัวอย่างน้ำธรรมชาติ พบว่าค่าร้อยละการได้กลับคืนของ As(III) อยู่ในช่วง 85-105 เปอร์เซ็นต์ As(V) อยู่ในช่วง 85-106 เปอร์เซ็นต์ และ As(total) อยู่ในช่วง 88-103 เปอร์เซ็นต์

ทำการศึกษาผลของการรบกวนจากแคตไอออนชนิดต่างๆ ที่พบในน้ำธรรมชาติโดยทั่วไป ได้แก่ Sb(III), Cd(II), Cu(II), Fe(II), Pb(II), Hg(II), Ni(II) และ Zn(II) รวมทั้งศึกษาระดับความเข้มข้นของตัวรบกวนต่อการวิเคราะห์ด้วย

วิธีการที่พัฒนาขึ้นได้นำไปประยุกต์ใช้ในการวิเคราะห์ตัวอย่างน้ำธรรมชาติจากบริเวณที่มีการปนเปื้อนของอาร์เซนิค ในตำบลร่อนพิบูลย์ อำเภอร่อนพิบูลย์ จังหวัดนครศรีธรรมราช ปริมาณของ As(III) ในตัวอย่างน้ำ มีค่าอยู่ในช่วงตั้งแต่ต่ำกว่าขีดจำกัดการตรวจวัดถึง 20.5 ไมโครกรัมต่อลิตร และ As(V) มีค่าอยู่ในช่วงต่ำกว่าขีดจำกัดการตรวจวัดถึง 1577 ไมโครกรัมต่อลิตร และเมื่อเปรียบเทียบปริมาณ As(total) ในตัวอย่างน้ำด้วยวิธีที่พัฒนาขึ้นกับวิธีมาตรฐาน อินดัคทีฟลี คอปเปิล พลาสมา ออปติคอล อิมีสชัน สเปคโทรเมตรี พบว่าทั้งสองวิธีให้ผลไม่แตกต่างกันที่ระดับความเชื่อมั่น 99 เปอร์เซ็นต์ (ที-เทสท์)

วิธีที่พัฒนาขึ้นประสบความสำเร็จในการนำไปประยุกต์ใช้ในตัวอย่างน้ำธรรมชาติ โดยเป็นวิธีที่สามารถเลือกใช้ในการวิเคราะห์ As(III) และ As(V) ได้อีกวิธีหนึ่ง ระบบนี้มีข้อดีคือ กึ่งอัตโนมัติ ใช้สารเคมีปริมาณน้อย การวิเคราะห์รวดเร็ว เหมาะสำหรับงานวิเคราะห์ประจำได้

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ABSTRACT

Flow injection – anodic stripping voltammetry for As(III) and As(V) determination was developed. Gold film coated glassy carbon was used as a working electrode, Ag/AgCl was used as a reference electrode and stainless block was used as an auxiliary electrode. The optimum conditions for pre-plated gold film electrode including the plating potential of -0.40 V, the plating time of 90 sec, the concentration of gold solution of 100 mg L^{-1} and the flow rate of gold solution of 1.0 mL min^{-1} were provided. The gold film electrode can be used up 8 consecutively analysis cycles without decline in sensitivity. For optimization of As(III) determination, a 0.2 M HCl was used as supporting electrolyte, the deposition potential of -0.30 V, scan rate of 90 mV sec^{-1}, flow rate of 2.5 mL min^{-1} and deposition time of 240 sec were obtained.

For As(V) determination, electroinactive As(V) was necessary reduced to As(III), some reducing agents, namely; sodium thiosulphate ($Na_2S_2O_3$), sodium dithionite ($Na_2S_2O_4$), L-cysteine, potassium iodide (KI) and a mixture of ascorbic acid and potassium iodide were investigated. Potassium iodide gave the best effectiveness. From the studies, 6.5 mM KI with reduction time of 45 minute was selected as optimum conditions to convert As(V) to As(III) effectively.

Analytical performance of the method, it was found that the linear ranges were 1.0-30 μg L^{-1} and 2.0-100 μg L^{-1} for As(III) and As(total), respectively. The limit of detection (LOD) was 0.42 μg L^{-1} for As(III) and 1.47 μg L^{-1} for As(III). The limit of quantification (LOQ) was 1.39 μg L^{-1} for As(III) and 4.90 μg L^{-1} for As(total). The relative standard deviations (n = 11) within 5% (at 2 and 20 μg L^{-1}) for As(III) and within 8% (at 10 and 100 μg L^{-1}) for As(total) were obtained.

In addition, the accuracy was performed by analysis of standard reference material (SRM 1640) and the results obtained were in good accuracy. The recoveries of method were in the range of 85-105% for As(III), 85-106% for As(V) and 88-103% for As(total) determinations.

Effect of interferences caused by cations commonly found in natural water, especially, Sb(III), Cd(II), Cu(II), Fe(II), Pb(II), Hg(II), Ni(II), Zn(II) and their interfering levels were investigated.

The developed method was applied to natural water samples which collected from arsenic contaminated area in Ron Phibun Sub-district, Ron Phibun District, Nakron Si Thammarat Province. Concentrations of As(III) in natural water samples were in the range of ND to 20.5 μg L^{-1} and As(V) in the range of ND to 1577 μg L^{-1}. The results of As(total) in natural water samples analyzed by the developed method (FI-ASV) were compared by Inductively Coupled Plasma – Optical Emission Spectrometric (ICP-OES) method. It was found that both methods were non-significant different at 99% confidence level (t-test).

The proposed method was successfully applied to natural water samples. It could be alternative method for As(III) and As(V) determination. The system provides the benefits of semi-automation, less amount of reagent consumption, short analysis time and suitability for routine analysis.

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Amornrat Suwan-in

THE RELEVANT OF THE RESEARCH WORK

Determination of As(III) and As(V) contaminated in natural water is a Master of Science Thesis in Analytical Chemistry. This research provides some new knowledge on the analysis technique relates to the environment. Organizations that apply use the outcome of this work include:

- Ministry of Public Health
- Ministry of Environment and Natural Resource
- Ministry of Education

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	=	Atomic absorption spectrometry
ACA	=	Ammonical copper arsenate
AES	=	Atomic emission spectrometry
AFS	=	Atomic fluorescence spectrometry
AsB	=	Arsenobetaine
AsC	=	Arsenochlorine
As(III)	=	Arsenite
As(V)	=	Arsenate
ASV	=	Anodic stripping voltammetry
CCA	=	Chromated copper arsenate
CCCSP	=	Constant current cathodic stripping potentiometry
CCSA	=	Constant current stripping analysis
CSV	=	Cathodic stripping voltammetry
dAsCP	=	Derivative anodic stripping chronopotentiometry
DMA	=	Dimethylarsenic acid
DPCSV	=	Differential pulse cathodic stripping voltammetry
DPP	=	Differential pulse polarography
Eh	=	Redox potential
EPA	=	Environmental protection agency
ETAAS	=	Electrothermal atomic absorption spectrometry
FAAS	=	Frame atomic absorption spectrometry
FeAsS	=	Arsenopyrite
FI-HGAAS	=	Flow injection - hydride generation atomic absorption spectrometry
$g\ cm^{-3}$	=	Gram per cubic centimeter
GC-MS	=	Gas chromatography - mass spectrometry

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

GFAAS	=	Graphite furnace atomic absorption spectrometry
$g\ mol^{-1}$	=	Gram per mol
HG	=	Hydride generation
HMDE	=	Hanging mercury drop electrode
HPLC - ICP-MS	=	High performance liquid chromatography - inductively coupled plasma - mass spectrometry
HPLC-MW-HGAAS	=	High performance liquid chromatography - microwave - hydride generation atomic absorption spectrometry
ICP-AES		Inductively coupled plasma - atomic emission spectrometry
ICP-MS	=	Inductively coupled plasma - mass spectrometry
ICP-OES	=	Inductively coupled plasma - optical emission spectrometry
$J\ mol^{-1}\ K^{-1}$	=	Joule per mol per Kelvin
$kJ\ mol^{-1}$	=	Kilo Joule per mol
LC-ICP-MS	=	Liquid chromatography - inductively coupled plasma - mass spectrometry
LD_{50}	=	50% lethal oral dose
LSV	=	Linear sweep voltammetry
MMA	=	Monomethylarsenic acid
$mg\ kg^{-1}$	=	Milligram per kilogram
$mg\ L^{-1}$	=	Milligram per liter
$\mu g\ L^{-1}$	=	Microgram per liter
NAA	=	Neutron activation analysis
$ng\ m^{-3}$	=	Nanogram per cubic meter
$n\Omega \cdot m$	=	Nano ohm meter
pm	=	Picometer
SRM	=	Standard reference material

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

SWCSV	=	Square wave cathodic stripping voltammetry
TMA	=	Trimethylarsenic acid
WHO	=	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Introduction

Arsenic is a naturally occurring element present in the environment in both inorganic and organic forms. It is known as a very toxic element and a carcinogen. The toxicity depends on chemical form, oxidation state and solubility in biological media (Somer and Almas, 2006). Inorganic arsenic is considered to be the most toxic form of the element and is found in groundwater and surface water, as well as in many foods. Arsenic toxicity could affect a wide variety such as skin and internal cancers, cardiovascular and neurological effects. Human exposure to inorganic arsenic occurs among smelter workers and the workers exposed in production and use of arsenic containing pesticides. Ingestion occurs mainly by drinking contaminated water. Most ingested arsenic is absorbed through the gastrointestinal tract and lungs and then into blood stream. It is distributed in lungs, liver, kidney, spleen, and intestine within 24 hours of ingestion and to skin, hair, and bone within two weeks. Harmful effects of arsenic on human health include acute and chronic toxicity (Smedley and Kinniburgh, 2002). People in many countries all over the world are suffering from the toxic effects of arsenic due to natural groundwater contamination as well as industrial effluent and drainage problems (Mandal and Suzuki, 2002).

In Thailand, one of the areas heavily contaminated with arsenic is at Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province of southern Thailand. The worst case of arsenic poisoning is related to mining activity. Health problems were first recognised in the area in 1987. By the late 1990s, around 1000 people had been diagnosed with arsenic related skin disorders, particularly in and close to Ron Phibun town. Arsenic concentrations up to 5000 mg L^{-1} have been found in shallow groundwaters from Quaternary alluvial sediment that has been extensively dredged during tin mining operations. Deeper groundwaters from an older limestone aquifer have been found to be less contaminated (Williams *et al*., 1996).

The WHO (1996) guideline for arsenic in drinking water was provisionally of 10 μg L^{-1}. Therefore, the development of method to arsenic determination at low level is important.

Arsenic in natural water can exist in two different oxidation states, As(III) and As(V), depending on the environment. The As(III) is more toxic than As(V). The ratio of As(V) to As(III) concentration in natural water is usually 0.1 : 1 to 10 : 1 (Li and Smart, 1996). Hence, the speciation analysis of arsenic species is more important than total arsenic determination. Speciation analysis is defined as analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample (Suzuki, 2005).

1.2 Background

1.2.1 Chemical and physical properties of arsenic

Arsenic is a metalloid with atomic number of 33 and symbol as “As”. It occurs in many ores and it is widely distributed in nature, being present in minute quantities in the soil, the sea and in living matter such as the human body. Arsenic is a naturally occurring element present in environment in both inorganic and organic forms and it is toxic and carcinogenic.

The physicochemical properties of arsenic are summarized in **Table 1-1**.

Table 1-1 Summary of some relevant physicochemical properties of arsenic

Atomic number	33
Electronic configuration	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^{10}\ 4s^2\ 4p^3$
Standard atomic weight	74.92160 g mol^{-1}
Density (near room temperature)	5.727 g cm^{-3}
Melting point	817 °C
Boiling point	614 °C (sublimes)
Heat of fusion	24.44 kJ mol^{-1}
Heat of vaporization	34.76 kJ mol^{-1}
Heat capacity (25 °C)	24.64 J mol^{-1} K^{-1}
Oxidation states	5, 3, 0, -3
Electronegativity	2.18 (Pauling scale)
Atomic radius	115 pm
Covalent radius	119 pm
van der Waals radius	185 pm
Electrical resistivity (20 °C)	333 nΩ·m
Mohs' hardness	3.5

Source: http//en.wikipedia.org/wiki/Arsenic

1.2.2 Arsenic in the environment

Arsenic is a rare element in the earth crust and it is found in various medias such as atmosphere, soils, rocks, natural waters and organisms. It is mobilized in the environment through a combination of natural processes such as weathering reactions, biological activity and volcanic emissions, as well as through a range of anthropogenic activities. Most environmental arsenic problems are the result of mobilization under natural conditions. However, man has had an important additional impact through mining activity, combustion of fossil fuels, the use of arsenical pesticides, herbicides and crop desiccants and the use of arsenic as an additive to livestock feed, particularly for poultry (Smedley and Kinniburgh, 2002).

As a result, arsenic is ubiquitous in environment, and humans are unavoidably exposed to this toxic metalloid. Under normal ecological conditions, the level of arsenic bioavailability is not a threat for human health. Soils may contain arsenic levels between 0.1 and 40 mg kg^{-1}, if the underlying bedrock is not disturbed or redistributed by natural or pedogenic processes. A large number of man-made arsenic compounds were used in agriculture as effective agents against pests, parasites or weeds and they gradually accumulated in the soil. Further, more concentrations of arsenic in the environment may be elevated due to certain other anthropological activities, resulting in significant increase in the human exposure to arsenic. Chemistry of inorganic arsenic in aquatic environment, especially of variable pH values and oxygen availability, is unusually complex. The important feature is that in highly aerated condition arsenate salts dissociated in all four arsenic acid (As^{5+}) species; H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-}. However, in mild reducing condition, arsenous acid (As^{3+}) species, H_3AsO_3, $H_2AsO_3^-$ and $HAsO_3^{2-}$ may be stable. Arsenic acid (As^{5+}) is the least toxic of the inorganic forms wheras arsenous acid (As^{3+}) is more toxic than arsenic acid. A large number of diverse chemical and biological reactions, namely; oxidation, reduction, adsorption, precipitation, methylation and volatilization participate actively in the cycling of this toxic element (**Figure 1-1**). These reactions control the availability of arsenic, and hence, arsenic concentrations effectively exposed to humans are governed by the arsenic speciation more than by the total amount of arsenic (Roy and Saha, 2002).

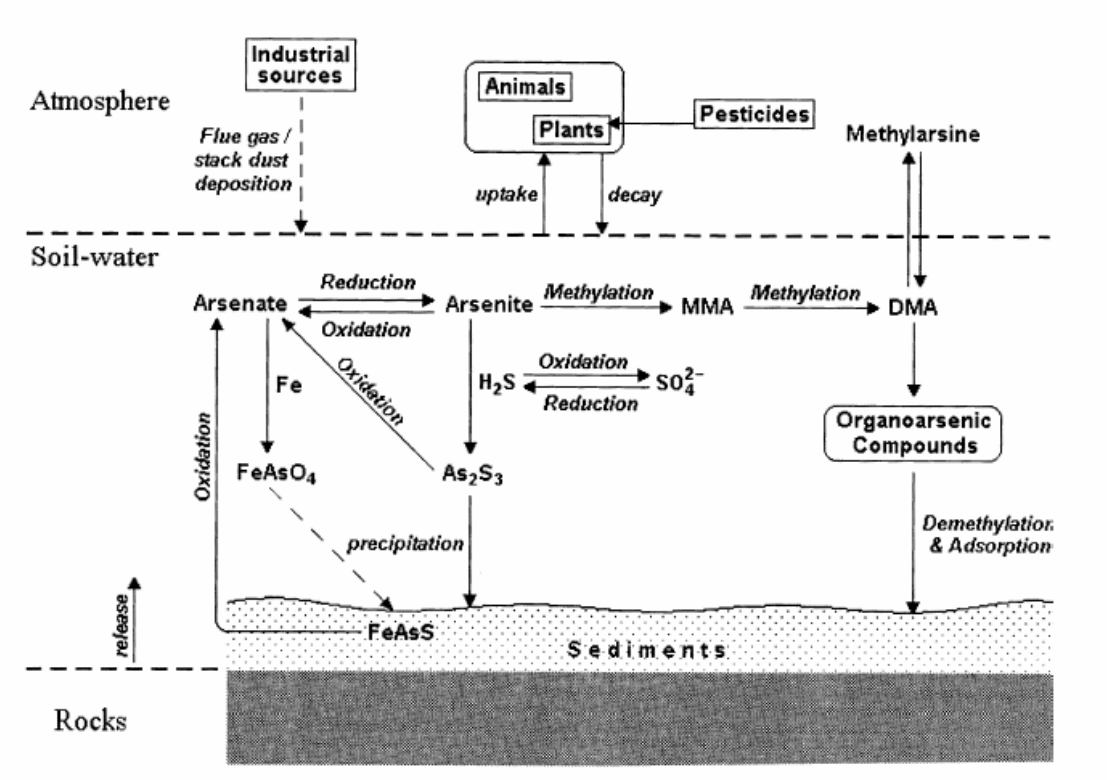

Figure 1-1 Arsenic cycle in the environment (Source: Roy and Saha, 2002)

Major reactions in the soil–water and sediment–rock systems influence the environmental transport, distribution and availability of arsenic. Oxygen availability controls the arsenate–arsenite redox reactions. Adsorption and precipitation of arsenate and arsenite immobilize the soluble arsenic. Slow release of arsenic from rocks and sediments or oxidative dissolution of arsenopyrite (FeAsS) from sediments contribute flux of arsenic in the environment. Methylation of arsenite to monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA) followed by other organoarsenic compounds, constitute the major biological reactions in the arsenic cycle (Roy and Saha, 2002).

In air, arsenic is predominantly absorbed on particulate matters, and usually present as a mixture of arsenite and arsenate, with the organic species being of negligible importance except in areas of arsenic pesticide application or biotic activity (Mandal and Suzuki, 2002). The human exposure of arsenic through air is generally

very low and normally arsenic concentrations in air ranges from 0.4 to 30 ng m^{-3} (WHO, 1996).

1.2.3 Arsenic in natural water

Arsenic is problematic in a number of major aquifers as a result of its relatively high mobility under the pH-redox conditions of most natural groundwater and its high toxicity. High concentration is found under both oxidizing and reducing conditions. Most problems are found in young aquifers with slow rates of groundwater flow such that aquifer flushing, and hence arsenic removal, has been restricted (Jindal, 2001).

Arsenic is perhaps unique among the heavy metalloid and oxyanion-forming elements in its sensitivity to mobilization at pH value typically found in groundwater (pH 6.5-8.5) and under both oxidizing and reducing conditions. Arsenic can occur in the environmental in several oxidation states (-3, 0, +3, +5) but in natural water is mostly found in inorganic form as oxidation of trivalent arsenite, As(III) or pentavalent arsenate, As(V). Organic arsenic forms may be produced by biological activity, mostly in surface waters, but are rarely quantitatively important. Organic forms may occur where waters are significantly impacted by industrial pollution (Smedley and Kinniburgh, 2002).

Most toxic trace metals occur in solution as cations, which generally become increasingly insoluble as the pH increases. At the near-neutral pH typical of most groundwater, the solubility of most trace-metal cations is severally limited by precipitation or coprecipitation with, an oxide, hydroxides clay or organic matter. In contrast, most oxyanions including arsenate tend to become less strongly sorbed as the pH increases. Under some conditions at least, the anions can persist in solution relatively high concentration (ten of $\mu g\ L^{-1}$) even at near-neutral pH values. Therefore the oxyanion-forming element, As, is some of the most common trace contaminants in groundwater. Arsenic is unique in being relatively mobile under reduced condition. It can be found at concentration in the mg L^{-1} range when all other oxyanion-forming metals are present in the $\mu g\ L^{-1}$ range (Smedley and Kinniburgh, 2002).

Redox potential (Eh) and pH are the most important factor controlling As speciation. Under oxidizing conditions, $H_2AsO_4^-$ is dominant at low pH (less than about pH 6.9), while at higher pH, $HAsO_4^{2-}$ becomes dominant (H_3AsO_4 and AsO_4^{3-} may be present in extremely acidic and alkaline conditions, respectively). Under reducing conditions at pH less than about pH 9.2, the uncharged arsenite species H_3AsO_3 will predominate **(Figure 1-2)**. The distributions of the species as a function of pH are given in **Figure 1-3**.

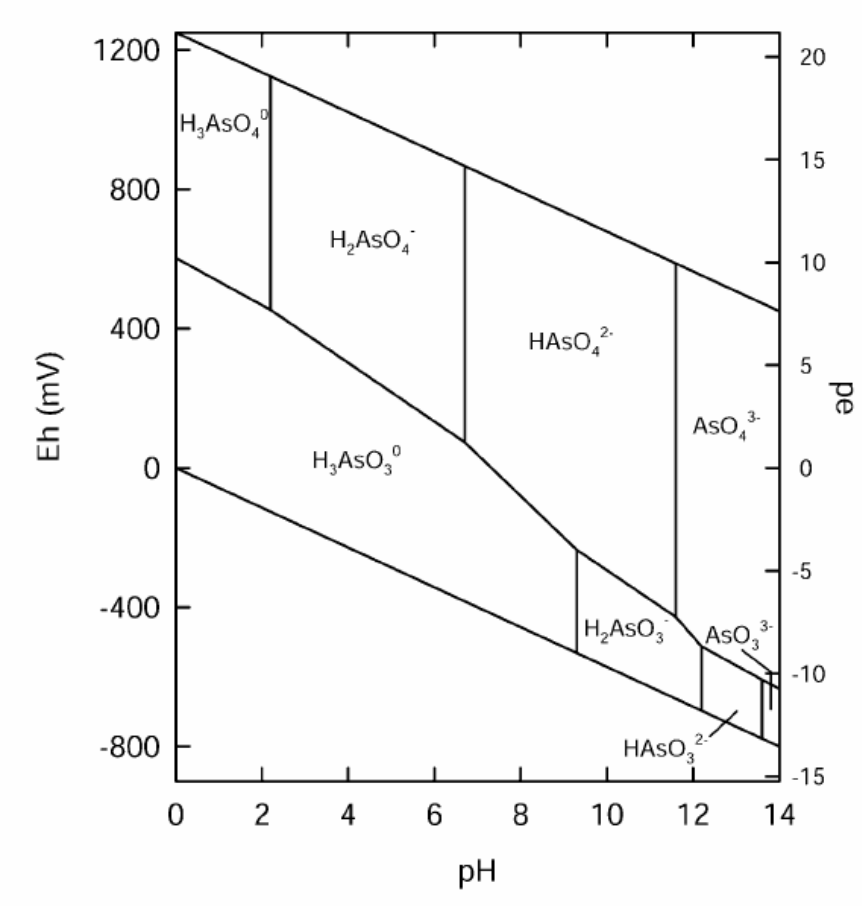

Figure 1-2 Eh-pH diagram for aqueous As species in the system As–O_2–H_2O (at 25°C and 1 bar total pressure)
(Source: Smedley and Kinniburgh, 2002)

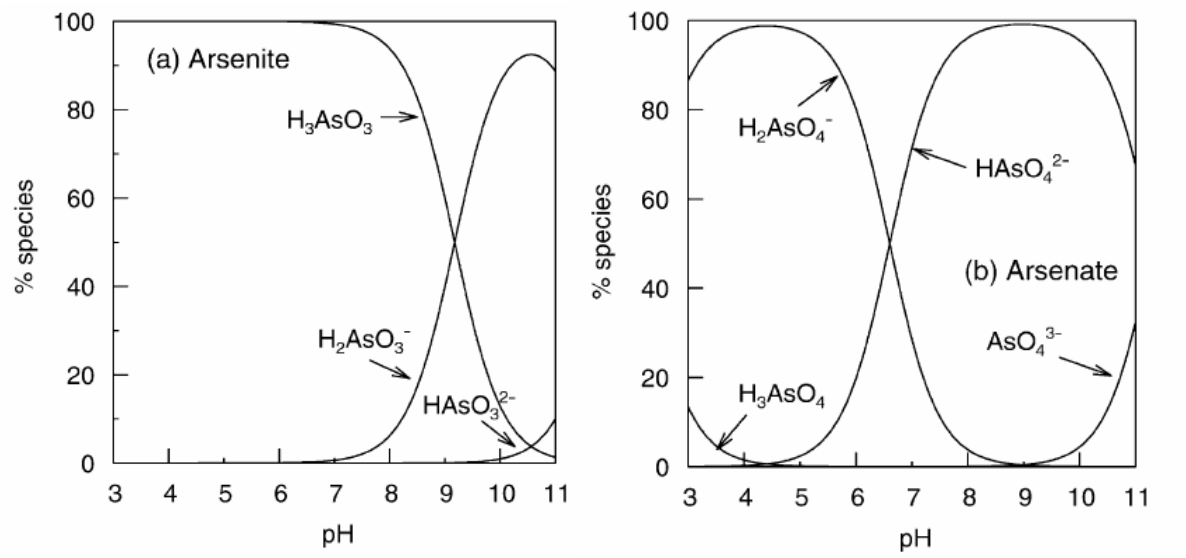

Figure 1-3 (a) Arsenite and (b) arsenate speciation as a function of pH (ionic strength of about 0.01 M). Redox conditions have been chosen such that the indicated oxidation state dominates the speciation in both cases (Source: Smedley and Kinniburgh, 2002).

1.2.4 Toxicity of arsenic

Exposure to environmental contaminants including arsenic can occur through one or more of three pathways; inhalation, ingestion and dermal absorption. In the case of arsenic, available evidence suggests that non-occupational exposure occurs primarily through the ingestion of food and water, with the inhalation pathway playing only a minor role. Food is more commonly the main contributor to total intake but in areas where drinking waters contain relatively high levels of arsenic, drinking water may be the most important source of arsenic intake. Intake via dermal absorption is believed to be negligible (Abernathy and Morgan, 2001).

Human exposure to inorganic arsenic occurs through inhalation, or skin absorption. Inhalation usually occurs occupationally or during cigarette smoking. In unpolluted areas the amount of arsenic inhaled is about 0.05 mg day^{-1} or less. Depending on the amount of arsenic in tobacco, an average smoker may inhale 20 μg day^{-1}. Skin exposure may occur among smelter workers and those exposed in the

production and use of arsenic containing pesticides. In most of food stuffs arsenic mainly occurs in the organic form and concentrations are usually less than 1 μg kg^{-1}. However, marine fish may contain arsenic up to 5 μg kg^{-1}. Ingestion occurs mainly by drinking contaminated water (Jindal, 2001).

Reactions of arsenic in human body are absorption, excretion and retention. These reactions are influenced by the amount and chemical forms in which it is ingested. Most ingested arsenic is absorbed through the gastrointestinal tract and lungs into blood stream. It is distributed in the lungs, liver, kidney, spleen and intestine within 24 hours of ingestion and to skin, hair and bone within 2 weeks. Inorganic arsenic entering the body undergoes methylation, which is a detoxification mechanism. First monomethylarsonate (MMA) is produced, which is then methylated to dimethylarsenate (DMA). The metabolites are less toxic than inorganic arsenic and bind less to tissues. In human body, trivalent arsenic is oxidized to the pentavalent state. The opposite can also take place. Arsenic (III) in the body combines with sulphydryl containing substances and inhibits the activity of many enzymes. It interferes with cell enzymes, cell respiration and mitosis (Jindal, 2001).

Toxicity of As(III) is explained in term of its great affinity for thiol group (S-H) in enzymes and proteins. Enzymes and protein are then inhibited (Jindal, 2001). The mechanism is shown in **Figure 1-4**.

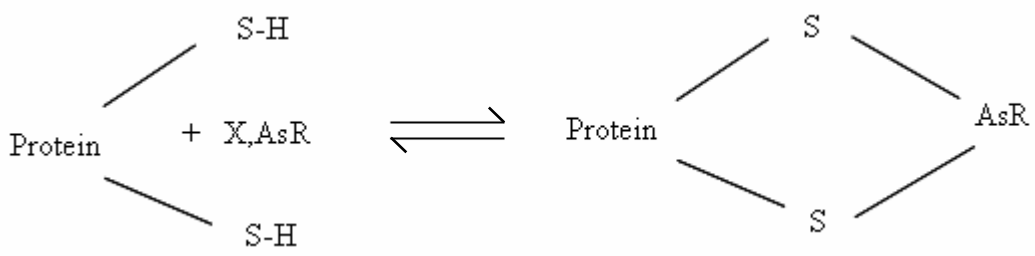

Figure 1-4 Affinity of As(III) for Thiol group in enzymes and proteins (Source: Jindal, 2001)

Arsenic affects all the organs and systems of the body. The toxicity of arsenic compounds depends on the chemical and physical forms of compound, the route by which it enters and the age and sex of the exposed individuals. Arsenite is

more toxic than arsenate. Arsenic in solution is more toxic than undissolved arsenic. Toxicity in order can be represented as follows; Arsine > inorganic arsenic > organic arsenic > arsonium compounds and elemental arsenic.

Among ingested arsenic, 5-10% is excreted in feces and 90-95% in urine. Small amounts are recovered in bile, feces, saliva and breast milk. The major metabolites found in the urine are methylarsenic acid and dimethylarsenic acid. A portion of the absorbed arsenic is deposited in the skin, hair and nails where it is firmly bound to keratin. Storage in these metabolically dead tissues is responsible for the slow elimination rate of arsenic. Arsenic in urine, hair and nails has been used as an index for monitoring the exposure of victim and urinary arsenic is generally reported as the most reliable indicator of recent exposure to inorganic arsenic. Blood arsenic is not considered a good indicator because it is cleared within a few hours of absorption. After administration, arsenic appears in urine within 2 to 8 hours. Approximately 10-30% of the ingested arsenic is directly excreted as the inorganic form, 10-20% as methylarsenic acid and 60-80% as dimethylarsenic acid. Unexposed people show arsenic concentration in urine 0.01-0.05 mg L^{-1}, in hair usually below 1 mg kg^{-1}, and in blood 0.0015-0.0025 mg L^{-1} (Abernathy and Morgan, 2001).

Arsenic has long been associated with toxic effects, producing marking impacts on health after both oral and inhalation exposure. Effects range from acute lethality to chronic effects, such as cancer and diseases of the vascular system. Studies in laboratory animals have demonstrated that the toxicity of arsenic is dependent on its form and its oxidation state. It is generally recognized that the soluble inorganic arsenicals are more toxic than the organic ones, and the trivalent form (arsenite) are more toxic than the pentavalent (arsenate). There are multiple end-points, with several different organ systems being affected, including the skin and respiration, cardiovascular, genitourinary, reproductive, gastrointestinal and nervous systems (Jindal, 2001).

Arsenic can cause acute and chronic toxicity. Acute toxicity occurs only from the ingestion of arsenic compounds; symptoms include severe vomiting and diarrhea, muscular cramps, facial edema and cardiac abnormalities. Ingestion dose of 70-180 mg of arsenic (III) oxide has been reported to be fatal in people. Symptoms may occur within a few minutes of exposure if the arsenic compound is in solution

but may be delayed for several hours if it is solid or taken with a meal. Chronic toxicity is best discussed in terms of organ systems affected, e.g. the skin, nervous system, liver, cardiovascular system and respiratory tract. Chronic effects develop very insidiously after six months to 2 years or more, depending on amount of arsenic ingested, the length of exposure, and the immunity level of the person (Jindal, 2001).

1.2.5 Techniques for arsenic determination

The important of arsenic detection is a well recognized fact that is emphasized by the extensive studies. At present, a plethora of detection methods have been developed. Most of them obtain limits of detection below the WHO arsenic guideline. There have been some reports on development of analytical methods for arsenic determination (**Table 1-2**), for example, hydride generation (HG) techniques in conjunction with atomic absorption spectrometry (AAS) (Nielson and Hansen, 1997) and atomic fluorescence spectrometry (AFS) (Semenova *et al*., 2000; Cava-Montesinos *et al*., 2003; Leal *et al*., 2006) , graphite furnace atomic absorption spectrometry (GFAAS) (Subramanian and Meranger, 1981; López-García *et al*., 1997; Hata *et al*., 1999; Liang and Liu, 2007), inductively coupled plasma mass spectrometry (ICP-MS) (Anderson and Pergantis, 2003) and neutron activation analysis (NAA) (Sun and Yang, 1999). But these techniques involve relatively expensive and complicate instruments, so that cost effective and reliable method with enough sensitivity is still needed.

Electrochemical methods can distinguish between the different oxidation states of arsenic and have a great sensitivity. The instrumentation required is relatively simple and low cost than those of techniques (Sun *et al*., 1997). Arsenic determination have been made using diferential pulse polarography (DPP) (Reed and Stolzberg, 1987), stripping chronopotentiometry (Dugo *et al*., 2005) cathodic stripping voltametry (CSV) (Li and Smart, 1996; Ferreira and Barros, 2002; Profumo *et al*., 2005; He *et al*., 2007) and anodic stripping voltammetry (ASV) (Sun *et al*., 1997; Huang and Dasgupta, 1999; Song and Swain, 2007; Yamada *et al*., 2008).

Table 1-2 Some reports of analytical methods for arsenic determination

Analytes	**Techniques**	**Detection limit**	**References**
As(III)	DPP	0.3 μg L^{-1}	Myers and Osteryoung, 1973
As(III), As(V	Amperometry (Pt wire flow through)	0.4 μg L^{-1}	Lown and Johnson, 1980
As(III), As(V)	ETAAS	0.7 μg L^{-1}	Subramanian and Meranger, 1981
As(III)	DPCSV (HMDE)	1 μg L^{-1}	Sadana, 1983
As(III) As(V)	HG-AES	0.62 μg L^{-1}	Chen *et al*., 1992
As(V)	DPCSV (HMDE)	4.4 μg L^{-1}	Greulach and Henze, 1995
As(III), As(V)	DPCSV (HMDE)	50 μg L^{-1}	Eguiart *et al*., 1996
As(total)	GFAAS	0.03 mg kg^{-1}	López-García *et al*., 1997
As(III), As(V)	FI-HGAAS	0.037 μg L^{-1} 5.0 μg L^{-1}	Nielson and Hansen, 1997
As(III), As(V)	DPASV (gold film electrode)	0.19 μg L^{-1}	Sun *et al*., 1997
As(III)	DPASV (Gold disc electrode)	0.15 μg L^{-1}	Kopanica and Novotny, 1998
As(III), As (V), MMA, DMA, AsB, AsC	HPLC-ICP-MS	0.04 – 0.28 ng	Moldovan *et al*., 1998
As(III)	CCCSP (mercury film electrode)	2.0 μg L^{-1}	Adeloju *et a*l., 1999
As(total)	ETAAS	0.04 μg L^{-1}	Hata *et al*., 1999

Table 1-2 (Continued)

Analytes	Techniques	Detection limit	References
As(III), As(V)	ASV (gold film electrode)	0.5 μg L^{-1}	Huang and Dasgupta, 1999
As(III) As(V)	NAA	1.0 ng L^{-1}	Sun and Yang, 1999
AsH_3 MMA DMA TMA	GC-MS	0.173 ng 20 μL^{-1} 0.024 ng 20 μL^{-1} 0.036 ng 20 μL^{-1} 0.100 ng 20 μL^{-1}	Pantsar-Kallio and Korpela, 2000
As(III) As(V)	HGAFS	0.67 μg L^{-1}	Semenova *et al*., 2000
As(III) As(V) MMA DMA	HPLC-ICP-MS	0.02 – 0.05 μg L^{-1}	Roig-Navarro *et al*., 2001
As(III) As(V)	SWCSV (HMDE)	0.2 μg L^{-1} 2.0 μg L^{-1}	Ferreira and Barros, 2002
As(III) As(V) MMA	LC-ICP-MS	0.021 – 0.025 μg L^{-1}	Nakazato *et al*., 2002
As(III) As(V)	CCSA (gold film carbon paste)	0.3 μg L^{-1} 0.5 μg L^{-1}	Svancara *et al*., 2002
As(III) As (V) MMA DMA AsB AsC	HPLC-MW-HGAAS	0.3 – 1.1 ng mL^{-1}	Villa-Lojo *et al*., 2002
As(total)	HG-ICP-MS	1 ng L^{-1}	Anderson and Pergantis, 2003

Table 1-2 (Continued)

Analytes	Techniques	Detection limit	References
As(III) As(V)	HG-AFS	8.1 μg L^{-1} 10.3 μg L^{-1}	Cava-Montesions *et al*., 2003
As(III) As(V)	DPCSV (HMDE)	0.5 μg L^{-1}	He *et al*., 2004
As(III) As(total)	HG-AAS	0.1 μg L^{-1} 0.06 μg L^{-1}	Anthemidis *et al*., 2005
As(III) AS(V)	HG-AAS	0.15 μg L^{-1}	Bortoleto and Cadore, 2005
As(III) As(V)	d ASCP	0.08 μg L^{-1}	Dugo *et al*., 2005
As(III) As(V)	SWCSV (HMDE)	0.01 μg L^{-1} 0.02 μg L^{-1}	Profumo *et al*., 2005
As(total)	HG-AFS	0.5 μg L^{-1}	Zhang *et al*., 2005
As(III) As(V)	HG-AFS	0.05 μg L^{-1}	Leal *et al*., 2006
As(III) As(V) MMA DMA	DPCSV (HMDE)	0.3 μg L^{-1}	He *et al*., 2007
As(III) As(V)	GF-AAS	24 ng L^{-1}	Liang and Liu, 2007
As(III)	DPCSV (Hanging copper amalgam drop electrode)	0.02 μg L^{-1}	Piech *et al*., 2007
As(III)	DPASV (Gold coated diamond thin film)	0.005 μg L^{-1}	Song and Swain, 2007

1.2.6 Anodic stripping voltammetry

Voltammetic techniques are based on the measurements of current as a function of potential. The current is produced at an electrode surface following the oxidation and reduction of the analyte at a characteristic potential. The oxidation or reduction at the electrode surface is essentially electron-transfer or charge-transfer. The current is measured in amperes or coulomb (Fifield and Haines, 1995). Voltammetric cell consists of three-electrode system immersed in electrolytic conductor (ionic cell solution). The electrolytic conductor consists of the sample solution with the electrochemically active analyte and an excess of an inert supporting electrolyte. The voltammetric measurements are essentially performed in a three-electrode system in an electrochemical cell, a typical configuration is illustrated in **Figure 1-5**.

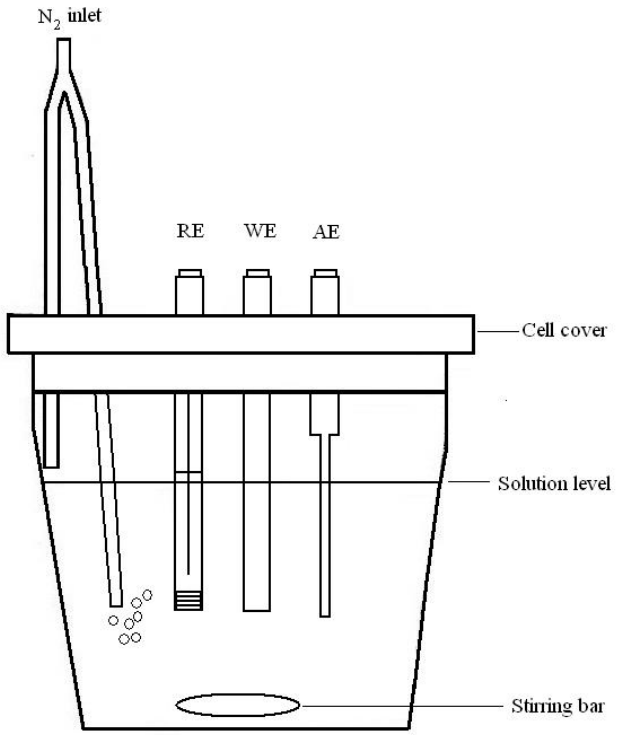

Figure 1-5 Typical configuration of a three-electrode system in an electrochemical cell
RE = reference electrode;
WE = working electrode;
AE = auxiliary electrode

The names of voltammetric techniques depend on the potential wave form applied. The excitation and resulting voltammograms are illustrated in **Figure 1-6**.

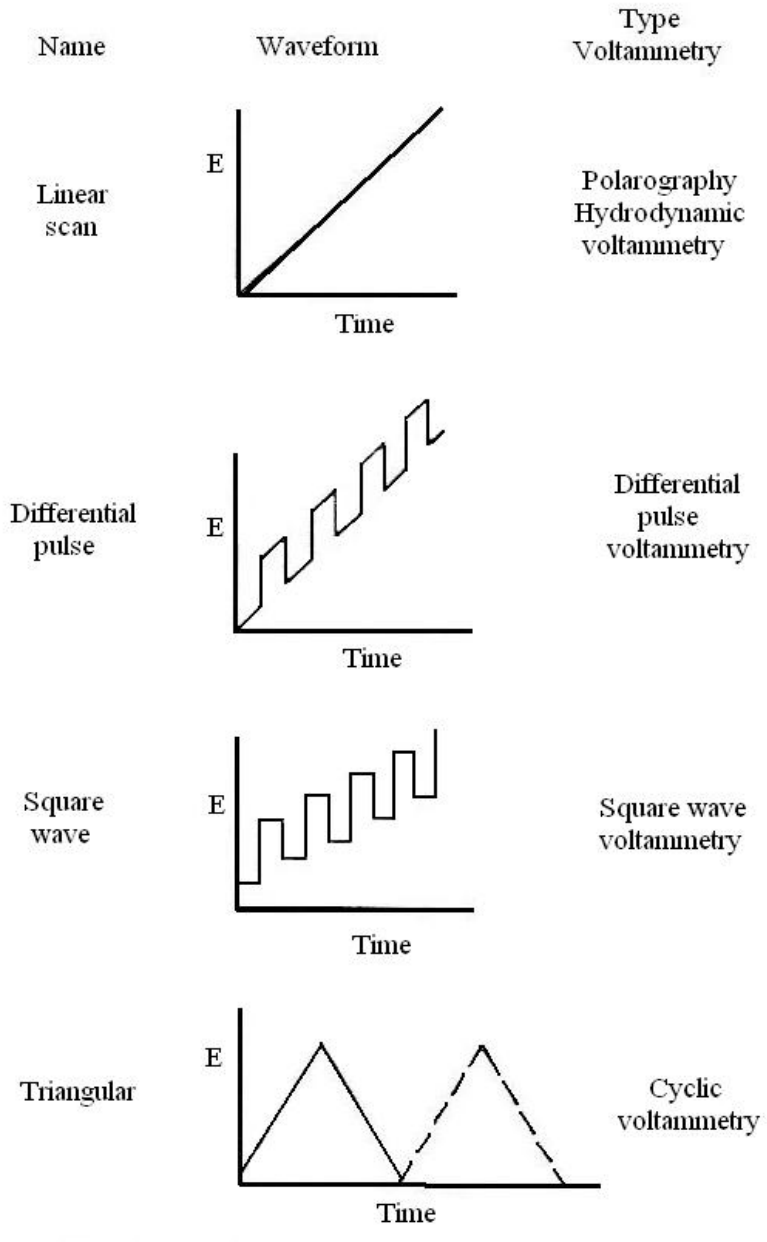

Figure 1-6 Potential excitation signals used in voltammetry (Fifield and Haines, 1995)

The simplest waveform is a steady increase or decrease of potential with time. This technique is known as linear scan and is the waveform applied in classical polarography. It is also the potential waveform applied in cyclic voltammetry (CV).

Stripping analysis is a sensitive electroanalytical technique for the determination of trace amounts of metal in solution. The technique consists of two steps. First, metal ions are deposited onto an electrode which is held at a suitable potential called deposition step. Second, the deposited metals are stripped from the electrode by scanning the potential called stripping step.

Deposition step: $M^{n+}_{(aq)} + ne^- \rightarrow M_{(s)}$

Stripping step: $M_{(s)} \rightarrow M^{n+}_{(aq)} + ne^-$

During stripping step, if the applied potential is scanned in a positive direction, it is called anodic stripping voltammetry. If the applied potential is scanned in a negative direction, it is called cathodic stripping voltammetry. The observed current during the stripping step can be related to amount of metal in solution.

In addition to varying the direction of the scan, the manner in which the potential is scanned may also differ. The simplest technique is Linear Sweep Voltammetry (LSV) where the potential is scanned linearly as a function of time. Another commonly used technique is Differential Pulse Voltammetry (DPV), which has a lower detection limit than LSV. This is due to its pulsed waveform which measures the current in pulses by taking two measurements and recording the difference as the potential is increased. This helps to reduce the background current. The differential waveform is depicted in **Figure 1-7**. It consists of small pulses (of constant amplitude) superimposed upon a staircase waveform. The current is sampled twice in each pulse period (before and at the end of the pulse), and the difference between these two current values is recorded and displayed. The differential pulse has the advantages of sensitive detection limits and more useful for routine quantitative analysis (http://www.bioanalytical.com/mans/EC_epsilon/Techniques/Pulse/pulse.htm).

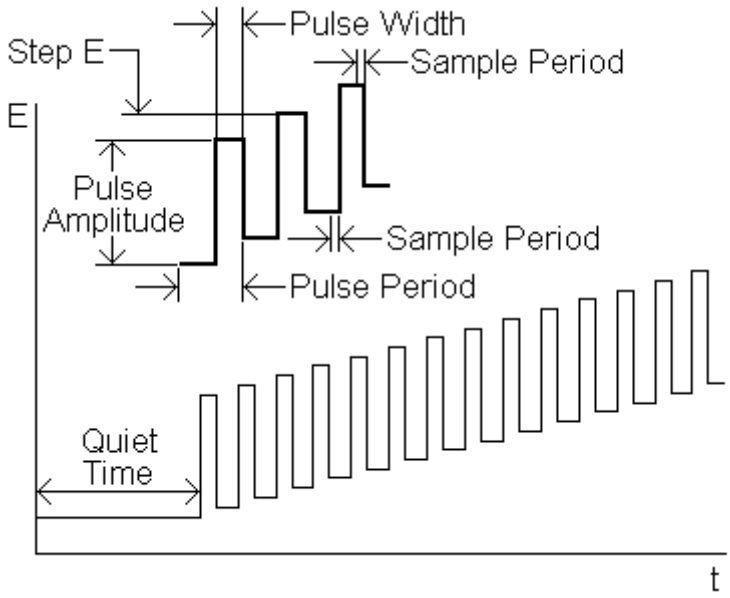

Figure 1-7 Potential wave form for differential pulse voltammetry (DPV) (Source: http://www.bioanalytical.com/mans/EC_epsilon/Techniques/Pulse/pulse.htm)

1.2.7 Flow injection analysis

Normally, any measurement in a chemical laboratory involving liquid materials comprises the following operations: solution handling, analyte detection, data collection, and computation of results. Solution handling is one of the most frequently performed laboratory tasks. In analytical laboratory it is also the most exacting and laborious one, as solutions have to be precisely metered, mixed, incubated, heated, separated and monitored in a reproducible way by optimum method for quantification of target analyte.

While a significant number of chemical assays worldwide is still carried out manually, by means of volumetric glassware that remained virtually unchanged during last 200 years, automation of reagent based chemical analysis has been implemented whenever many samples have to be processed, or when a continuous monitoring is desired. Downscaling of automated assays is the current trend, as it will decrease sample and reagent consumption, and waste generation. Also, processing smaller volumes of chemically or biologically hazardous materials within a closed system improves laboratory safety. In the batch processing mode each sample is assigned an individual container (such as vial, or microwell) in which reactants are pipetted, mixed, incubated and transported to a detector. Therefore a discrete (batch) analyzer has many complex mechanical parts.

Flow processing is characterized by its versatility and ease of automation, as the apparatus often comprises only two mechanical components; a pump and a valve. Sample is metered into a working channel by means of an injection valve and is transported through all steps of an analytical protocol by a flowing stream.

Flow injection analysis is based on a combination of three principles: sample injection, controlled dispersion of the injected sample zone and reproducible timing of its movement from the injection point toward and into the detector.

The simplest flow injection analyzer (**Figure 1-8a**) consists of a pump which is used to propel the carrier stream through a narrow tube, an injection port by means of which a well-defined volume of a sample solution is injected into the carrier stream in a reproducible manner, and a microreactor in which a sample zone disperses and reacts with the components of the carrier stream, forming a species that is sensed

by a flow through detector and recorder. A typical recorder output has the form of a peak (**Figure 1-8b**), the height (***H***), width (***W***), or area (***A***) of which is related to the concentration of the analyte (Ruzicka and Hansen,1988). Peak height is the most frequency measured peak dimension, since it is easily identified and directly related to the detector response, such as absorbance, potential or current, that is

$$H = kC \qquad \text{……..equation 1.1}$$

Where ***k*** is a proportionality constant.

C is the concentration of the analyte.

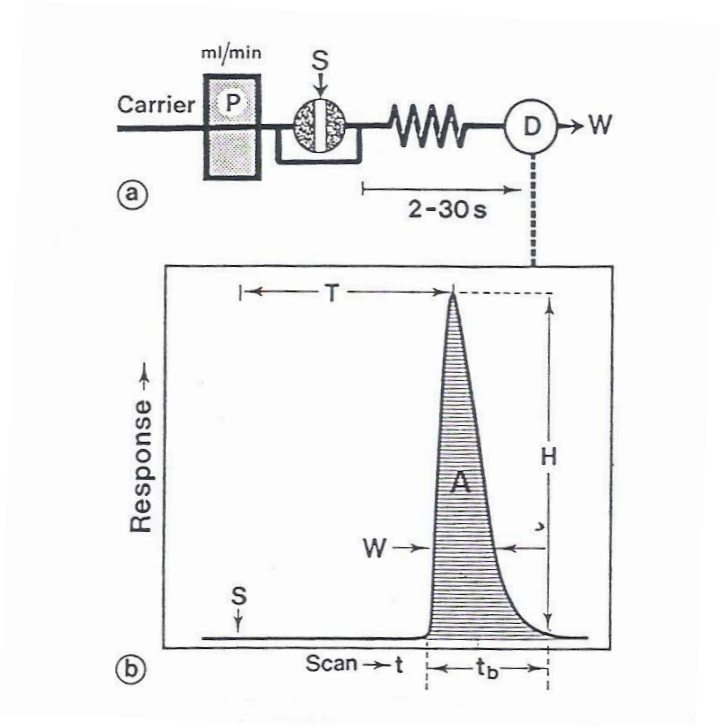

Figure 1-8 (a) The simplest single line FIA manifold utilizing a carrier stream of reagent; ***P*** is the pump, ***S*** is the injection port, ***D*** is the detector and **W** is the waste.

(b) The analog output has the form of a peak, the recording starting at ***S*** (time of injection t_o), ***H*** is the peak height, ***W*** is the peak width at a selected level, and ***A*** is the peak area, ***T*** is the residence time corresponding to the peak height measurement and t_b is the peak width at the baseline.

(Source: Ruzicka and Hansen, 1988)

The most important variables involved in the design of flow system are dispersion coefficient, residence time, and dispersion factor.

A homogeneous sample solution has the original concentration ***C°*** (**Figure 1-9,** left). When the sample zone is injected, it follows from the movement of the carrier stream, forming a dispersed zone whose form depends on the geometry of the channel and the flow velocity. Therefore, the response curve has the shape of a peak reflacting a continuum of concentration (**Figure 1-9**, right), forming a concentration gradient. However, to view this continuum of concentrations as being composed of individual elements of fluid, each of them having a certain concentration of sample material ***C***,

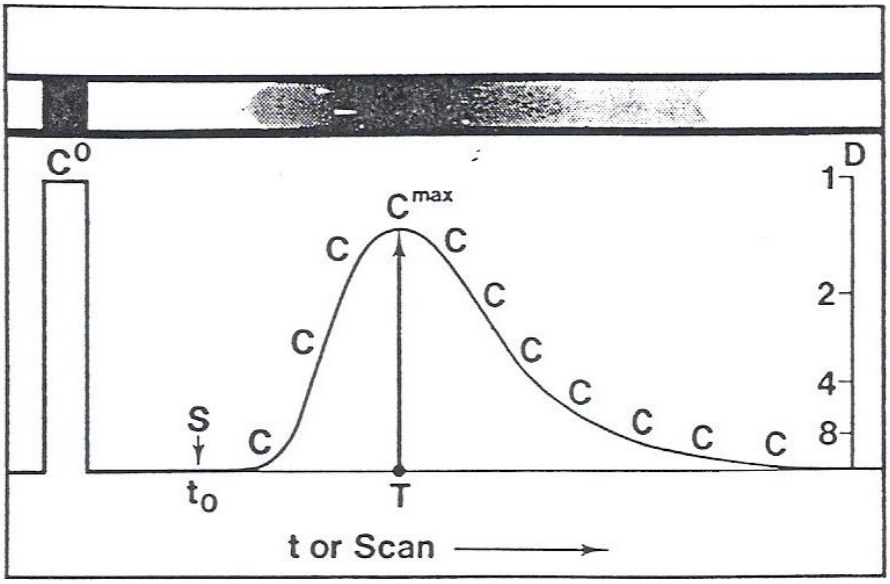

Figure 1-9 An originally homogeneous sample zone (top left) disperses during its movement through a tubular (top center), thus changing from an original square profile (bottom left) of original concentration C° to a continuous concentration gradient with maximum concentration C^{max} at the apex of the peak.
(Source: Ruzicka and Hansen, 1988)

The dispersion coefficient ***D*** has been defined as the ratio of concentrations of sample material before and after the dispersion process has taken place in that element of fluid that yields the analytical readout, as shown in **equation 1.2**.

$$D = C^{\circ}/C \qquad \text{……..equation 1.2}$$

Where C° is the concentration of sample before the dispersion

C is the concentration of sample after the dispersion

If the analytical readout is based on maximum-peak-height measurement, the concentration of element, which corresponds to the maximum of recorded curve (C^{max}) has to be considered, Thus, by relating C^{max} to the original concentration of injected sample solution C°, the ***D*** is obtained as shown in **equation 1.3**.

$$D = C^{\circ}/C^{max} \qquad \text{……..equation 1.3}$$

Where C^{max} is the concentration of the sample at peak maximum of the dispersed zone

Dispersion coefficient can be classified into 3 categories. There are limited, medium and large dispersion. These adjectives refer to dispersions of 1 to 3, 3 to 10 and greater than 10, respectively. The limited-dispersion FI- techniques have been found considerable applications for high speed feeding of such detector systems as FAAS, ICP-AES, pH or conductivity measurements and so on. The medium-dispersion FI-techniques are often designed for many techniques, for instance, spectrophotometry, which sample and reagent must be mixed to form a detectable product. The large-dispersion is suitable for highly concentrated samples, which must be diluted into the detection range.

1.3 Literature reviews

Arsenic (As), a metalloid, occurs naturally in the earth's crust and is widely distributed in the environment. Sources of arsenic in the environment include both natural and anthropogenic. The major cause of human arsenic toxicity is from contamination of drinking water from natural geological sources rather than from mining smelting, or agricultural sources (Ratnaike, 2003). In natural sources, arsenic was distributed ubiquitously throughout earth crusts, soil, sediments, water, air and living organisms. Arsenic is a rare crystal element comprising about 0.00005% of the earth's crust and the average concentration of arsenic in igneous and sedimentary rocks is 2 mg kg^{-1} (Mandal and Suzuki, 2002).

Arsenic naturally occurs in over 200 different mineral forms, of which approximately 60% are arsenates, 20% sulfides and sulfosalts and the remaining 20% includes arsenides, arsenites, oxides, silicates and elemental arsenic (As) (Mandal and Suzuki, 2002). The levels of arsenic in the soils of various countries are reported to range from 0.1 to 40 mg kg^{-1}. Arsenic is present in soils in higher concentrations than those in rocks. Uncontaminated soils usually contain 1–40 mg kg^{-1} of arsenic, with lowest concentrations in sandy soils and those derived from granites, whereas larger concentrations are found in alluvial and organic soils.

The principal factors influencing the concentration of elements in soils are the parent rock and human activities. Factors such as climate, the organic and inorganic components of the soils and redox potential status also affect the level of arsenic in soils. The forms of arsenic present in soils depend on the type and amounts of sorbing components of the soil, the pH and the redox potential. Arsenates of Fe and Al ($AlAsO_4$, $FeAsO_4$) are the dominant phases in acid soils and are less soluble than calcium arsenate (Ca_3AsO_4), which is the main chemical form in any alkaline and calcareous soils. The adsorbed arsenate fraction in soils is closely related to soil pH and redox potential. It is also varied with soil type under the same pH conditions, increasing in order from brown soil to chestnut soil (Mandal and Suzuki, 2002).

Arsenic is found at low concentration in natural water. The maximum permissible concentration of arsenic in drinking water is 50 μg L^{-1} and recommended value is 10 μg L^{-1} by EPA and WHO.

The seawater ordinarily contains 0.001– 0.008 mg L^{-1} of arsenic. The major chemical form in which arsenic appears to be thermodynamically stable is arsenate ion. The ratio of As(V) to As(III) based on thermodynamic calculation should be 10^{26}:1 for oxygenated seawater at pH 8.1. In reality, it is 0.1:1 to 10:1. The concentration of arsenic in unpolluted fresh waters typically ranges from 1–10 μg L^{-1}, rising to 100–5000 μg L^{-1} in areas of sulfide mineralization and mining. At moderate or high redox potentials arsenic can be stabilized as a series of pentavalent (arsenate) oxyanions, H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-}. However, under most reducing (acid and mildly alkaline) conditions and lower redox potential, the trivalent arsenite species (H_3AsO_3) predominate. As^0 and As^{3-} are rare in aquatic environments (Mandal and Suzuki, 2002). Complex organic arsenic compounds such as tetramethylarsonium salts, arsenocholine, arsenobetaine, dimethyl(ribosyl)arsine oxides and arsenic containing lipids are identified in the marine environment (Townshend, 1995).

In air, arsenic exists predominantly absorbed on particulate matters, and is usually present as a mixture of arsenite and arsenate, with the organic species being of negligible importance except in areas of arsenic pesticide application or biotic activity. The human exposure of arsenic through air is generally very low and normally arsenic concentrations in air ranges from 0.4 to 30 ng m^{-3} (WHO, 1996).

The anthropogenic sources exceed natural sources in the environment by 3:1. Man in his utilization of natural resources releases arsenic into the air, water and soil. The emissions can ultimately affect the accumulation in soil through use of arsenical pesticides, application of fertilizers, dust from the burning of fossil fuels, disposal of industrial and animal wastes.

During 1970s, about 80% of the consumption of arsenic were for agricultural purpose. Arsenic was widely used for preparation of insecticides and pesticides. Most of arsenic in the form of pesticides, such as lead arsenate, Ca_3AsO_4, copper acetoarsenite, H_3AsO_4, monosodium methanearsonate, disodium methanearsonate and cacodylic acid are used in cotton production as pesticides. At present, agricultural use of arsenic declines (Mandal and Suzuki, 2002).

The inorganic arsenicals, primarily, sodium arsenite, were widely used for preparation of herbicides as weed killers, particularly as non-selective soil sterilants.

Arsenic acid (H_3AsO_4) is used extensively as a cotton desiccant. The combination of Chromated Copper Arsenate (CCA) and Ammonical Copper Arsenate (ACA) were used in 99% of the arsenical wood preservatives. Zinc and chromium arsenate are also used as wood preservative. Many arsenic compounds, such as H_3AsO_4, 3-nitro-4-hydroxy phenylarsenic acid, 4-nitrophenylarsenic acid are used for feed additives. Common medical preparations, which contained arsenic, include potassium arsenite, arsenic and mercuric iodides, arsenic trioxide and black pepper, liquor arsenic chloride, sodium cacodylate, arsphenamine, neoarsphenamine, oxophenarsine hydrochloride, arsthinol, acetarsone, tryparsamide, and carbarsone. In addition, Arsenic compounds are infamous as very potent poisons and are preferred to homicidal and suicidal agents (Mandal and Suzuki, 2002).

Humans are exposed to many different forms of inorganic and organic arsenic species (arsenicals) in food, water and other environmental media. The toxicity of an arsenic containing compound depends on the valence state of arsenic (-3, 0, 3, 5), its form (organic and inorganic), and the physical aspects governing its absorption and elimination. In general, inorganic arsenic is more toxic than organic arsenic, and trivalent arsenic is more toxic than pentavalent and zero-valent arsenic (http://www.manbir-online.com/diseases/arsenic.htm). Extensive toxicity studies of arsenic have shown that different forms exhibit different toxicities (**Table 1-3**). Acute toxicity generally decreases with increasing degree of methylation, and inorganic arsenic is more toxic than organic arsenic. Therefore, the speciation analysis of arsenic is more important than total arsenic determination.

Table 1-3 The LD_{50} values in rats for some arsenic species

Types of arsenic	symbol	LD_{50} (mg kg^{-1})
Arsine	AsH_3	3
Arsenite	As(III)	14
Arsenate	As(V)	20
Monomethyl arsenic acid	MMA(V)	700-1,800
Dimethyl arsenic acid	DMA(V)	700-2,600
Trimethyl arsine oxide	TMAO	10,600
Arsenobetaine	AsB	> 10,000
Arsenocholine	AsC	> 10,000

Source: Ng, 2002

There have been certain reports on the development of analytical method for arsenic determinations (**Table 1-2**) including spectrometric techniques, separation techniques, and electrochemical techniques as follows:

Spectrometric techniques for arsenic determination include hydride generation atomic absorption spectrometry (HG-AAS), hydride generation atomic fluorescence spectrometry (HG-AFS), graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma mass spectrometry (ICP-MS).

Hydride generation is, perhaps, the most popular sample derivatization method used for inorganic arsenic detection, since Holak first reported it in 1969 (Hung *et al*., 2004). Initially it was developed as a method for AAS, whereby sodium or potassium tetrahydroborate (III) is used for arsine production (Scheme 1).

$$As(OH)_3 + 3BH_4^- + 3H^+ \rightarrow AsH_3 + 3BH_3 + 3H_2O$$

$$BH_3 + 3H_2O \rightarrow H_3BO_3 + 3H_2$$

Scheme 1 Hydride generation reaction.

The hydride generation procedure can be also used for differential determination of As(III) and As(V), based on the fact that As(III) reacts with tetrahydroborate at a higher pH than As(V). Thus tetrahydroborate is acting as a reductant for As(V) as well as a hydride source (Hung *et al*., 2004).

Semanova *et al*. (2000) reported for total inorganic arsenic determination by hydride generation-atomic fluorescence spectrometry, using sodium tetrahydroborate as a reducing agent. These methods increase the sensitivity of detection, the detection limit of 0.67 $\mu g\ L^{-1}$. The inclusion of on-line HG generally increases the sensitivity of detection in the preconcentration step and reduces the precipitation of interferences such as sulfate (SO_4^{2-}), phosphate (PO_4^{3-}) and chloride (Cl^-) in water samples (Narcise, 2005).

Graphite furnace atomic absorption spectrometry (GFAAS) or electrothermal atomic absorption spectrometry (ETAAS) is one of the spectrometric methods, which can be run without HG. The technique is based on the absorption of free atoms produced from the sample deposited in a small graphite tube, which can be heated by the application of high temperatures. However, most of reported methods for arsenic detection based on GFAAS require pre-concentration in order to increase sensitivity.

Hata *et al*. (1999) proposed a soluble membrane filter technique for the solid-phase extraction of trace elements, including arsenic, in water before determination using electrothermal atomic absorption spectrometry. In this technique, the analyte is converted into hydrophobic species, which are retained on a membrane filter. Further material collected with the membrane filter is dissolved in sulfuric acid or organic solvent. Finally, the sample is pre-concentrated and analysed with ETAAS. This simple and rapid method provides similar limit of detection to FI-HG-AAS limit of detection and has been successfully applied in river water sample analysis.

Plasma is used to ionize compounds in ICP techniques, whereby the sample is acidified and sprayed into the plasma. The high temperature of the plasma atomizes and ionizes all forms of arsenic so that the response does not vary with species as in the more traditional AAS methods which require through digestion prior analysis. Often, ICP is used in conjunction with other analytical techniques, such as MS and AES. ICP–AES is a less used technique and normally applied for a

comparison and more accurate analysis of a multi-element sample. In contrast, the ICP-MS technique is one of the most widely applied analytical protocols for arsenic detection (Hung *et al*., 2004).

The main advantages of ICP-MS over ICP-AES are isotope analysis capability of high precision and lower detection limits. The possible drawback of ICP-MS equipped with a direct nebulizer is the possible interference from high levels of chloride due to the formation of argon chloride ($^{40}Ar^{35}Cl$) in the plasma, which has the same mass as arsenic (^{75}As) (Moldovan *et al*., 1998).

However, the determination of low concentrations of arsenic in real samples suffers from low sensitivity due to the poor ionization efficiency in ICP. In order to overcome this, several ICP-MS applications combined with hydride generation and a cold vapour mercury sample introduction technique have been applied for arsenic determination.

The separation techniques including HPLC and GC-MS were used for organic arsenic determination based on properties of molecular structure, solubility and boiling point which could be separated in column.

Pantsar-Kallio and Korpela (2000) reported developed method based on GC-MS for analysis volatile arsenic species include arsine, monomethylarsine (MMA), dimethylarsine (DMA) and trimethyl arsine (TMA). The proposed method gaseous arsenic species could be determined in less than 2 min and no pretreatment for gas phase samples was needed, which minimized the risks of species conversion before analysis. The detection limits for different species were 24-174 pg.

A coupled system including HPLC and ICP-MS give a suitable method for the determination of non-volatile species of element such as arsenic. Although, ICP-MS has multi-element capability, this coupled technique has hitherto mainly been used for speciation analysis of single elements (Hung *et al*., 2004).

Moldovan *et al*. (1998) reported the comparison of two analytical methods, HPLC-MO-HG-AAS and HPLC-ICP-MS. It was found that, the HPLC-ICP-MS method is the better alternative for monitoring arsenic at trace levels because it has detection limits about 20 times lower than those of the HPLC-MO-HG-AAS.

A comparison between using HPLC-HG-AFS and HPLC-HG-ICP-MS performance has been reported by Gomez-Ariza *et al*. (2000) for the speciation of

arsenite, arsenate and other arsenic compounds in fresh water. It was found that the limit of detection were similar for both techniques, however, AFS presented the benefits of much lower running costs, shorter warm up times prior to analysis (15–90 min) and easy handling.

Electrochemical techniques can differentiate the oxidation states of arsenic and have a great sensitivity (Sun *et al*., 1997). These techniques provide a low-cost, rapid and portable option for routine in-field (Jia *et al*., 2006). These techniques have been applied to the determination of arsenic include polarography, cyclic voltammetry, potentiometry, cathodic stripping voltammetry (CSV) and anodic stripping voltammetry (ASV).

Polarography is the oldest electrochemical method for the determination of trace inorganic metals, which, suffers from low limits of detection due to high capacitive currents. Differential pulse polarography (DPP) offers the same benefits of selectivity as well as lower capacitive currents and as a result improved of limits of detection. DPP was popular for routine analysis of trace metals due to its high sensitivity for a wide range of elements, including arsenic, which do not form mercury amalgams readily and because of the availability of inexpensive commercial instruments (Hung *et al*., 2004). However, only few works related to the determination of arsenic in water using differential pulse polarography have been published recently, mainly because of the limited sensitivity at ultratrace concentration (Kumaresan and Riyazuddin, 2001).

Stripping voltammetry techniques combined with an extra preconcentration step has also been used. The analyte is deposited onto the electrode from a solution and then determined by being ‘stripped’ from the electrode in the process.

Generally, stripping analysis is better suited than direct polarography for trace determination in real samples because the substance of interest is pre-concentrated on the working electrode.

The anodic stripping voltammetry (ASV) involves electrolytically depositing the analyte onto an electrode behaving as a cathode. In cathodic stripping voltammetry (CSV), the analyte is determined by reduction.

Cathodic stripping analysis of arsenic at the HMDE is based on arsenic preconcentration in highly acidic media with further scanning in the cathodic direction to obtain peak due to the formation of arsine. In order to increase sensitivity, intermetallic complexes of arsenic are stripped from HMDE, whereby As(III) reacts with copper or selenium to form the relevant complex, which can be stripped cathodically (**Scheme 2**).

Deposition step: $2As^{3+} + 3MHg + 6e^- \rightarrow M_3As_2 + 3Hg$

Stripping step: $M_3As_2 + 3Hg + 12H^+ + 12e^- \rightarrow AsH_3 + 3MHg + H_2$

Where M = Cu or Se

Scheme 2. Processes occur during cathodic stripping voltammetry

Li and Smart (1996) reported the measurement of As(III) in natural waters using square wave cathodic stripping voltammetry at a hanging mercury drop electrode. Pre-concentration was carried out in 2 M HCl in the presence of 0.8 mM $CuCl_2$ at a potential of -0.4 V vs. Ag/AgCl. The detection limit is approximately (5 ng L^{-1}) when a 10 min deposition time was used. The deviation was calculated as 8% (*n =11*) at 1×10^{-9} M As for 1 min period of deposition.

Profumo *et al*. (2005) reported a cathodic stripping voltammetric method for the determination of As(III) and total inorganic arsenic. This method is based on the formation of a copper-arsenic intermetallic at HDME during the preconcentration step. Sodium dithionite was used for the reduction of As(V) to As(III). As(III) was then determined in HBr supporting electrolyte. Quantification limits of 0.010 and 0.020 μg L^{-1} for As(III) and As(V) respectively was obtained.

Anodic stripping voltammetry techniques for trace arsenic analysis, which is based on the deposition of metal arsenic on the electrode surface with subsequent anodic stripping (**Scheme 3**).

Deposition step: $As^{3+} + 3e^- \rightarrow As^0$

Stripping step: $As^0 \rightarrow As^{3+} + 3e^-$

Scheme 3. Processes occur during anodic stripping voltammetry

Forsberg *et al*. (1975) investigated in detail the determination of arsenic by ASV and differential pulse anodic stripping voltammetry (DPASV) at various electrode materials (HMDE, Pt and Au). It was found that the arsenic oxidation peak appeared as a shoulder on the mercury oxidation wave on a HMDE and was of little analytical utility. Platinum was a suitable electrode material and was employed in the initial studies. Gold was found to be superior to platinum as a working electrode material due to a higher hydrogen overvoltage. This experiments showed that ASV proved to be an exceedingly sensitive technique as for the determination arsenic, while the use of DPASV greatly shortened deposition times.

Bodewig *et al*. (1982) used a gold rotating disk electrode, which removed the hydrogen bubbles mechnically, allowing good reproducibility of results. The determination of arsenite by anodic stripping voltammetry using gold disk or gold film electrode does not offer sufficient reproducibility (Greulach and Henze, 1995).

Sun *et al*. (1997) reported a new method of gold film deposition on a rotating glassy carbon electrode. Factors likely to affect the stability of an electrode including, acidity, deposition time, rotation rate, scan rate, the electrode reaction, and the reduction step of As(V) to As(III) were investigated. These techniques capable to determine the nanogram level of arsenic. The determination limit was approximately 0.19 μg L^{-1} for a deposition time of 4 min. Precision of method was 2-0.6% at 1-5 μg L^{-1}.

Kopanica and Novotny (1998) reported the determination of As(III) in aqueous solution by differential pulse anodic stripping voltammetry (DPASV) using disc gold electrode. It was found that good reproducibility of measurement is achieved by a programmed electrochemical treatment of the electrode surface before each measurement. The detection limit of 0.15 μg L^{-1} was obtained.

The total inorganic arsenic content detection is normally based on the concentration of As(III) after converting all arsenic species to the trivalent arsenic form. This is especially applied in electrochemical techniques, as pentavalent arsenic is electroinactive (Hung *et al*., 2004).

The most popular prereductant of As(V) to As(III) is potassium iodide, which can be used with ascorbic acid, in order to prevent the oxidation of iodide to triiodide by air (Chen *et al*., 1992). However, potassium iodide can reduce arsenic

only in a strong acidic media. Other reagents used for reduction of As (V) are mercaptoacetic acid (Anderson *et al*., 1986) and L-cysteine (Chen *et al*., 1992), which was also found to reduce interferences and increase the sensitivity. Some techniques require a specific reagent, thus sodium meta bisulfite along with sodium thiosulphate has been used (He *et al*., 2004), gaseous sulfur dioxide, sodium sulphite in stripping voltammetry (Sun *et al*., 1997) and the use of hydrazine in flow injection analysis (Lown and Johnson, 1980) has been reported.

Some reducing agents in electrochemical techniques are shown in **Table 1-4**.

Table 1-4 Some reducing agents in electrochemical techniques

Techniques	**Reducing agent**	**Working electrode**	**References**
CCSA	α-cysteine	gold-plated carbon paste	Švancara *et al*., 2002
CSP	L-cysteine	Mercury film electrode	Adeloju *et al*., 1999
CSV	KI	HMDE	Equiarte *et al*., 1996
CSV	$Na_2S_2O_3$	HMDE	Ferreira and Barros, 2002
CSV	$Na_2S_2O_4$	HMDE	Profumo *et al*., 2005
CSV	L-cysteine	HMDE	He *et al*., 2007
ASV	Na_2SO_3	gold film coated glassy carbon electrode	Hamilton *et al*., 1980
ASV	SO_2	gold film carbon paste	Sun *et al*., 1997
ASV	Na_2SO_3	gold film coated glassy carbon electrode	Rasul *et al*., 2002
ASV	Na_2SO_3	Gold film coated diamond electrode	Song and Swain, 2007

However, the drawbacks of the batch analysis include long time analysis, a large volume of the sample and reagent and necessity for homogeneous mixing solutions before analysis. However, contamination and poor reproducible measurement can be taken.

Therefore, the application of flow cell with gold film electrode by using anodic stripping voltammetry for As(III) and As(V) determination provided the detection limit the range of μg L^{-1} or sub-μg L^{-1} levels (Sun *et al*., 1997), which closed to arsenic level was found in natural water system. The coupling of flow cell with stripping analysis is a simple that utilizes anodic stripping voltammetry with a renewable gold film electrode to measure As(III) and As(V) (Huang and Dasgupta, 1999).

In this work, FI-system coupling with ASV for As(III) and As(V) determination in natural water samples was set up. Natural water samples were collected from arsenic contaminated areas in Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province (Williams *et al*., 1996). The literature review for arsenic determination at Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province was shown in **Table 1-5**.

The FI system provides the benefit of semi-automation, less amount of reagent, reduced waste, short time analysis, good precision and suitable for routine analysis.

Table 1-5 Literature reviews on the concentration of arsenic in natural water samples at Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province

Sampling period	Location	Sample	Concentration of arsenic ($mg\ L^{-1}$)	References
May 1990-	High risk area	Shallow well		
March 1991	Village 1	water	0.320 (n = 46)	
	Village 2		0.365 (n = 39)	
	Village 12		2.785 (n = 49)	
	Village 13		1.705 (n = 46)	Na Chiangmai,
	Low risk area	Shallow well		1990
	Village 8	water	0.048 (n = 2)	
	Village 9		1.945 (n = 45)	
	Village 11		0.014 (n = 13)	
	Village 14		0.013 (n = 19)	
March 26,	Village 2	stream water	0.145 -1.25	
1990		Water in mine	0.09 - 0.28	
		Shallow well water	0.135 - 0.160	Suwanmanee, 1991
May 10,	Village 2	Stream water	0.042 - 0.29	
1990		Water in mine	0.026 - 0.75	
August 1996	Village 2	Stream water	0.246	Vitayavirasuk and Thongboriboon, 1997
January 1997		Stream water	0.038	
April 1997		Stream water	0.162	
May 2, 2001	Village 2	Surface water	0.020 – 0.150	
		Shallow ground water	> 0.700	Jindal, 2001
	Village 12	Shallow well water	0.010	

1.4 Objectives

The aims of this research are:

1.4.1 To develop flow injection-anodic stripping voltammetry for determination of As(III) and As(V).

1.4.2 To optimize conditions of the method for determination of As(III) and As(V).

1.4.3 To apply the method to determine As(III) and As(V) in natural water samples.

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

All chemicals used throughout the analysis were of high purity commercially available as shown in **Table 2-1**. They were used without further purification.

Table 2-1 List of the chemicals used in this research

No.	Chemicals	Formula	Source	Grade	Remarks (Assay)
1.	Arsenic (III) oxide	As_2O_3	Fluka, Switzerland	C.R.M.	99.5%
2.	Sodium arsenate heptahydrate	$Na_2HAsO_4 \cdot 7H_2O$	Fluka, Switzerland	Puriss	99.5%
3.	Potassium tetrachloroaurate	$KAuCl_4$	Fluka, Switzerland	High purity	99.995%
4.	Hydrazinium chloride	$(N_2H_6)Cl_2$	Merck, Germany	Pure	>99%
5.	Sulfuric acid	H_2SO_4	J.T.Baker, USA	A.R.	96.2%
6.	Hydrochloric acid	HCl	Lab-scan, Thailand	A.R.	37%
7.	Nitric acid	HNO_3	J.T.Baker, USA	A.R.	69.3%
8.	L-ascorbic acid	$C_6H_8O_6$	Poch, Poland	Pure	99.0-100.0%
9.	Potassium iodide	KI	J.T.Baker, USA	A.C.S.	101%
10.	L-cysteine	$C_3H_7NO_2S$	Fluka, Japan	Puriss	>99%

Table 2-1 (Continued)

No.	Chemicals	Formula	Source	Grade	Remarks (Assay)
11.	Sodium thiosulphate	$Na_2S_2O_3 \cdot 5H_2O$	Ajax, Australia	A.R.	99.5-101%
12.	Sodium dithionite	$Na_2S_2O_4$	Sigma-aldrich, USA	Purified	86%
13.	Stock solution of cadmium	Cd^{2+}	Carlo Erba, USA	AAS	1,000 mg L^{-1}
14.	Stock solution of copper	Cu^{2+}	Carlo Erba, USA	AAS	1,000 mg L^{-1}
15.	Stock solution of lead	Pb^{2+}	Merck, Germany	AAS	1,000 mg L^{-1}
16.	Stock solution of nickel	Ni^{2+}	J.T. Baker, USA	AAS	1,000 mg L^{-1}
17.	Stock solution of iron	Fe^{2+}	J.T. Baker, USA	AAS	1,000 mg L^{-1}
18.	Stock solution of mercury	Hg^{2+}	Merck, Germany	AAS	1,000 mg L^{-1}
19.	Stock solution of antimony	Sb^{3+}	J.T. Baker, USA	AAS	1,000 mg L^{-1}
20.	Stock solution of Zinc	Zn^{2+}	J.T. Baker, USA	AAS	1,000 mg L^{-1}

C.R.M. = Certified reference material

A.R. = Analytical reagent

2.2 Preparation of reagents and standard solutions

Distilled de-ionized water was used for reagent preparation. All plasticwares and glasswares were immersed in 10% nitric acid at least 24 hours, then washed with de-ionized water. Each stock solution was kept in a polyethylene bottle and stored in a refrigerator (4°C) for a month. Working standard solutions were daily prepared.

2.2.1 As(III) stock solution (1000 mg L^{-1})

As(III) stock solution (1000 mg L^{-1}) was prepared by dissolving 0.1320 g of As_2O_3 in the minimum amount of 5.0 M NaOH. The stock solution was adjusted to the pH about 3.5 with 0.1 M HCl and diluted to 100 mL with de-ionized water. A 0.5 mL of 1000 mg L^{-1} hydrazinium chloride was added to prevent oxidation of As(III) to As(V). The As(III) stock solution was stored at 4°C and was kept to stable at least 1 month (Sun *et al*., 1997). As(III) working standard solutions were prepared by appropriate dilutions of the stock solution with 0.2 M HCl.

2.2.2 As(V) stock solution (1000 mg L^{-1})

As(V) stock solution (1000 mg L^{-1}) was prepared by dissolving 0.4162 g of $Na_2HAsO_4 \cdot 7H_2O$ in 100 mL of de-ionized water (Greulach and Henze, 1995). Stock solution was stored at 4°C and was kept to stable at least 1 month. As(V) working standard solutions were prepared by appropriate dilution of the stock solution with 0.2 M HCl.

2.2.3 Au(III) solution (100 mg L^{-1})

Au(III) solution (100 mg L^{-1}) was prepared by dissolving 0.0960 g of $KAuCl_4$ in 1 M H_2SO_4 and made up to 500 mL with 1 M H_2SO_4 (Sun *et al*., 1997).

2.2.4 H_2SO_4 (1 M)

A 1 M H_2SO_4 solution was prepared by diluting 27.78 mL of conc. H_2SO_4 (96%) to 500 mL with de-ionized water.

2.2.5 HCl (0.2 M)

A 0.2 M HCl solution was prepared by diluting 16.67 mL of conc. HCl (37%) to 1000 mL with de-ionized water.

2.2.6 Ascorbic acid (0.75 M)

A 25 mL amount of 0.75 M ascorbic acid was prepared by dissolving 3.3024 g of L-ascorbic acid with de-ionized water.

2.2.7 Potassium iodide (0.75 M)

3.1125 g of potassium iodide was dissolved with de-ionized water and the volume was made up to 25 mL.

2.2.8 Potassium iodide (1.0 M)

4.1500 g of potassium iodide was dissolved with de-ionized water and the volume was made up to 25 mL.

2.2.9 Sodium thiosulphate (0.02 M)

0.1581 g of sodium thiosulphate was dissolved with de-ionized water and the volume was made up to 50 mL.

2.2.10 Sodium dithionite (0.5 M)

4.3528 g of sodium dithionite was dissolved with de-ionized water and the volume was made up to 50 mL.

2.2.11 L-cysteine (0.1 M)

0.6058 g of L-cysteine was dissolved with de-ionized water and the volume was made up to 50 mL.

2.3 Instruments and apparatus

2.3.1 **Autolab** model PGSTAT 100 (Metrohm, Switzerland)

The Autolab PGSTAT 100 combined with the GPES software is a computer controlled electrochemical measurement system. It consists of a data acquisition system and a potentiostat/galvanostat as shown in **Figure 2-1**.

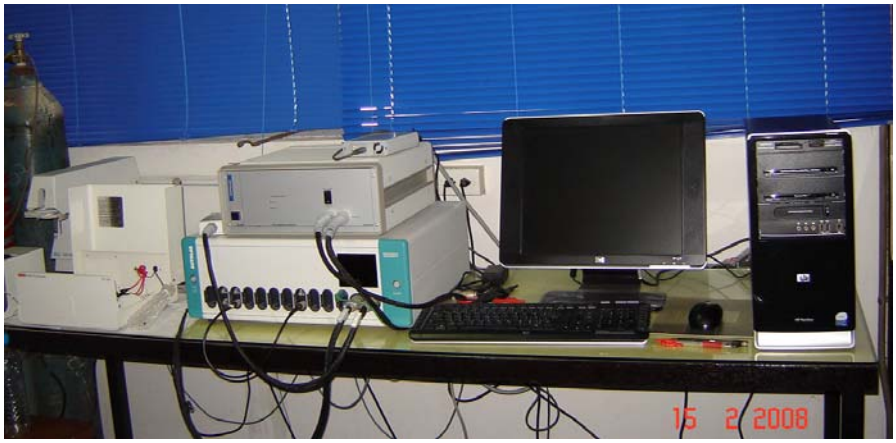

Figure 2-1 Autolab model PGSTAT 100 (Metrohm, Switzerland)

2.3.2 Electrochemical flow cell model CC-5E (Bioanalytical system, USA)

It consists of a glassy carbon electrode (2 millimeter diameter) served as the support working electrode, a Ag/AgCl electrode served as a reference electrode and a stainless steel block served as an auxiliary electrode as shown in **Figure 2-2**.

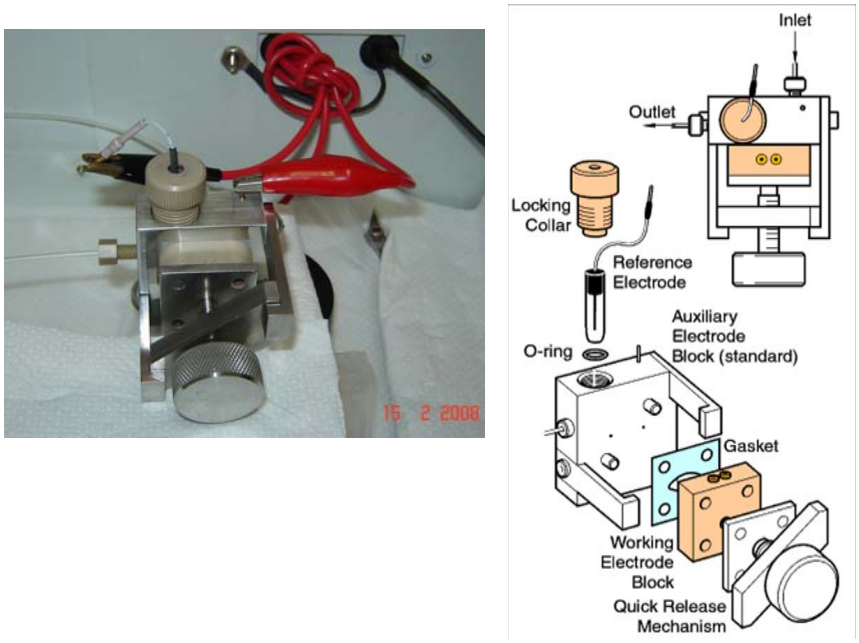

Figure 2-2 Electrochemical flow cell model CC-5E (Bioanalytical system, USA)

2.3.3 Inductively coupled plasma - optical emission spectrometer (ICP-OES)

Total As determination was performed using ICP-OES model optima 4300 DV (Perkin Elmer, USA) which located at Central Equipment Division, Faculty of Science, Prince of Songkla University.

2.3.4 Apparatus and materials

- Analytical balance model TC 254 (Denver Instrument, USA)
- pH meter model 225 (Denver Instrument, USA)
- Global positioning system model GPS (III) plus (Garmin, USA)
- Peristaltic pump model REGLO Analog ISME 27 (Ismatec, Switzerland)
- Tygon tubing (Ismatec, Switzerland)
- Teflon tubing (Upchurch, USA)
- Microliter pipette: 200, 1000 μL (Rainin, USA)
- Nitrogen gas 99.99% (TIG, Thailand)

2.4 Instrument set up

The set up of flow injection – anodic stripping voltammetry (FI-ASV) system for As(III) and As(V) determination is depicted in **Figure 2-3**. It consists of peristaltic pump (Ismatec, Switzerland), electrochemical flow cell (Bioanalytical system model CC-5E, USA) and electrochemical detector (Autolab model PGSTAT 100, Metrohm, Switzerland).

Sample or standard solution was aspirated into the system by a peristaltic pump. An electrochemical flow cell consists of 3-electrode system. A glassy carbon electrode was used as a substrate to plate gold film which served as a working electrode. A Ag/AgCl electrode was served as a reference electrode and a stainless steel block was served as an auxiliary electrode. The electrochemical flow cell was connected to the Autolab model PGSTAT 100 served as the electrochemical detector and stripping voltammetry was controlled by computer running GPES 4.9 software.

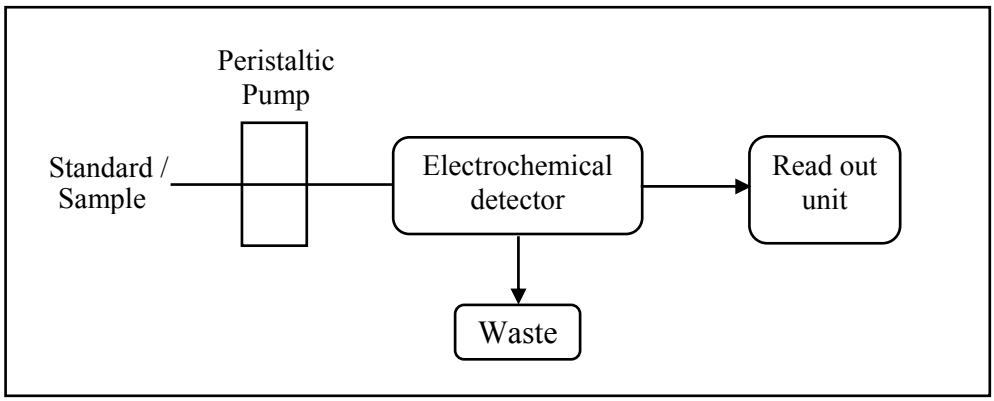

Figure 2-3 The set up of flow injection - anodic stripping voltammetry (FI-ASV)

2.5 Operation procedure

2.5.1 Pre-plated gold film electrode

The procedure of pre-plated gold film electrode was adapted from a report of Sun *et al*. (1997). A glassy carbon electrode and a stainless steel block were polished as described in **Appendix A-1**. In order to plate gold film, Au(III) solution was aspirated into the system with the optimum flow rate. The potential was applied to the working electrode. The Au(III) solution was deposited on the glassy carbon electrode as a brown powder. In order to obtain a good gold film, parameters such plating potential, plating time, concentration of Au(III) and flow rate were optimized. Initial conditions for the optimization are illustrated in **Table 2-2**.

Table 2-2 Initial conditions for pre-plating gold film electrode

Conditions	**Values**
Plating potential (V)	-0.40*
Plating time (sec)	90
Concentration of Au(III) (mg L^{-1})	200*
Flow rate of Au(III) solution (mL min^{-1})	2.0

* Jarunsri, 2006

2.5.2 As(III) determination

After the gold film electrode was prepared, As(III) determination step was carried out. As(III) standard solution was deaerated with pure nitrogen gas all the time of analysis. An optimum deposition potential was applied to the electrode while As(III) solution was aspirated to the system. After deposition, the pump was switched off and 10 sec of equilibration time was applied. Finally, stripping process was performed in the differential pulse mode starting at -0.30 V to 0.50 V. In order to obtain a good sensitivity, various parameters such as concentration of supporting electrolyte, deposition potential, deposition time, scan rate and flow rate were optimized. Initial conditions for the optimization of As(III) determination are illustrated in **Table 2-3**.

Table 2-3 Initial conditions for As(III) determination

Conditions	**Values**
Concentration of supporting electrolyte (HCl) (M)	0.10*
Deposition potential (V)	-0.30*
Scan rate (mV sec^{-1})	100**
Flow rate (mL min^{-1})	2.0
Deposition time (sec)	180**

*Kopanica and Novotný, 1998, **Jarunsri, 2006

2.5.3 As(V) determination

In general, the determination of As(V) by ASV requires to reduce As(V) to As(III), because As(V) is electrochemically inactive (He *et al*., 2004). In this work, several reducing agents such as KI, a mixture of ascorbic acid and KI, sodium thiosulphate, sodium dithionite and L-cysteine were tested as described in section **2.6.3**. After As(V) was off-line reduced to As(III) using optimum conditions, then As(total) in form of As(III) was carried out using aforementioned procedure of As(III) determination (section **2.5.2**). Then As(V) can be calculated from the different amount of As(total) and As(III).

2.6 Optimization of the FI-ASV system

2.6.1 Optimization of pre-plated gold film electrode

The conditions of the pre-plated gold film electrode for As(III) and As(V) determination were investigated. There were plating potential, plating time, concentration and flow rate of Au(III) solution. Au(III) solution was deaerated with pure nitrogen gas before plating. The optimum conditions were obtained by considering the sensitivity for As(III) determination. Optimization was carried out by varying one parameter at a time while the others were kept constant. When an optimized value of one parameter was obtained, it was used in the optimization of the next parameters following the sequence in this section.

2.6.1.1 Plating potential

In order to optimize the plating potential, the conditions of plating were fixed as shown in **Table 2-2**. The plating potentials were varied in the range of -0.50 to -0.20 V versus Ag/AgCl as reference electrode.

The efficiency of gold film electrode was considered from signal of 20 μg L^{-1} As(III) determination using conditions in **Table 2-3**. Three replicates were performed for each.

2.6.1.2 Plating time and concentration of Au(III) solution

Effect of plating time and concentration of Au(III) solution were investigated simultaneously. The conditions of pre-plated gold film electrode as shown in **Table 2-2** and conditions of As(III) determination as shown in **Table 2-3** were exploited. The plating times were varied in the range of 60 to 120 sec and concentrations of Au(III) solution were varied in the range of 50 to 200 mg L^{-1}. Three replicates were carried out for each. The plating time and concentration of Au(III) solution which gave the highest signal (current) of 20 μg L^{-1} As(III) determination was selected as optimum condition.

2.6.1.3 Flow rate of Au(III) solution

Effect of flow rate of Au(III) solution was studied using the conditions of pre-plated gold film electrode as shown in **Table 2-2** and using the conditions of As(III) determination as shown in **Table 2-3**. The flow rates of Au(III) solution were varied in the range of 0.5 to 2.0 mL min^{-1}. Three replicates of 20 μg L^{-1} As(III) determination were performed for each. The sensitivity and precision of the determination were taken into account.

2.6.1.4 Stability of the gold film electrode

The overall optimum conditions for pre-plated gold film electrode were employed for studying the stability of the gold film electrode. After gold film electrode was prepared, 20 μg L^{-1} of As(III) and As(total) standard solutions were analyzed consecutively until signal decreased more than 5%. The number of consecutive runs without error (%decrease less than 5%) of the same gold film electrode was obtained. %Decrease in signal was calculated as **equation 2.1**.

$$\% \text{ Decrease} = \frac{I_{\text{mean (No.1–No.5)}} - I(\mu A)}{I_{\text{mean (No.1–No.5)}}} \times 100 \qquad \textbf{..... equation 2.1}$$

Where $I_{mean(No.1-No.5)}$ = Average current of the entry No.1 to No.5

$I(\mu A)$ = Current measured in each analysis

2.6.2 Optimization of As(III) determination

According to the optimization of gold film electrode, the optimum conditions were exploited in this section. In order to enhance the performance of the system, various parameters for As(III) determination such as concentration of supporting electrolyte, deposition potential, scan rate, flow rate of As(III) solution and deposition time were investigated. The optimum conditions were obtained by considering the sensitivity, precision, peak shape and analysis time. The optimizations of those parameters were carried out by varying one parameter at a time while the others were kept constant. The initial operating conditions of As(III) determination were employed as shown in **Table 2-3**.

2.6.2.1 Concentration of supporting electrolyte

In anodic stripping determinations of arsenic, the stripping medium must be acidic in order to avoid the formation of hydrolysed species during stripping step (Munoz and Palmero, 2005). Hydrochloric acid was found to be the most suitable, sensitivity and narrowest peak achieved using gold film electrode as working electrode (Sun *et.al*., 1997). The influence of the concentration of the supporting electrolyte solution was studied. In this work, the concentrations of HCl as supporting electrolyte were varied in the range of 0.01 to 0.60 M. The optimum concentration of HCl was obtained by considering current peak of 10 and 20 μg L^{-1} As(III) determination using initial conditions in **Table 2-3**. Duplicate determinations were performed for each.

2.6.2.2 Deposition potential

Basically, anodic stripping voltammetry consists of a series of controlled-potential electrolysis steps, the sensitivity is depended on the concentration of an interested element deposited on the electrode, and therefore the efficiency of the deposition (preconcentration) step becomes critical (Sun *et al*., 1997).

The amount of arsenic deposited on electrode depends on deposition potential. Hence, the effect of the deposition potential on signal was investigated. The deposition potentials were varied in the range of -0.10 to -0.40 V versus Ag/AgCl as reference electrode. The sensitivity of As(III) determination was considered from a slope of calibration curve of 5 to 20 μg L^{-1} As(III) determinations using initial conditions in **Table 2-3**. Duplicate determinations were performed for each.

2.6.2.3 Scan rate

It is stated that the stripping peak current is proportional to the square root of scan rate (Sun *et al*., 1997). The effect of scan rate on peak current of As(III) determination was therefore studied. The scan rates were varied in the range of 40 to 100 mV sec^{-1}. The optimum scan rate was obtained by considering peak shape and a slope of calibration curve of 5 to 20 μg L^{-1} As(III) determinations using initial conditions in **Table 2-3**. Duplicate determinations were performed for each.

2.6.2.4 Flow rate of As(III)

The flow rate of As(III) solution is one of the factors affecting the amount of analyte deposited on the gold film electrode. Consequently, optimization of the flow rate is necessary. The flow rates were varied in the range of 0.5 to 2.5 mL min^{-1}. The optimum flow rate was considering the determination of sensitivity (a slope of calibration curve of 5 to 20 μg L^{-1} As(III)) using initial conditions in **Table 2-3**. Duplicate determinations were performed for each.

2.6.2.5 Deposition time

In order to obtain the highest sensitivity, deposition time was optimized. The deposition times were varied in the rage of 120 to 300 sec. Sensitivity of As(III) determination was considered a slope of calibration curve of 5 to 20 μg L^{-1} As(III) using initial conditions in **Table 2-3**. Duplicate determinations were performed for each.

2.6.3 Optimization for reduction of As(V) to As(III)

In general, the determination of total inorganic arsenic requires a preliminary step to reduce As(V) to As(III), followed by the determination of As(III). In order to achieve the best conversion, three parameters, namely, type of reducing agent, concentration of reducing agent, and reduction time were investigated.

2.6.3.1 Type of reducing agent

Various reducing agents such as potassium iodide, a mixture of ascorbic acid and potassium iodide, sodium thiosulphate, sodium dithionite and L-cysteine were investigated. The reducing agent which can convert As(V) to As(III) effectively and does not interfere to As(III) determination was considered. The list of the aforementioned reducing agents and their procedures is shown in **Table 2-4**.

Table 2-4 The list of reducing agents and their procedures

Entry	Reducing agents	Procedures	References
1.	Potassium iodide (KI)	1.0 mL of 12 M HCl and 0.5 mL of 1.2 M KI were added to 30 μg L^{-1} As(III) solution and volume was made up to 100 mL. The solution was left at room temperature for 30 min before analysis.	Eguiarte *et al.*, 1996
2.	Ascorbic acid / Potassium iodide (KI)	10 mL of 0.75 M ascorbic acid and 10 mL of 0.75 M KI were added to 30 μg L^{-1} As(III) solution and volume was made up to 100 mL. The solution was left and shaken occasionally at room temperature for 30 min before analysis.	Duangthong, 2004
3.	Sodium thiosulphate ($Na_2S_2O_3$)	10 mL of 1.0 M H_2SO_4 and 0.3 mL of 0.02 M sodium thiosulphate were added to 30 μg L^{-1} As(III) solution and volume was made up to 100 mL. The solution was left at room temperature for 5 min before analysis.	Matsubara *et al.*, 1987
4.	Sodium dithionite ($Na_2S_2O_4$)	60 μL of 0.5 M sodium dithionite was added to 100 mL of 30 μg L^{-1} of As(III) solution and purging with nitrogen for 300 sec before analysis.	Profumo *et al.*, 2005
5.	L-cysteine	20 μL of 0.1 M L-cysteine was added to 100 mL of 30 μg L^{-1} As(III) solution and then the solution was placed into a water bath. The solution was cooled to room temperature before determination.	He *et al.*, 2007

2.6.3.2 Concentration of reducing agent

Possible reducing agents for reduction of As(V) to As(III) were potassium iodide and a mixture of ascorbic acid and potassium iodide. Concentration of each reducing agent was optimized. The concentrations of potassium iodide were varied in the range of 5.5 to 7.5 mM and the concentrations of a mixture of ascorbic acid and potassium iodide were varied in the range of 2.5 to 10.0 mM. The optimum concentration of the reducing agent was obtained by considering percent conversion of As(V) reduction. The percent conversion can express how effective reducing agent can convert As(V) to As(III). If the reduction of As(V) to As(III) occurs completely, signal of As(V) after reduction will have to equal the signal of As(III) determination at the same concentration. The conversion percentage can be assumed by **equation 2-2**.

$$\% \text{ Conversion} = \frac{A}{B} \times 100 \qquad \text{.....equation 2-2}$$

where A = Current of As(total) with reducing agent

B = Current of As(III) with reducing agent

2.6.3.3 Reduction time

The reduction time is one of the important factors which affect the conversion percentage. Consequently, the reduction time was studied. The optimum concentration of potassium iodide (from the previous study) was employed. The reduction times were varied in the range of 5 to 60 min. The efficiency of the reduction time was considered from percentage of conversion of 100 $\mu g\ L^{-1}$ As(V) standard solution. Duplicate determinations were performed for each.

2.6.3.4 The effect of ratios of [As(III)/As(V)] to percentage of conversion

The effect of ratios of [As(III)/As(V)] to the reduction of As(V) to As(III) was studied by considering percentage of conversion which was evaluated from the analysis of As(III)/As(V) standard solutions with various ratios (total arsenic concentration, 30 $\mu g\ L^{-1}$). Duplicate determinations were performed for each.

2.7 Analytical performances of FI-ASV

2.7.1 Linear dynamic range

The standard solutions in the range of 1.0 to 60 μg L^{-1} As(III) and 2.0 to 140 μg L^{-1} As(total) were prepared. Each concentration was analyzed using the optimum conditions as mentioned before. The linear dynamic range was obtained by plotting the current versus the concentration. The linearity of the response was determined by considering the correlative coefficient of the linear curve.

2.7.2 Limit of detection (LOD)

The limit of detection is expressed as the smallest concentration of analyte that can be reliably detected with a specified degree of certainty (IUPAC definition). In this work, the limit of detection was calculated as in **equation 2-3** (Li and Smart, 1996).

$$LOD = 3\sigma \qquad \text{.....equation 2-3}$$

Where σ is the standard deviation of 11 measurements of signal of 1 μg L^{-1} for As(III) and 3 μg L^{-1} for As(total)

In this study, the signal of blank can not be measured, the limit of detection can be calculated from signal of 1 μg L^{-1} As(III) and 3 μg L^{-1} As(total) determination, respectively, because the signal of 1 μg L^{-1} As(III) and 3 μg L^{-1} As(total) as least concentration can be detectable.

2.7.3 Limit of quantitation (LOQ)

The limit of quantitation is expressed as the smallest concentration of analyte that can be reliably quantified with an acceptable level of precision and accuracy (IUPAC definition). Usually, the limit of quantitation is evaluated as the signal to noise ratio that equivalent to 10 times of the standard deviation of the noise (S/N) = 10σ). However, the limit of quantitation in this work was calculated based on 10σ (**equation 2-4**) from signal of 1 μg L^{-1} As(III) and 3 μg L^{-1} As(total) determination, respectively.

$$LOQ = 10\sigma \qquad \text{.....equation 2-4}$$

Where σ is the standard deviation of 11 measurements of signal of 1 μg L^{-1} for As(III) and 3 μg L^{-1} for As(total)

2.7.4 Precision

Precision is a measurement of the degree of repeatability of an analytical method under normal operation and it is normally expressed as the percent relative standard deviation for a statistically significant number of samples. To assess the precision of the method, %RSD of As(III) and As(V) determination was evaluated. Concentrations of 2 and 20 μg L^{-1} of As(III) solutions and 10 and 100 μg L^{-1} of As(V) solutions were used. Eleven replicates at each concentration were performed. The %RSD was then calculated as **equations 2-5** (Miller and Miller, 1993).

$$\%\,RSD = \frac{s}{\overline{X}} \times 100 \qquad \text{.....equation 2-5}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}} \qquad \text{.....equation 2-6}$$

where s is the standard deviation

n is total number of measurements

X_i is each individual value used to calculate the mean

$\overline{X}$ is the mean of n measurements

2.7.5 Accuracy

The accuracy term is the measurement of exactness value of the analyte concentration or agreement between measured value and certified value or accepted reference value. The accuracy from this research was studied using Standard Reference Material (SRM) 1640 natural fresh water (NIST, USA). The difference in value between the measured value and certified value was express in term of relative percent error (% Error).

$$\% \text{ Error} = \frac{\text{Measured value} - \text{Certified value}}{\text{Certified value}} \times 100 \quad \text{.....equation 2-7}$$

2.7.6 % Recovery

The aim of this experiment was to establish validity of this method and then to apply for determination of As(III) and As(V) in natural water samples. Percentage of recovery was assessed by spiking As(III) standard solution at concentration 5, 10 and 20 μg L^{-1}, As(V) standard solution at concentration 20, 30 and 40 μg L^{-1} and mixture of As(III) and As(V) standard solutions at concentration 30 μg L^{-1} (various ratios of As(III)/As(V) concentrations; 1:1, 1:2 and 1:5) in natural water samples. Percentage of recovery was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Measured value}}{\text{Real value}} \times 100 \quad \text{.....equation 2-8}$$

2.8 The effect of interferences

Possible interferences in the arsenic determination by stripping voltammetry are cation metals which be able to reduce at the gold electrode. The cation metals including Sb(III), Cd(II), Cu(II), Fe(II), Pb(II), Hg(II), Ni(II) and Zn(II) were investigated. Various concentrations of those cation metals were added to 30 μg L^{-1} of As(III) and 30 μg L^{-1} of a mixture of As(III) and As(V) standard solutions. Duplicate determinations were carried out. Peak currents of the As determination with and without interference were compared. The interfering level was defined as the minimum concentration of interfering ion which produced % relative error exceeding ±5% (as **equation 2.9**).

$$\% \text{ Relative error} = \frac{A - B}{A} \times 100 \quad \text{.....equation 2-9}$$

where A = Current of As determination without interfering ion

B = Current of As determination with interfering ion

2.9 Application of the method

2.9.1 Sampling

Natural water samples were collected from different areas in Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat Province. According to previous report (Na Chaingmai, 1999), it was concluded that village 1 (Hudan), village 2 (Ronna), village 12 (Talad Ron Phibun) and village 13 (Sala Ki Lek) in Ron Phibun sub-district were identified as high arsenic contaminated areas. In this research, the sampling points were restricted to the high risk arsenic contaminated regions. Therefore, those mentioned villages were focused. Natural water samples were collected from four different sites each village on 9 February 2008. The sampling sites are illustrated in **Figure 2-4**. The information of latitude and longitude for the sites using global positioning system (GPS) is presented in **Appendix C-1**. The details of the samples including pH, temperature and resource are also reported.

2.9.2 Sample pretreatment

A polyethylene bottle was used to store the sample. It was washed, soaked in 10% HNO_3 at least 24 hours and then rinsed with de-ionized water. The volume of approximately 2 L of the sample was collected from each contaminated site. In order to preserve the sample, it was acidified by adding 0.5 mL of conc. HNO_3 into 500 mL water sample (pH < 2). All sample bottles were kept into a cold box at 4°C during transportation. Inorganic arsenic species in the sample were stable for up to 2 weeks under the mentioned preservation (Ariza *et al*., 2000).

2.9.3 Determination of As(III) and As(V) in natural water samples by FI-ASV

2.9.3.1 Comparison of the calibration and standard addition method

The aim of this experiment was to study the effect of matrix present in the natural water sample affect on As(III) and As(V)determination. The samples were determined using the optimum conditions for both calibration and standard addition

methods. A series of concentrations ranging from 5 to 20 μg L^{-1} of As(III) and 5 to 40 μg L^{-1} of As(V) standard solutions were added to de-ionized water for calibration method and natural water samples for standard addition method. Slope of calibration curve was used in compared.

2.9.3.2 As(III) determination

A 50 mL sample aliquot was adjusted to 0.2 M HCl by adding 0.83 mL of 12 M HCl prior to the analysis. The concentration of As(III) was calculated by calibration curve method. Duplicate determinations were performed.

2.9.3.3 As(total) determination

2.50 mL of 12 M HCl and 0.65 mL of 1.0 M KI were added into 10 mL of natural water sample. The solution were left and shaken occasionally at room temperature for 45 min. As(V) can be reduced to As(III) in a strong hydrochloric acid solution (Dugo *et al*., 2005). The volume was finally made up to 150 mL (concentration of HCl as supporting electrolyte was diluted to 0.2 M) and then the sample was analyzed using the optimum conditions. The concentration of As(total) was calculated using standard addition method. Duplicate determinations were performed.

2.9.3.4 As(V) determination

As(V) concentration was calculated from the difference of the amount of As(total) and As(III), as follows:

$$As(V) = As(total) - As(III) \quad \text{.....equation 2-9}$$

2.9.4 Determination of As(total) in natural water samples by ICP-OES

In order to evaluate the accuracy of the proposed method, As(total) in natural water sample was analyzed by ICP-OES. The determinations were conduct by an official at Central Equipment Division, Faculty of Science, Prince of Songkla University. The operating conditions are summarized in **Table 2-5**.

Table 2-5 Operating conditions for ICP-OES

Conditions	**Values**
Wavelength	188.979 nm
RF power	1.3 kW
Argon gas flow rates	
Plasma	15 L min^{-1}
Auxiliary	0.2 L min^{-1}
Nebulizer (carrier gas)	0.8 L min^{-1}
Measurements integration time	
Maximum	10 sec
Minimum	5 sec
Delay time	30 sec
Generator	40 MHz
Sample flow rate	1.5 mL min^{-1}

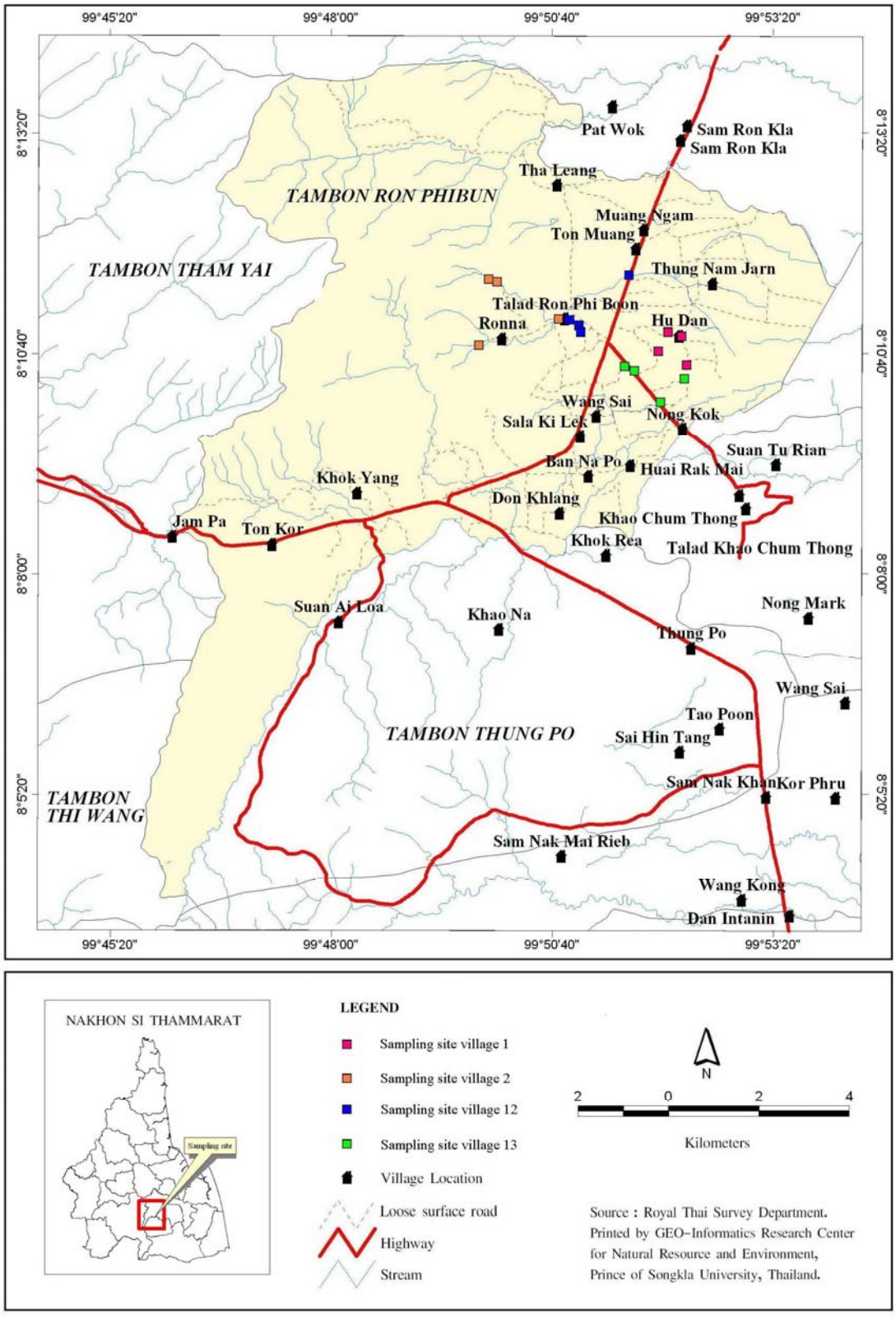

Figure 2-4 Sampling sites in Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Cyclic voltammogram of As(III) at pre-plated gold film electrode

Cyclic voltammetry was employed for preliminary study of electrochemical reaction of As(III) in 0.2 M HCl as supporting electrolyte. Potentials in the range of -0.30 to 0.50 V were applied to the gold film working electrode versus Ag/AgCl at a scan rate of 50 mV sec^{-1}. The cyclic voltammogram of 10 mg L^{-1} As(III) in 0.2 M HCl is shown in **Figure 3-1**. From the cyclic voltammogram, oxidation and reduction peaks were observed at peak potential of 0.091 V and 0.017 V, respectively. The reduction peak is attributed to the three-electron reduction of As(III) to As(0) while the oxidation peak is registered to the oxidation of As(0) to As(III). The well-defined oxidation peak indicates the potential of using the gold film electrode for As(III) determination by anodic stripping voltammetry.

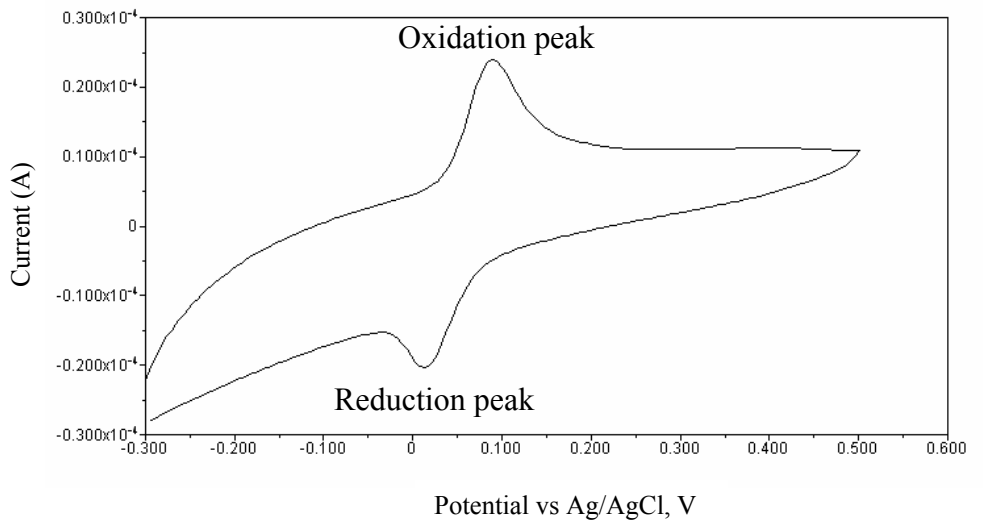

Figure 3-1 The cyclic voltammogram of 10 mg L^{-1} As(III) standard solution in 0.2 M HCl at pre-plated gold film electrode with the scan rate of 50 mV sec^{-1}

3.2 Optimization of the FI-ASV system

3.2.1 Optimization of pre-plated gold film electrode

Au(III) solution in 1.0 M sulfuric acid was used as plating solution (Sun *et al.,* 1997). Before plating, the working electrode and auxiliary electrode were polished as described in **Appendix A-1**. The conditions for pre-plated gold film electrode, i.e., plating potential, plating time, concentration of Au(III) solution and flow rate of Au(III) were optimized.

3.2.1.1 Plating potential

The effect of plating potential on 20 μg L^{-1} As(III) determination by using conditions in **Table 2-1** and **Table 2-2** was investigated. The plating potential of -0.20, -0.30, -0.40 and -0.50 V versus Ag/AgCl were studied. The results are presented in **Table 3-1** and **Figure 3-2.**

Table 3-1 Effect of plating potential on 20 μg L^{-1} As(III) determination

Plating potential (V)	Current (μA)*	% RSD
-0.20	5.278 ± 0.437	8.29
-0.30	6.993 ± 0.094	1.35
-0.40	7.868 ± 0.179	2.28
-0.50	7.720 ± 0.070	0.96

* Values given are the means (n = 3)

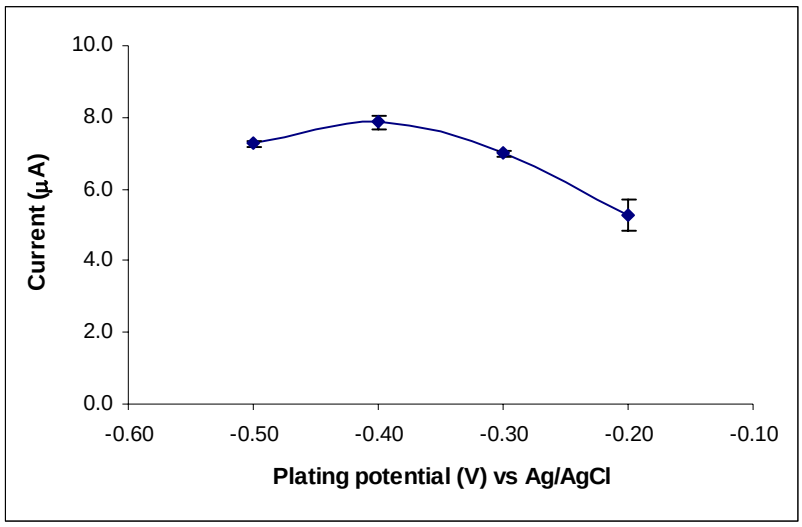

Figure 3-2 Effect of plating potential on 20 µg L^{-1} As(III) determination; deposition potential, -0.30 V; deposition time, 180 sec; scan rate, 100 mV sec^{-1}

According to the results, it was found that the plating potential which applied to gold film electrode had significant effect on As(III) determination. With higher negative potential, higher current signal was obtained. The peak current slightly increased with the increase of plating potential and then continuously decreased at the potential greater than -0.40 V. The plating potential of -0.40 V, which gave the highest signal with reasonable precision, was selected for further studies.

3.2.1.2 Plating time and concentration of Au(III) solution

The plating time and concentration of Au(III) solution, both affected the amount of gold deposited on the electrode surface, were examined simultaneously. The plating times and the concentrations of Au(III) solution were varied in the range of 60 to 120 sec and 50 to 200 mg L^{-1}, respectively. The sensitivity of 20 µg L^{-1} As(III) determination was considered for this purpose. The results are illustrated in **Table 3-2** and **Figure 3-3.** It was indicated that the plating time of 90 sec and the concentration of Au(III) solution of 100 mg L^{-1} provided the highest current response. Hence, those mentioned conditions were selected for the following experiments.

Table 3-2 Effect of plating time and concentration of Au(III) solution on 20 μg L^{-1} As(III) determination

Concentration of Au(III) (mg L^{-1})	Current (μA)*		
	60 sec	**90 sec**	**120 sec**
50	6.217 ± 0.124	6.340 ± 0.084	6.049 ± 0.092
100	6.495 ± 0.080	6.949 ± 0.046	6.353 ± 0.084
150	5.744 ± 0.244	6.425 ± 0.170	5.250 ± 0.196
200	5.814 ±0.051	6.082 ± 0.141	5.264 ± 0.089

* Values given are the means (n = 3), % RSD < 5

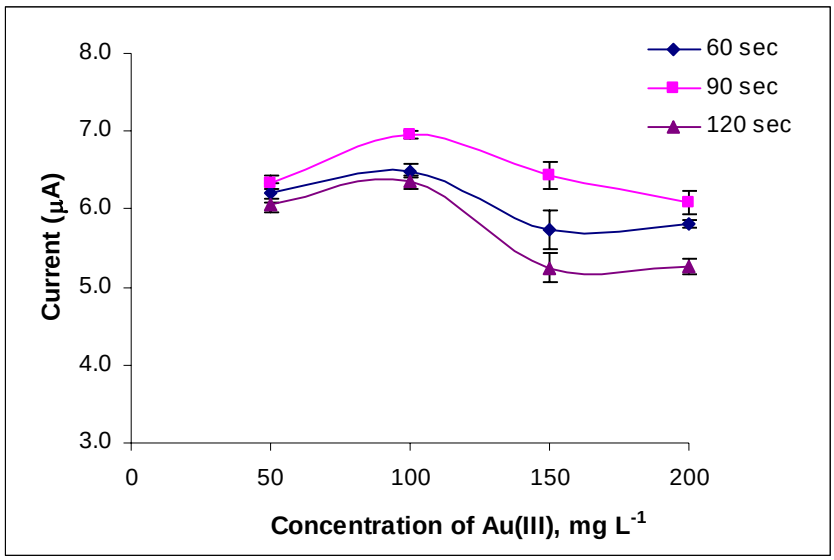

Figure 3-3 Effect of plating time and concentration of Au(III) on 20 μg L^{-1} As(III) determination; deposition potential, -0.30 V; deposition time, 180 sec; scan rate, 100 mV sec^{-1}

3.2.1.3 Flow rate of Au(III) solution

According to the previous optimizations in plating step, 100 mg L^{-1} Au(III) solution was aspirated to the flow cell while applying potential of working electrode at -0.40 V vs Ag/AgCl for 90 sec. The flow rates of Au(III) solution were varied in the range of 0.5 to 2.0 mL min^{-1}. The influence of the flow rate on 20 μg L^{-1} As(III) determination is illustrated in **Table 3-3** and **Figure 3-4.**

Table 3-3 Influence of the flow rate of Au(III) solution on 20 μg L^{-1} As(III) determination

Flow rate of Au(III) (mL min^{-1})	Current (μA)*	% RSD
0.5	7.832 ± 0.261	3.33
1.0	8.495 ± 0.133	1.57
1.5	8.367 ± 0.242	2.89
2.0	7.686 ± 0.188	2.44

* Values given are the means (n = 3)

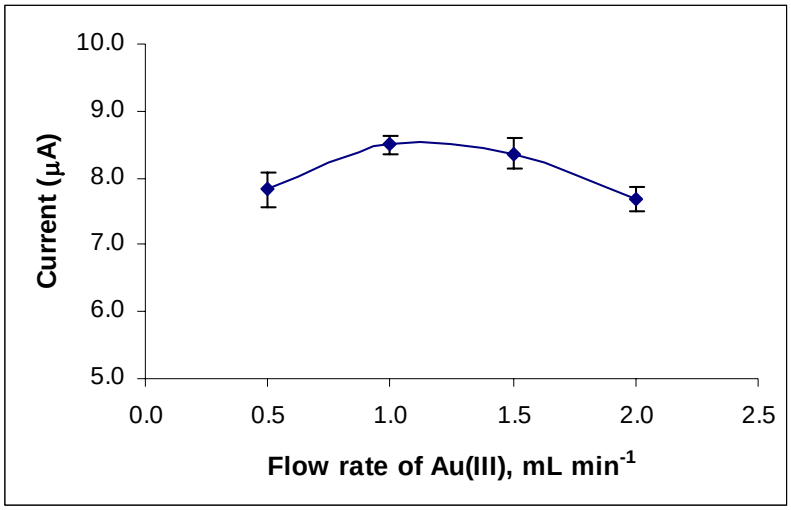

Figure 3-4 Influence of the flow rate of Au(III) solution for pre-plated gold film electrode on 20 μg L^{-1} As(III) determination; deposition potential, -0.30 V; deposition time, 180 sec; scan rate, 100 mV sec^{-1}

The response of current reached maximum value at a flow rate of 1.0 mL min^{-1} and then fell off at faster flow rate. It was noteworthy that slow plating of gold from solution gave smooth surface (Davis *et al*., 1978). With consideration to the response as well as precision, a flow rate of Au(III) at 1.0 mL min^{-1} was employed.

3.2.1.4 Stability of gold film electrode

The pre-plated gold film electrode was prepared on-line by adopting the optimum conditions as displayed in **Table 3-4**.

Table 3-4 Optimum conditions for pre-plated gold film electrode

Conditions	**Value**	**% RSD**
Plating potential (V)	-0.40	2.28
Plating time (sec)	90	0.66
Concentration of Au(III) solution (mg L^{-1})	100	0.66
Flow rate of Au(III) solution (mL min^{-1})	1.0	1.57

In order to explore the stability of the gold film electrode, 20 μg L^{-1} As(III) and As(total) determinations were repeatedly carried out at the same film. The results are shown in **Table 3-5** and **Table 3-6**. As the results, the decrease in the signal of As(III) and As(total) determination was observed when reusing the gold film. %Decrease in signal was calculated (as **equation 2.1**) to indicate the deterioration of the gold film.

Table 3-5 The stability of the gold film electrode for 20 μg L^{-1} As(III) determination

Entry	Experiment 1*		Experiment 2**	
	Current (μA)	% Decrease	Current (μA)	% Decrease
1	8.376	-	9.957	-
2	8.546	-	10.248	-
3	8.935	-	10.044	-
4	8.972	-	10.070	-
5	8.970	-	9.880	-
6	8.821	0.70	9.785	2.54
7	8.613	1.67	9.768	2.71
8	8.389	4.23	9.734	3.05
9	8.008	8.58	9.480	5.58
10	7.931	9.46	9.237	8.00

* Current mean (No. 1-5) of experiment 1 was 8.760 μA

** Current mean (No. 1-5) of experiment 2 was 10.040 μA

Table 3-6 The stability of the gold film electrode for 20 μg L^{-1} As(total) determination

Entry	Experiment 1*		Experiment 2**	
	Current (μA)	% Decrease	Current (μA)	% Decrease
1	5.508	-	5.107	-
2	5.555	-	4.982	-
3	5.505	-	4.820	-
4	5.430	-	4.796	-
5	5.261	-	4.881	-
6	5.201	4.61	4.879	0.77
7	5.186	4.88	4.796	2.46
8	5.182	4.95	4.713	4.16
9	5.167	5.23	4.593	6.60
10	4.607	15.5	4.223	14.1

* Current mean (No. 1-5) of experiment 1 was 5.452 μA

** Current mean (No. 1-5) of experiment 2 was 4.917 μA

From the decrease in sensitivity, it can be concluded that the gold film on the glassy carbon electrode was lost (dissolved) because of the tendency for complex formation between the gold and chloride ion (from the supporting electrolyte) as $AuCl_4^-$ (Sun *et al*., 1997). Thereby, the active gold surface for arsenic deposition was diminished.

The proposed gold film was experimentally proven that it can be repeatedly applied up to 8 consecutively analysis cycles without significant decline in sensitivity (%decrease less than 5%).

3.2.2 Optimization of As(III) determination

After pre-plated gold film procedure, gold film electrode was provided for As(III) determination. In order to achieve maximum sensitivity for As(III) determination, various parameters including concentration of supporting electrolyte, deposition potential, deposition time, scan rate and flow rate were optimized.

3.2.2.1 Concentration of HCl as a supporting electrolyte

A number of supporting electrolytes for As(III) determination by anodic stripping voltammetry were studied by Sun *et al*. (1997). It was found that HCl was the most suitable. The highest sensitive and the narrowest peaks were obtained. In this study, HCl was chosen as a supporting electrolyte. The concentrations of HCl were varied in the range of 0.01 to 0.60 M as shown in **Table 3-7** and **Figure 3-5**. It was remarkable that signal of As(III) determinations became apparently higher by increasing in HCl concentration. However, in such conditions containing high Cl^- concentration, the stability of gold film electrode can be reduced owing to the $AuCl_4^-$ formation (as mentioned in section **3.2.1.4**). In order to compromise between sensitivity and stability of the gold film electrode, 0.2 M HCl was selected for subsequent experiments.

Table 3-7 Effect of HCl concentration as a supporting electrolyte on 10 and 20 μg L^{-1} As(III) determination

HCl concentration (M)	Current (μA)* of 10 μg L^{-1} As(III)	%RSD	Current (μA)* of 20 μg L^{-1} As(III)	%RSD
0.01	1.150 ± 0.035	3.0	2.070 ± 0.037	1.8
0.05	2.776 ± 0.013	0.5	5.478 ± 0.086	1.6
0.10	3.852 ± 0.155	4.0	8.000 ± 0.046	0.6
0.20	5.203 ± 0.063	1.2	10.066 ± 0.048	0.5
0.30	5.804 ± 0.171	2.9	11.137 ± 0.101	0.4
0.40	6.286 ± 0.068	1.1	11.418 ± 0.065	0.6
0.50	6.416 ± 0.033	0.5	12.363 ± 0.136	1.1
0.60	6.728 ± 0.036	0.5	12.981 ± 0.052	0.4

* Values given are the means (n = 2)

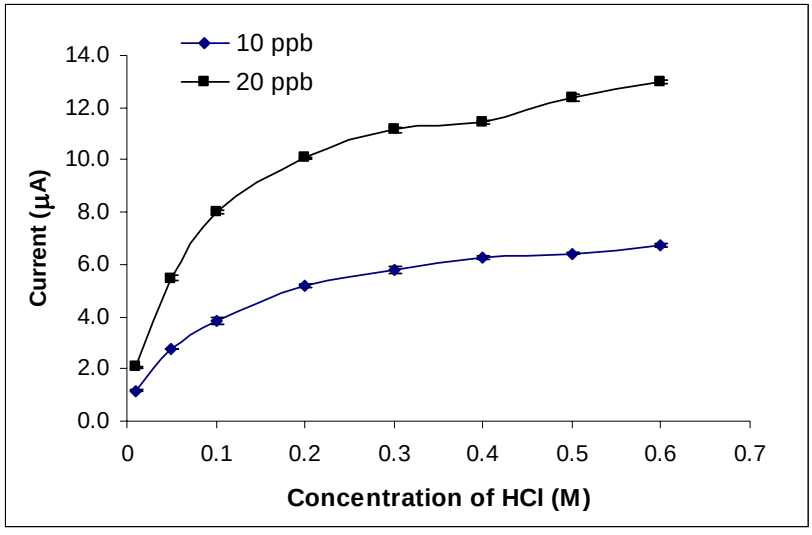

Figure 3-5 Effect of HCl concentration as supporting electrolyte on 10 and 20 μg L^{-1} As(III) determination; deposition potential, -0.30 V; deposition time, 180 sec; scan rate, 100 mV sec^{-1}

3.2.2.2 Deposition potential

Deposition potential is one of considerable factors affecting sensitivity of As(III) determination. Then the optimization was examined by varying deposition potential from -0.10 to -0.40 V vs Ag/AgCl while deposition time of 180 sec and scan rate of 100 mV sec^{-1} were fixed. The sensitivity of As(III) was evaluated from a slope of calibration curve for As(III) determination. The dependence of peak current on deposition potential is illustrated in **Table 3-8** and **Figure 3-6**. It was found that the sensitivity increased linearly when more negative deposition potential was applied. Nevertheless, it slightly cut down at deposition potential over -0.30 V. From the previous report (Sun *et al*. 1997), the formation of H_2 bubbles could be possible after deposition potential of -0.35 V. Therefore, the deposition potential of -0.30 V was chosen due to the highest sensitivity and bubble avoidance.

Table 3-8 Effect of the deposition potential on As(III) determination

Deposition potential (V)	**Current (μA)* at various concentrations of As(III), μg L^{-1}**				**Equation, R^2**
	5	**10**	**15**	**20**	
-0.10	2.366	4.906	7.272	8.533	y = 0.417x + 0.552, R^2 = 0.9796
-0.20	2.813	5.693	8.176	9.534	y = 0.453x + 0.893, R^2 = 0.9770
-0.30	2.769	5.473	7.983	10.036	y = 0.486x + 0.488, R^2 = 0.9963
-0.40	2.648	4.951	7.546	9.639	y = 0.471x + 0.304, R^2 = 0.9985

* Values given are the means (n = 2), %RSD < 6

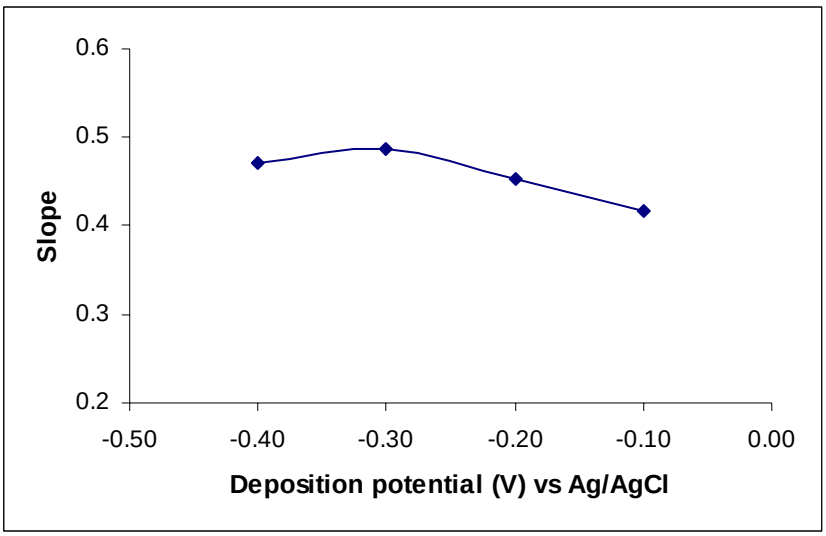

Figure 3-6 Effect of the deposition potential on 5-20 μg L^{-1} As(III) determination; deposition time, 180 sec; scan rate, 100 mV sec^{-1}

3.2.2.3 Scan rate

Basically, stripping current is proportional to the square root of scan rate (Sun *et al.* 1997). The influence of scan rate on As(III) determination was studied by varying scan rate from 40 to 100 mV sec^{-1}. A slope of 5-20 μg L^{-1} As(III) determination was evaluated. Results are presented in **Table 3-9**, **Figure 3-7, Figure 3-8** and **Figure 3-9**. As the results, influence of scan rate on peak current was observed. The higher scan rate, the more increasing in signal was obtained. On the other hand, current peaks became broader when higher scan rate applied. In order to meet criterions of high sensitivity and well-defined stripping peak, scan rate of 90 mV sec^{-1} was chosen for subsequent experiments.

Table 3-9 Effect of the scan rate on 5-20 μg L^{-1} As(III) determination

Scan rate (mV sec^{-1})	Current (μA)* at various concentrations of As(III), μg L^{-1}				Equation, R^2
	5	10	15	20	
40	0.964	2.890	4.494	6.039	y = 0.337x - 0.610, R^2 = 0.9972
50	1.372	3.584	5.412	6.631	y = 0.352x - 0.151, R^2 = 0.9842
60	1.956	3.991	5.993	7.430	y = 0.368x + 0.237, R^2 = 0.9939
70	2.284	4.516	6.584	8.200	y = 0.396x + 0.442, R^2 = 0.9950
80	2.555	4.681	7.015	8.696	y = 0.415x + 0.548, R^2 = 0.9960
90	2.643	4.759	7.130	9.018	y = 0.430x + 0.514, R^2 = 0.9983
100	2.659	4.338	7.188	9.340	y = 0.458x + 0.158, R^2 = 0.9913

* Values given are the means (n = 2), %RSD < 8

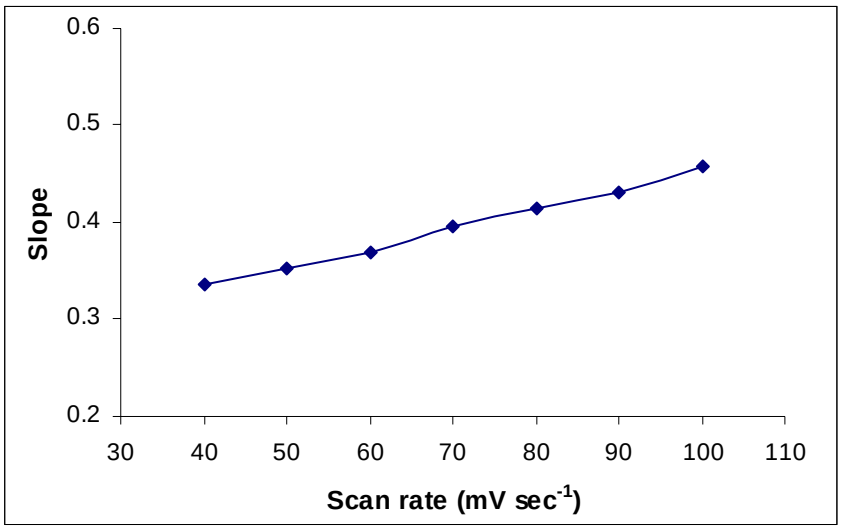

Figure 3-7 Effect of the scan rate on 5-20 μg L^{-1} As(III) determination; deposition potential, -0.30 V; deposition time, 180 sec

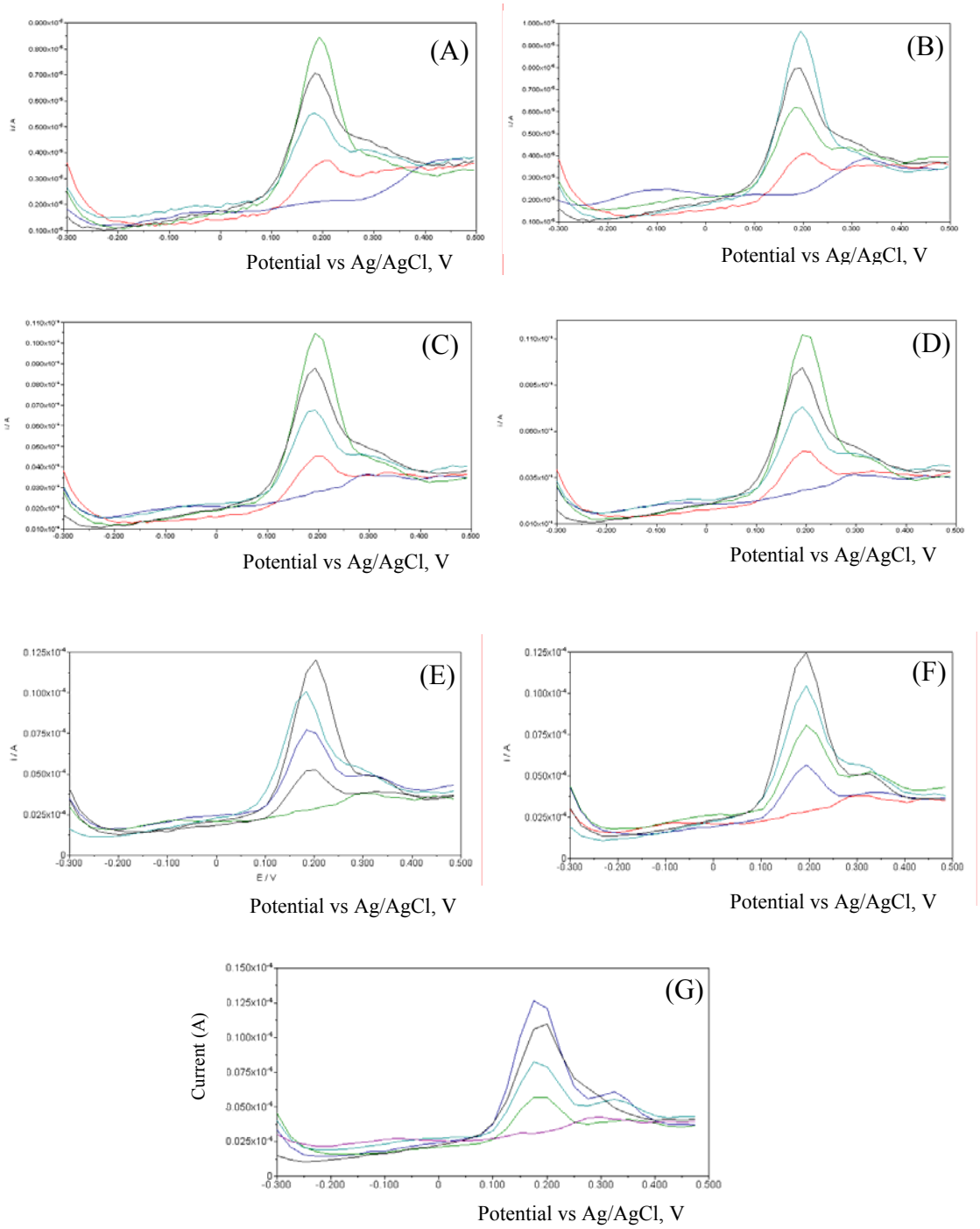

Figure 3-8 Voltammograms of 0, 5, 10, 15 and 20 µg L^{-1} As(III) determination at various scan rates; (A) 40 mV sec^{-1}, (B) 50 mV sec^{-1}, (C) 60 mV sec^{-1}, (D) 70 mV sec^{-1}, (E) 80 mV sec^{-1}, (F) 90 mV sec^{-1}, (G) 100 mV sec^{-1}

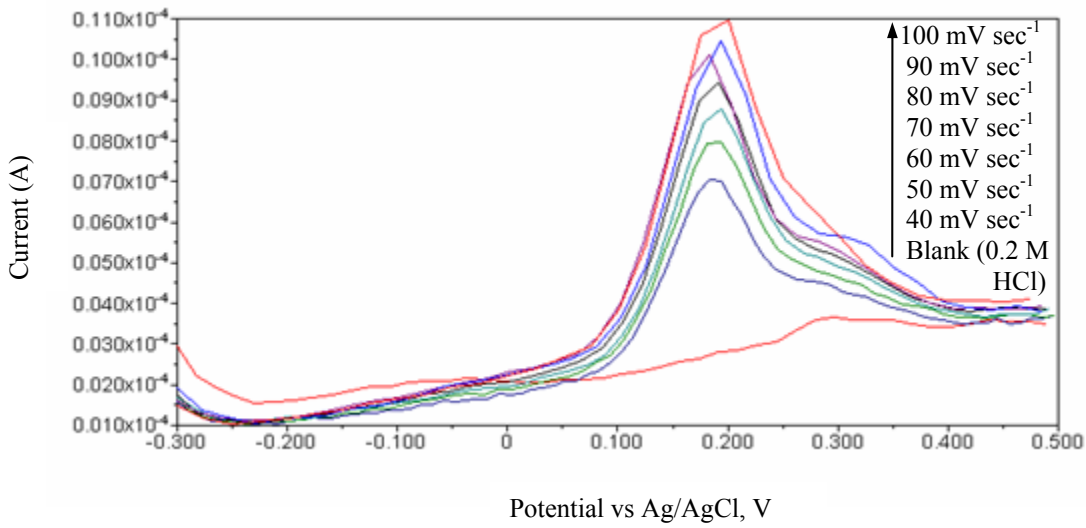

Figure 3-9 Voltammograms of 15 μg L^{-1} As(III) determination by FI-ASV at various scan rates; deposition potential, -0.30 V; deposition time, 180 sec

3.2.2.4 Flow rate of As(III)

The effect of flow rate on As(III) determination investigated by considering the slopes of calibration curve at different flow rate is shown in **Table 3-10**, **Figure 3-10** and **Figure 3-11.** It can be seen that the sensitivity is increased by increasing flow rate. A flow rate of 2.5 mL min^{-1} giving the maximum sensitivity was selected as optimum flow rate. However, at higher flow rate than 2.5 mL min^{-1}, the experiment could not be performed due to the leakage. Additionally, deterioration of the gold film electrode may be obtained with high flow rate.

Table 3-10 Effect of the flow rate on As(III) determination

Flow rate (mL min^{-1})	Current (μA)* at various concentrations of As(III), μg L^{-1}				Equation, R^2
	5	10	15	20	
0.5	1.247	2.970	4.269	5.629	y = 0.291x – 0.115, R^2 = 0.9951
1.0	2.014	3.856	5.476	7.112	y = 0.346x + 0.250, R^2 = 0.9976
1.5	2.069	3.983	6.173	8.083	y = 0.405x + 0.019, R^2 = 0.9992
2.0	2.195	4.433	6.751	9.135	y = 0.469x – 0.264, R^2 = 1
2.5	1.966	4.550	7.279	9.429	y = 0.502x – 0.473, R^2 = 0.9977

* Values given are the means (n = 2), % RSD < 7

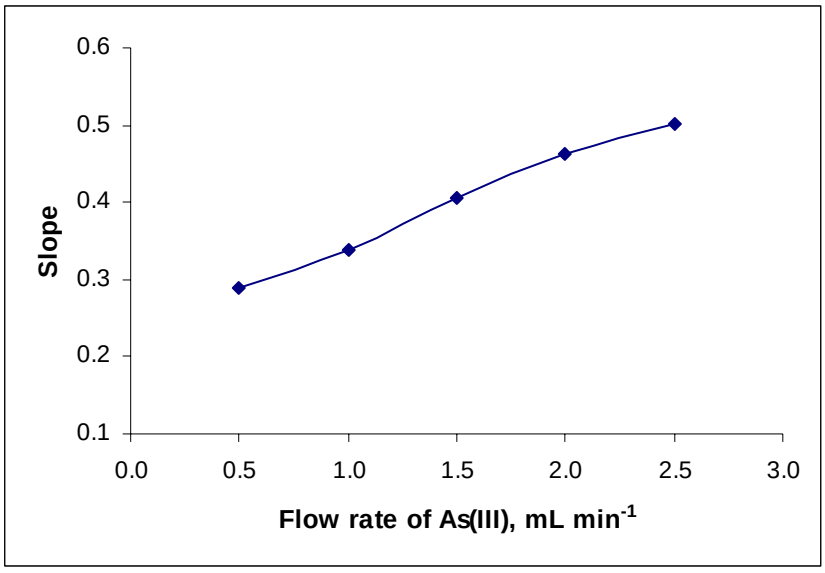

Figure 3-10 Effect of the flow rate on 5-20 μg L^{-1} As(III) determination; deposition potential, -0.30 V; scan rate 90 mV sec^{-1}; deposition time, 180 sec

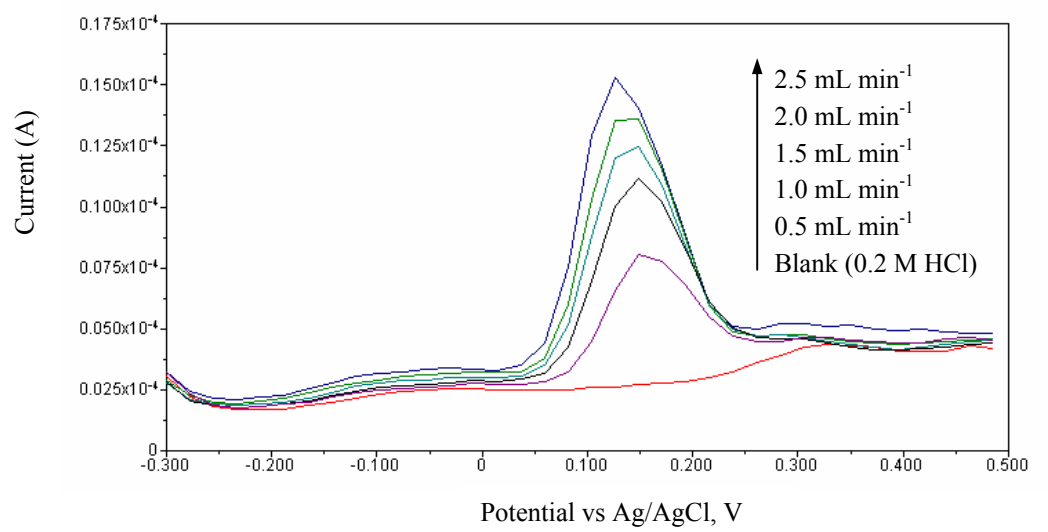

Figure 3-11 Voltammograms of 20 μg L^{-1} As(III) determination at various flow rates; deposition potential, -0.30 V; scan rate 90 mV sec^{-1}; deposition time, 180 sec

3.2.2.5 Deposition time

The effect of deposition time on As(III) determination was examined by varying deposition time within a range of 120 to 300 sec. The sensitivity of As(III) determination was considered from a slope of calibration curve. The variation of peak current as a function of deposition time is illustrated in **Table 3-11**, **Figure 3-12** and **Figure 3-13.**

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 the gold film electrode with increasing deposition time. For the detection of high concentrations of As(III), short deposition time can be used to avoid the saturation of electrode surface. For low concentrations of As(III), longer deposition time can be applied. However, longer analysis time which resulted decrease of sample throughput was not suitable for routine analysis. To compromise sensitivity and analysis time, the deposition of 240 sec was selected to be the optimum condition.

Table 3-11 Effect of the deposition time on As(III) determination

Deposition time (sec)	Current (μA)* at various concentrations of As(III), μg L^{-1}				Equation, R^2
	5	10	15	20	
120	1.621	3.504	5.311	7.331	y = 0.379x - 0.293, R^2 = 0.9995
150	1.908	4.288	6.485	8.510	y = 0.440x - 0.203, R^2 = 0.9987
180	2.614	5.229	7.870	10.427	y = 0.522x + 0.016, R^2 = 1
210	2.723	5.782	8.707	11.690	y = 0.596x - 0.231, R^2 = 0.9999
240	3.321	6.828	10.376	13.663	y = 0.691x - 0.096, R^2 = 0.9997
270	3.643	7.674	11.135	14.759	y = 0.736x + 0.101, R^2 = 0.9990
300	4.113	8.033	11.752	15.473	y = 0.756x + 0.394, R^2 = 0.9998

*Values given are the means (n = 2), %RSD < 8

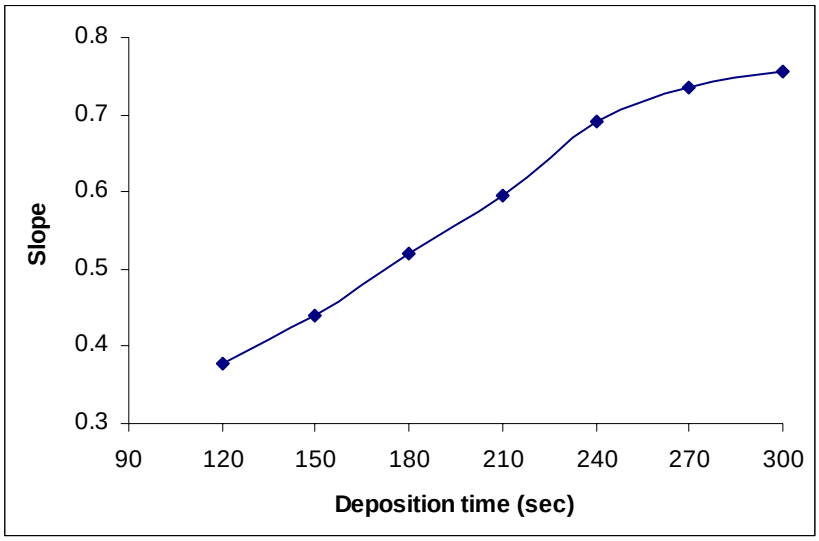

Figure 3-12 Effect of the deposition time on 5-20 μg L^{-1} As(III) determination; deposition potential, -0.30 V; scan rate, 90 mV sec^{-1}

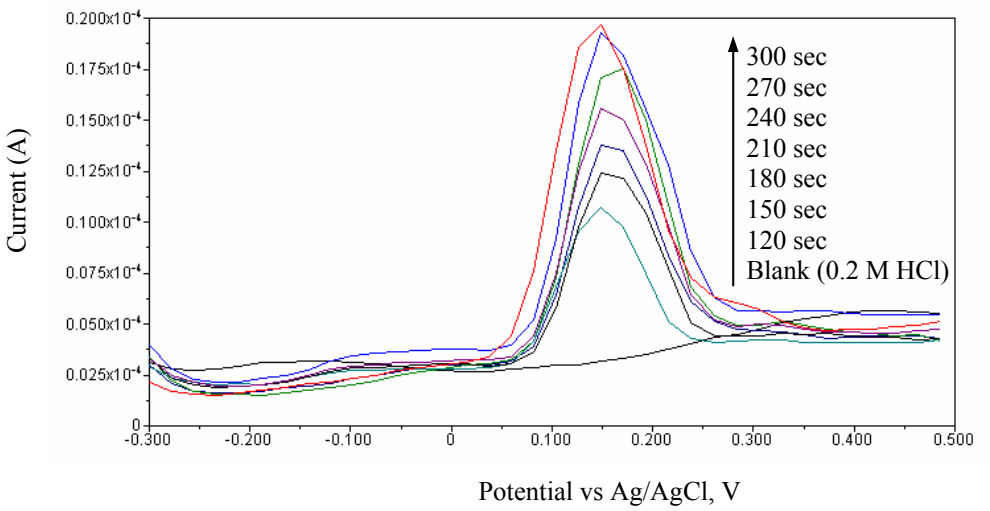

Figure 3-13 Voltammograms of 20 μg L^{-1} As(III) determination at various deposition times; deposition potential, -0.30 V; scan rate, 90 mV sec^{-1}; flow rate, 2.5 mL min^{-1}

The optimum conditions of As(III) determination can be summarized in **Table 3-12**.

Table 3-12 The optimum conditions for As(III) determination

Conditions	**Optimum values**
Concentration of HCl as supporting electrolyte (M)	0.20
Deposition potential (V)	-0.30
Scan rate (mV sec^{-1})	90
Flow rate (mL min^{-1})	2.5
Deposition time (sec)	240

3.2.3 Optimization for reduction of As(V) to As(III)

In general, the determination of total inorganic arsenic requires a preliminary step to reduce As(V) to As(III), followed by the determination of As(III). Then As(V) can be calculated from the different amount between As(total) and As(III).

In order to reach the maximum reduction efficiency, parameters such type of reducing agent, concentration and reduction time were optimized.

3.2.3.1 Type of reducing agent

Certain reducing agents including potassium iodide (KI), a mixture of ascorbic acid and KI, sodium thiosulphate ($Na_2S_2O_3$), sodium dithionite ($Na_2S_2O_4$) and L-cysteine were investigated. For this purpose, 30 µg L^{-1} As(III) determinations with and without reducing agent were also compared. Results are presented in **Figure 3-14** to **Figure 3-18**.

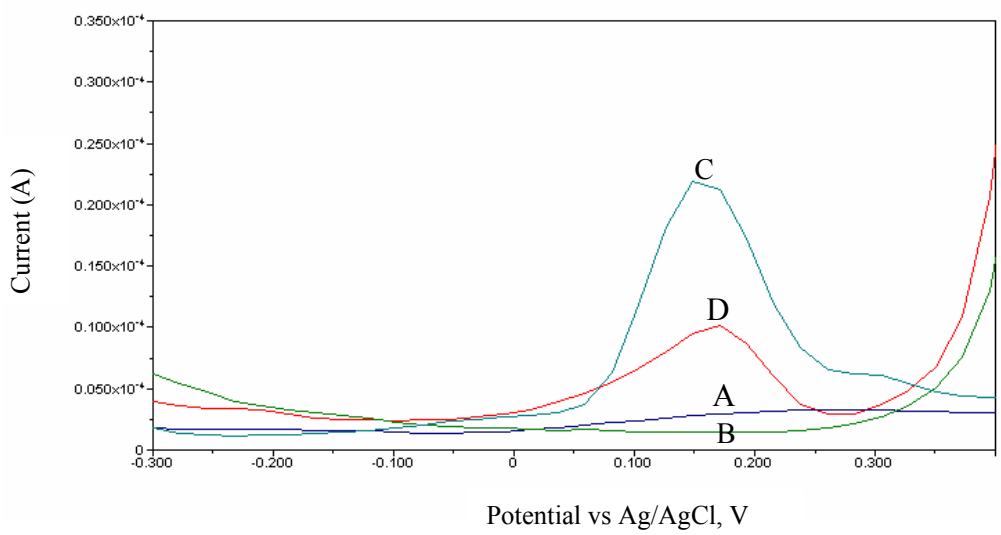

Figure 3-14 Voltammograms of As(III) determination; (A) Blank (0.2 M HCl); (B) Blank (0.2 M HCl) + 6.0 mM KI; (C) 30 µg L^{-1} As(III); (D) 30 µg L^{-1} As(III) + 6.0 mM KI; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

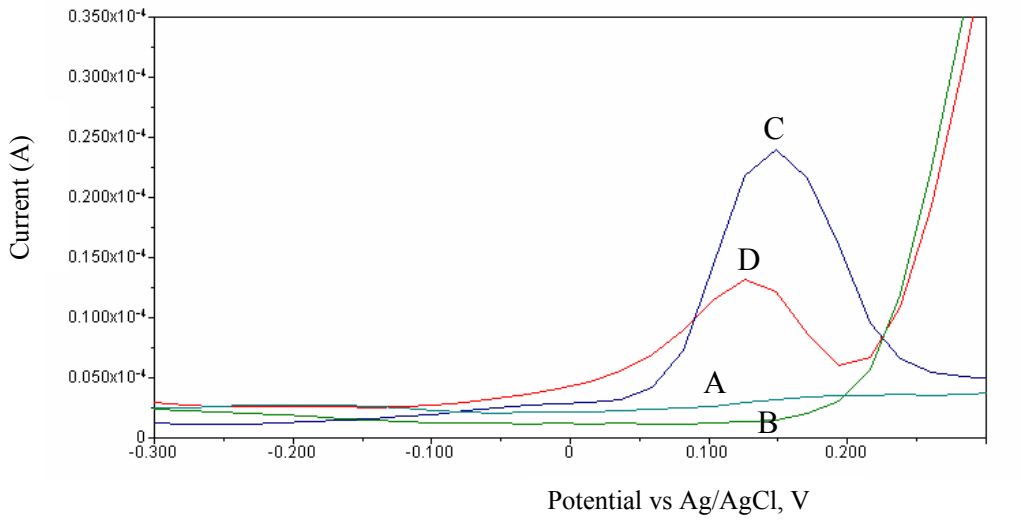

Figure 3-15 Voltammograms of As(III) determination; (A) Blank (0.2 M HCl); (B) Blank (0.2 M HCl) + a mixture of 7.5 mM ascorbic acid and KI; (C) 30 µg L^{-1} As(III); (D) 30 µg L^{-1} As(III) + a mixture of 7.5 mM ascorbic acid and KI; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

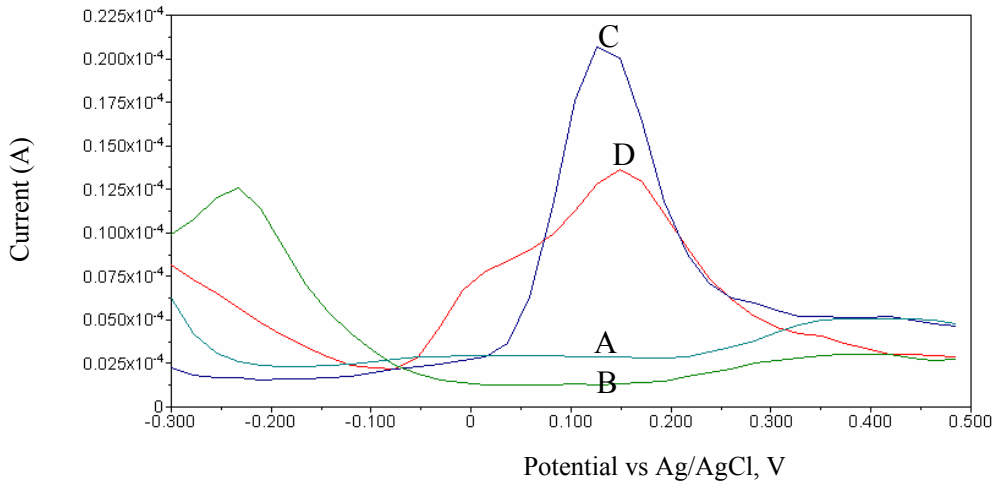

Figure 3-16 Voltammograms of As(III) determination; (A) Blank (0.2 M HCl); (B) Blank (0.2 M HCl) + 0.06 mM $Na_2S_2O_3$; (C) 30 µg L^{-1} As(III); (D) 30 µg L^{-1} As(III) + 0.06 mM $Na_2S_2O_3$; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

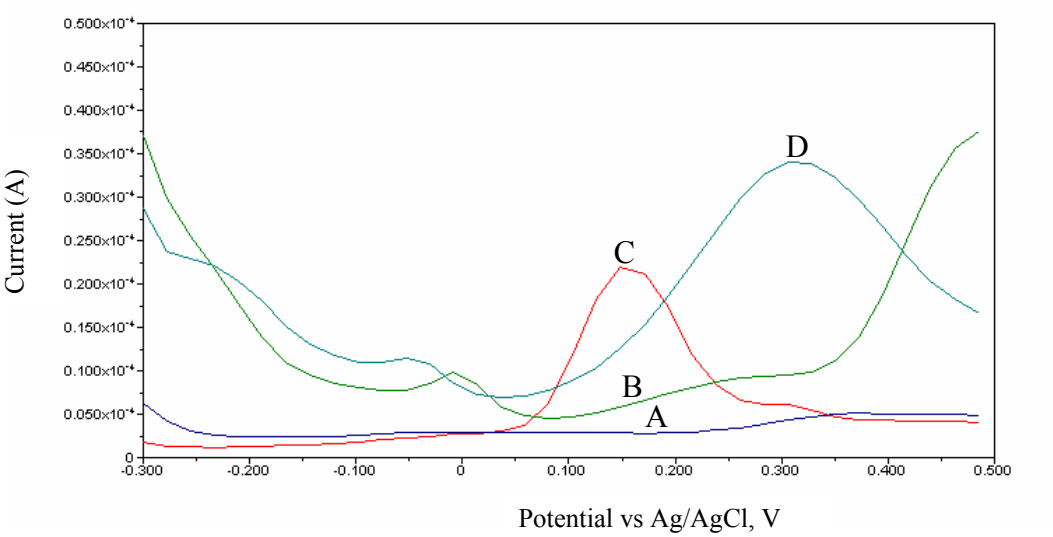

Figure 3-17 Voltammograms of As(III) determination; (A) Blank (0.2 M HCl); (B) Blank (0.2 M HCl) + 0.3 mM $Na_2S_2O_4$; (C) 30 µg L^{-1} As(III); (D) 30 µg L^{-1} As(III) + 0.3 mM $Na_2S_2O_4$; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

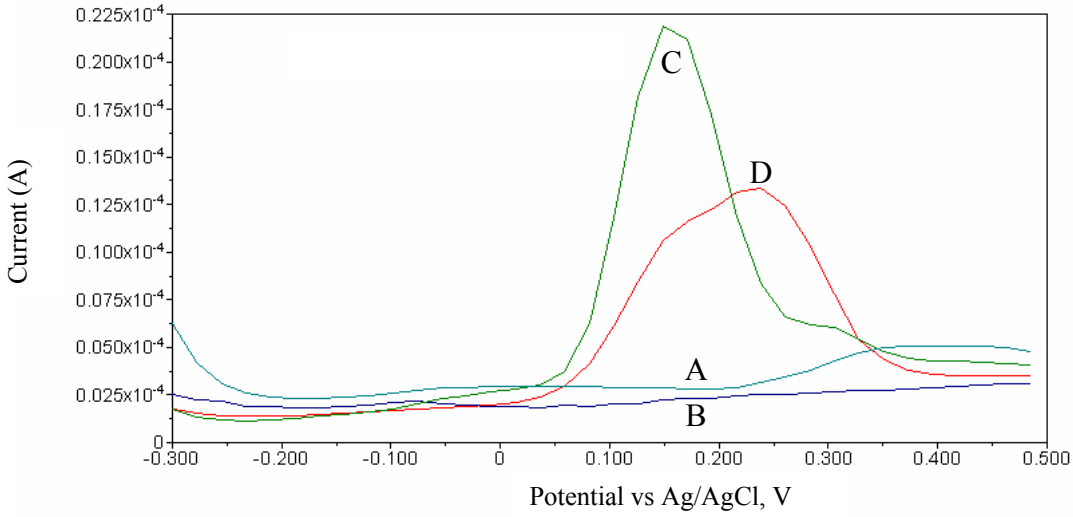

Figure 3-18 Voltammograms of As(III) determination; (A) Blank (0.2 M HCl); (B) Blank (0.2 M HCl) + 0.02 mM L-cysteine; (C) 30 µg L^{-1} As(III); (D) 30 µg L^{-1} As(III) + 0.02 mM L-cysteine; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

By using $Na_2S_2O_3$ as the reducing agent, the result is illustrated in **Figure 3-16**. It was found that a high-current peak of the reducing agent, at approximately -0.22 V (B), was observed. The lower peak current and broader peak shape of 30 μg L^{-1} As(III) in the reducing agent (D) was obtained comparing with As(III) without reducing agent (C).

By using $Na_2S_2O_4$ as reducing agent, the result is presented in **Figure 3-17**. It can be seen that current peak of the reducing agent (B) was different from current peak of blank (0.2 M HCl) (A). Moreover, the higher peak current, broader and positive shifted peak of As(III) with reducing agent (D) was observed comparing with As(III) without reducing agent (C). It can be concluded that current peak of the reducing agent interfered As(III) determination.

By using L-cysteine as reducing agent, the result is shown in **Figure 3-18**. It was found that lower peak current and broader peak shape of As(III) with reducing agent (D) was observed comparing with As(III) without the reducing agent (C).

As in **Figure 3-18**, with L-cysteine as reducing agent, lower peak current and broader peak shape of As(III) with reducing agent (D) was observed compared with As(III) without the reducing agent (C). Additionally, a shift of the peak potential toward more positive potential was exhibited.

By using KI and a mixture of ascorbic acid and KI as reducing agent, the results are depicted in **Figure 3-14** and **Figure 3-15**. It was found that the peak shapes of 30 μg L^{-1} As(III) with and without reducing agent were similar and appeared at the same position. However, in case of with reducing agent, the peak current decreased. From the peak shape and peak current, KI and a mixture of ascorbic acid and KI were suitable as reducing agent, whereas $Na_2S_2O_3$, $Na_2S_2O_4$ and L-cysteine were ignored. Therefore, KI and a mixture of ascorbic acid and KI were selected for further experiments to increase the efficiency of the reduction.

3.2.3.2 Concentration of reducing agent

The effect of concentration of reducing agent on efficiency of the reduction of As(V) to As(III) was studied. Various concentrations of the reducing agents, KI and a mixture of ascorbic acid and KI, were optimized with a concentration of 100 μg L^{-1} As(V). The influence of the concentration of the reducing agent on percentage of conversion is expressed in **Table 3-13**, **Table 3-14**, **Figure 3-19** and **Figure 3-20**.

It was observed that percentage of conversion increased for the concentration of KI of up to 6.5 mM and then decreased at higher concentrations. Similarly, %conversion increased for concentration of a mixture of ascorbic acid and KI of up to 7.5 mM and then decreased at higher concentrations. Due to higher percentage of conversion, 6.5 mM KI was chosen for further experiments.

Table 3-13 Effect of concentration of KI as reducing agent for reduction of 100 μg L^{-1} As(V)

Concentration of KI (mM)	Current (μA)* of As(III)	Current (μA)* of As(V)	%Conversion
5.5	20.122 ± 0.245	17.136 ± 0.267	85.2
6.0	19.053 ± 0.156	18.934 ± 0.431	99.4
6.5	18.314 ± 0.706	19.528 ± 0.227	106
7.0	18.431 ± 1.038	18.064 ± 1.131	98.0
7.5	18.879 ± 0.338	16.153 ± 1.359	86.0

* Values given are the means (n = 2), %RSD < 7

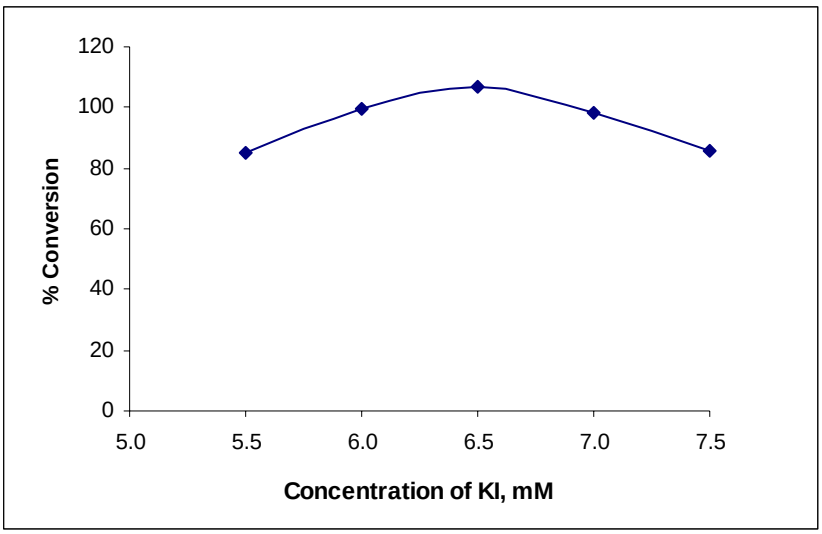

Figure 3-19 Effect of concentration of KI as reducing agent for reduction of 100 μg L^{-1} As(V); deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-14 Effect of concentration of a mixture of ascorbic acid and KI as reducing agent for reduction of 100 μg L^{-1} As(V)

Concentration of ascorbic acid + KI (mM)	Current (μA)* of As(III)	Current (μA)* of As(V)	%Conversion
2.5	17.253 ± 0.663	2.259 ± 0.122	13.1
5.0	14.771 ± 0.816	3.063 ± 0.366	20.7
7.5	15.843 ± 0.373	5.138 ± 0.029	32.4
10.0	15.191 ± 1.074	2.886 ± 0.144	19.0

*Values given are the means (n = 2), %RSD < 7

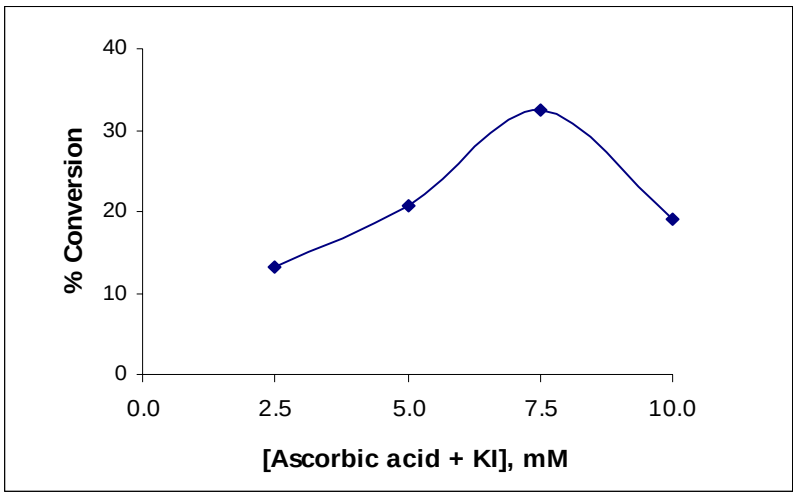

Figure 3-20 Effect of concentration of a mixture of ascorbic acid and KI as reducing agent for reduction of 100 μg L^{-1} As(V); deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

3.2.3.3 Reduction time

To determine the time required to quantitatively reduce As(V) to As(III), the effect of the reduction time was studied. 100 μg L^{-1} As(V) standard solution with 6.5 mM KI as reducing agent was determined with different reduction times (5 to 60 min). Effect of the reduction time is shown in **Table 3-15** and **Figure 3-21**.

The reduction time of 45 min was chosen as optimum condition due to the highest percentage of conversion.

Table 3-15 Effect of the reduction time on 100 μg L^{-1} As(V) reduction

Reduction time (min)	Current (μA)* of As(III)	Current (μA)* of As(V)	%Conversion
5	23.472 ± 0.107	11.823 ± 0.038	50.4
30	25.247 ± 0.413	20.419 ± 0.486	80.9
45	22.707 ± 1.100	22.272 ± 0.841	98.1
60	23.174 ± 0.826	20.620 ± 0.706	89.0

* Values given are the means (n = 2), %RSD < 5

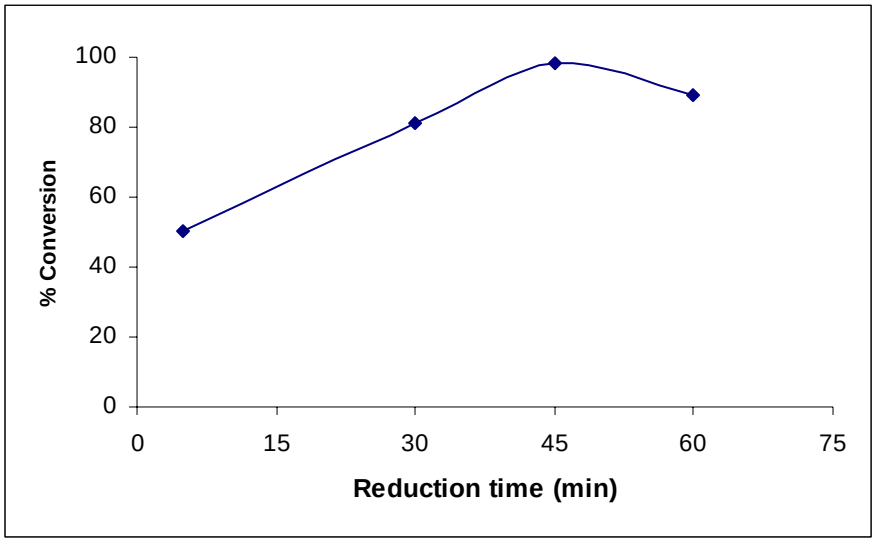

Figure 3-21 Effect of the reduction time on 100 μg L^{-1} As(V) reduction; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

3.2.3.4 The effect of ratios of [As(III)/As(V)] to percentage of conversion

The effect of ratios of [As(III)/As(V) to the reduction of As(V) to As(III) was tested by comparing the results obtained in the analysis of solutions with the same total arsenic concentration (30 μg L^{-1}) but with different ratios of As(III)/As(V). The reduction step was carried out using 6.5 mM KI as reducing agent with reduction time of 45 min. The percentage of conversion As(V) to As(III) was considered. Results are shown in **Table 3-16**.

From the results, it can be seen that percentage of conversion of As(V) to As(III) at different ratios were in the range of 93-100%. Therefore, the reduction by using these conditions was quantitative.

Table 3-16 %Conversion of As(V) to As(III) at various ratios

Ratios of As(III) : As(V)	**Ratio of As(III) : As(V)**	**Current (μA)***	**%Conversion****
30 : 0	30 : 0	7.562 ± 0.224	-
25 : 5	5 : 1	7.253 ± 0.028	95.9
20 : 10	2 : 1	7.067 ± 0.334	93.5
15 : 15	1 : 1	7.066 ± 0.164	93.5
10 : 20	1 : 2	7.420 ± 0.307	98.1
5 : 25	1 : 5	7.581 ± 0.342	100
3 : 27	1 : 9	7.301 ± 0.240	96.6
2 : 28	1 : 14	7.582 ± 0.177	100
0 : 30	0 : 30	7.064 ± 0.195	93.4

* Values given are the means (n = 2), % RSD < 5

** Calculated by compare with 7.562 μA (current of As(III) with reducing agent)

3.3 Analytical performances of FI-ASV

3.3.1 Linear dynamic range

The linear dynamic range is generally determined by plotting the signals (current) versus the concentrations of arsenic standard solutions. It is desirable to work within the linear region of the resulting of calibration curve.

The optimized conditions were used to established the linear dynamic range. Results are shown in **Table 3-17**, **Table 3-18** and **Figure 3-22** to **Figure 3-27**. It was found that the linear dynamic range of As(III) concentration was obtained from 1.0 to 30 μg L^{-1} with a good correlation coefficient, $R^2 = 0.995$. The equation is:

$$Ip(\mu A) = 0.656\ [As(III)]\ (\mu g\ L^{-1}) - 0.074$$

The linear range of As(total) concentration from 2.0 to 100 μg L^{-1} with $R^2 = 0.995$, was provided. The equation is:

$$Ip(\mu A) = 0.230\ [As(total)]\ (\mu g\ L^{-1}) + 0.359$$

The different slopes of the two curves (As(III) and As(total)) are related to the presence of 6.5 mM KI as reducing agent when As(total) is to be determined. The decreasing sensitivity at high concentration of As(III) and As(V) are caused by a coating of the electrode with the deposited As(0) which is non-conductive and gold film may become saturated.

Table 3-17 Study of linear dynamic range for As(III) determination by using FI-ASV

As(III) concentration ($\mu g\ L^{-1}$)	**Current* (μA)**	**%RSD**
0	0.000 ± 0.000	-
1	0.780 ± 0.077	9.89
2	1.118 ± 0.034	3.09
3	1.752 ± 0.030	1.72
4	2.342 ± 0.045	1.93
5	3.217 ± 0.040	1.25
8	5.458 ± 0.023	0.41
10	7.340 ± 0.173	2.36
15	10.012 ± 0.088	0.88
20	14.022 ± 0.166	1.99
25	16.428 ± 0.129	0.78
30	19.044 ± 0.264	1.39
35	20.650 ± 0.001	0.01
40	22.831 ± 0.263	1.15
50	28.867 ± 0.445	1.66
60	28.973 ± 0.259	0.89

* Values given are the means (n = 2)

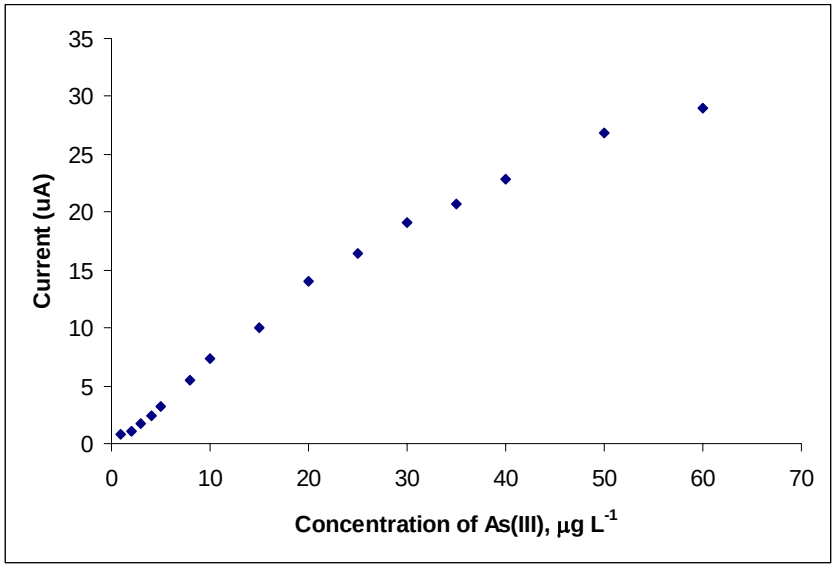

Figure 3-22 The current response of As(III) as a function of concentration

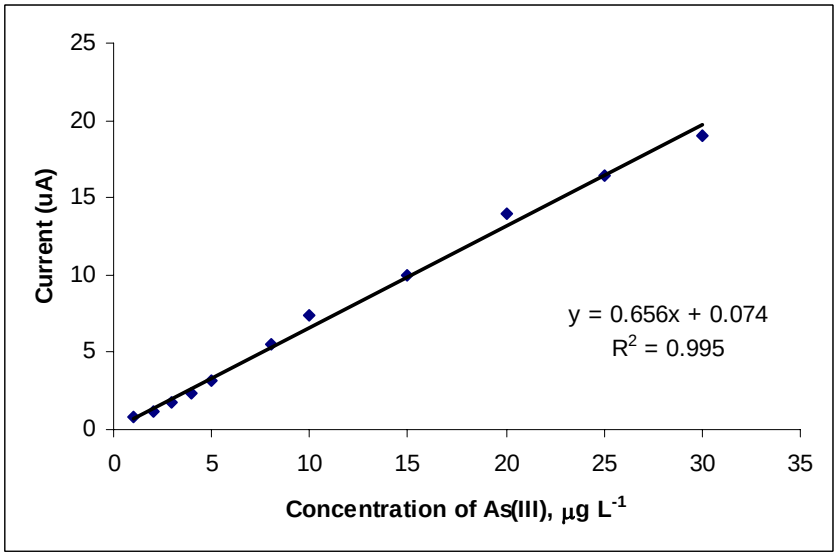

Figure 3-23 The linear dynamic range of As(III) determination from 1.0 to 30 µg L^{-1}

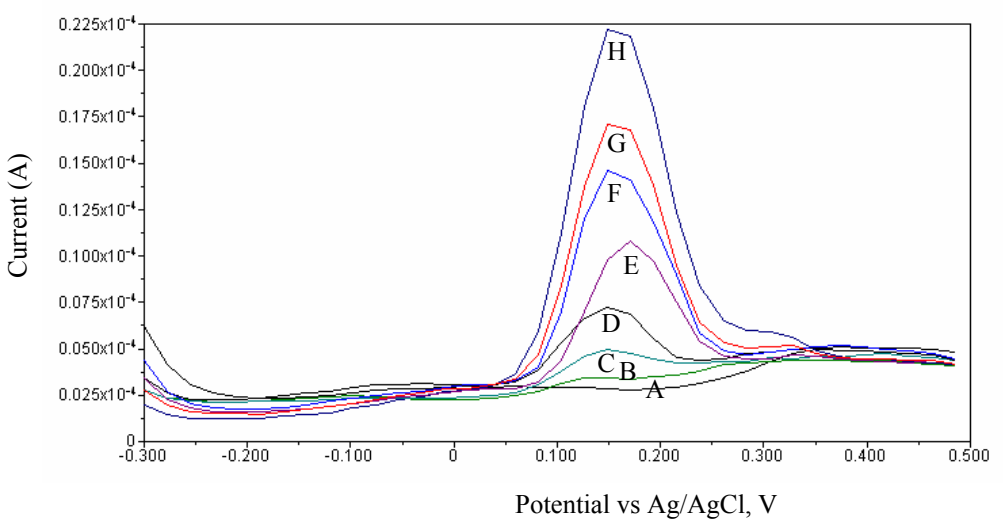

Figure 3-24 Voltammograms of various concentrations of As(III) standard solutions; (A) Blank 0.2 M HCl; (B) 1.0 μg L^{-1}; (C) 3 μg L^{-1}; (D) 5 μg L^{-1}; (E) 10 μg L^{-1}; (F) F 15 μg L^{-1}; (G) 20 μg L^{-1}; (H) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-18 Study of linear dynamic range for As(total) determination by using FI-ASV

As(V) concentration (μg L^{-1})	**Current* (μA)**	**%RSD**
0	0.000 ± 0.000	-
2	0.274 ± 0.027	9.93
5	0.917 ± 0.000	0.05
10	2.493 ± 0.132	5.28
20	5.765 ± 0.142	2.46
40	10.425 ± 0.297	2.85
60	14.454 ± 0.319	2.21
80	18.631 ± 0.412	2.21
100	22.856 ± 1.556	6.81
120	23.977 ± 0.376	1.57
140	24.902 ± 1.476	5.93

*Values given are the means (n = 2)

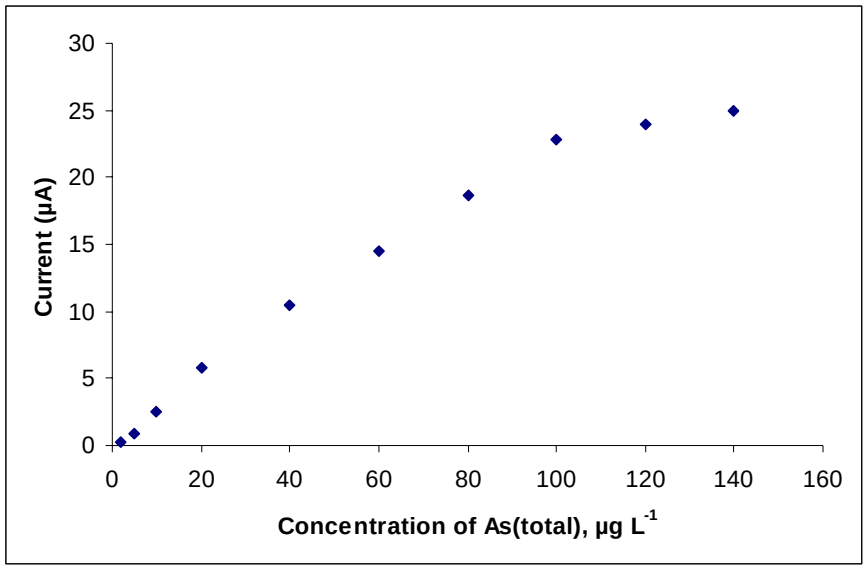

Figure 3-25 The current response of As(total) as a function of concentration

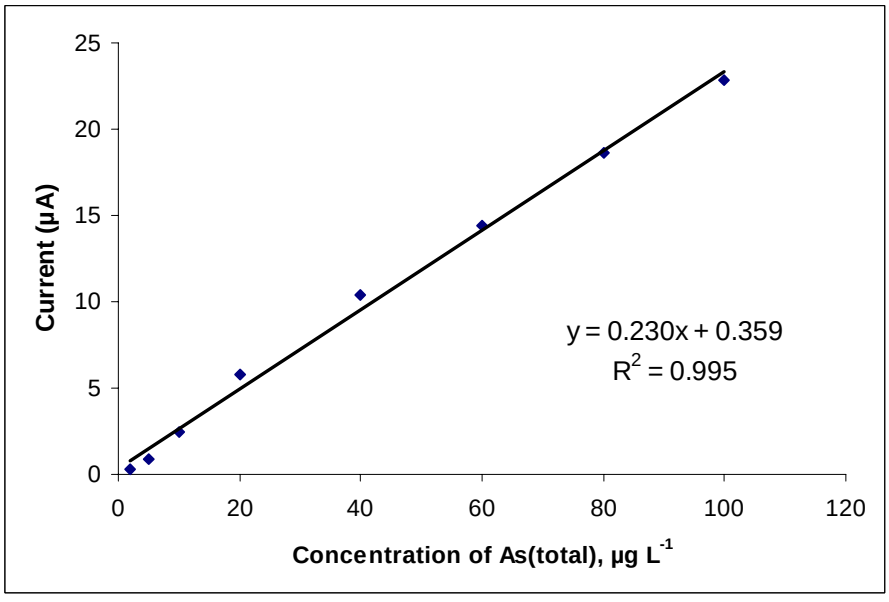

Figure 3-26 The linear dynamic range of As(total) determination from 2.0 to 100 µg L^{-1}

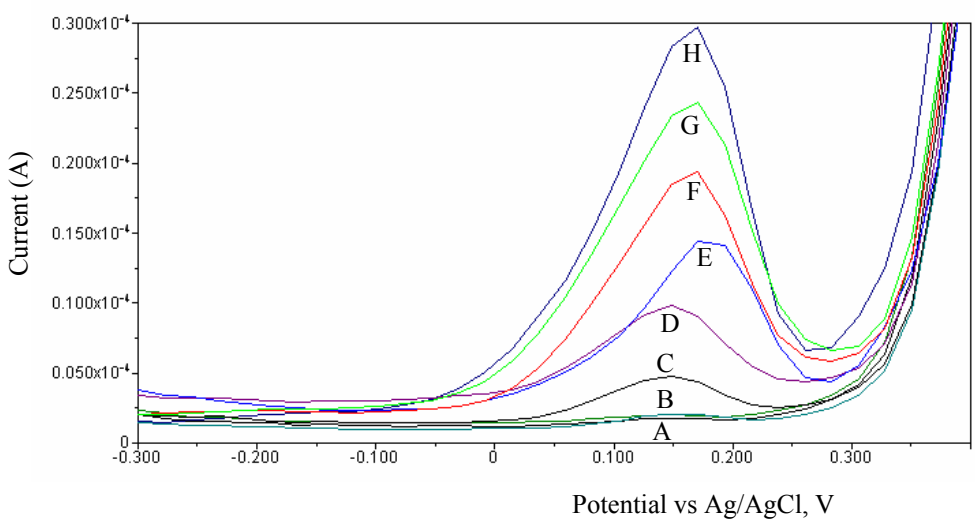

Figure 3-27 Voltammograms of various concentrations of As(total) standard solutions; (A) Blank 0.2 M HCl; (B) 2.0 µg L^{-1}; (C) 10µg L^{-1}; (D) 20µg L^{-1}; (E) 40µg L^{-1}; (F) 60 µg L^{-1}; (G) 80 µg L^{-1}; (H) 100 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

3.3.2 The limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined as described in section **2.7.2** and section **2.7.3**. For this purpose, optimum conditions for As(III) determination mentioned before were employed. Three times of the standard deviation (3σ) associated with the mean of 11 measurements of a solution containing 1 µg L^{-1} As(III) for the determination of As(III) and 3 µg L^{-1} As(V) for the As(total) determination were calculated to obtain the LOD (at 4 min of deposition time). In case of LOQ, 10σ was evaluated. Results are presented in **Table 3-19**. It was found that LOD and LOQ of As(III) determination were 0.42 µg L^{-1} and 1.39 µg L^{-1}, respectively. For As(total) determination, LOD of 1.47 µg L^{-1} and LOQ of 4.90 µg L^{-1} were obtained.

Table 3-19 The LOD and LOQ of As(III) and As(total) determinations

Entry	As(III) ($\mu g\ L^{-1}$)	As(total) ($\mu g\ L^{-1}$)
1	1.120	2.396
2	0.943	3.761
3	0.715	3.353
4	0.916	3.462
5	1.240	2.671
6	1.078	2.485
7	1.073	2.457
8	0.953	2.441
9	1.058	2.509
10	0.957	2.517
11	0.890	3.020
Mean ± SD	**0.995 ± 0.139**	**2.825 ± 0.490**
LOD (3σ)	**0.42**	**1.47**
LOQ (10σ)	**1.39**	**4.90**

3.3.3 Precision

The precision of the analysis was presented in the term of percent relative standard deviation (%RSD) of 11 measurements. The precisions of As(III) and As(total) determination are shown in **Table 3-20**.

The relative standard deviations of As(III) were within 5% for 2 and 20 $\mu g\ L^{-1}$ As(III) determination. For 10 and 100 $\mu g\ L^{-1}$ As(total) determinations, their %RSDs within 8% were obtained.

Table 3-20 The precision of As(III) and As(total) determination

Entry	As(III)		As(total)	
	2 μg L^{-1}	**20 μg L^{-1}**	**10 μg L^{-1}**	**100 μg L^{-1}**
1	1.852	19.385	10.702	91.871
2	1.851	19.856	11.270	77.777
3	1.962	19.267	9.882	89.151
4	1.852	19.076	9.108	81.886
5	1.709	18.755	9.271	79.964
6	1.735	19.266	10.237	74.178
7	1.730	19.113	9.608	76.446
8	1.748	19.439	9.035	75.802
9	1.721	19.454	10.472	81.146
10	1.808	19.064	9.743	82.198
11	1.831	18.056	9.294	74.586
Mean ± SD	1.800 ± 0.079	19.198 ± 0.363	9.899 ± 0.748	80.455 ± 5.749
% RSD	4.36	1.89	7.55	7.15

3.3.4 Accuracy

The accuracy of the proposed method was studied through analysis of Standard Reference Material (SRM) 1640 natural fresh water (NIST, USA). The analytical results for the SRM 1640 revealed that As(III) is non-detectable. The SRM 1640 was reduced with KI using optimum conditions for As(total) determination. The obtained accuracy was expressed as the %error from the certified values, as shown in **Table 3-21**. It was found that analytical results obtained by the proposed method were in good agreement with the certified value.

Table 3-21 Determination of SRM 1640 natural fresh water (NIST, USA)

SRM 1640	Certified value ($\mu g\ L^{-1}$)	Measured value* ($\mu g\ L^{-1}$)	% Recovery	% Error	% RSD
	26.67 ± 0.41	25.18 ± 0.74	94.42	5.58	2.93

* Values given are the means (n = 3)

3.3.5 % Recovery

For inorganic arsenic speciation, no certified value of concentration has been provided for arsenic species. Therefore, the %recovery value of inorganic arsenic species was quantified by spiking As(III), As(V) and As(total) at various concentrations to natural water samples. The analytical recovery percentages of As(III), As(V) and As(total) are shown in **Table 3-22** to **Table 3-24**, which can be concluded as follows:

The recovery percentages of As(III) at 10.0, 20.0 and 30.0 $\mu g\ L^{-1}$ were in the range of 85 to 105%. The recovery percentages of As(V) at 10.0, 20.0, 30.0 and 40.0 $\mu g\ L^{-1}$ were in the range of 85 to 106%. The recovery percentages of As(total) at 30.0 $\mu g\ L^{-1}$ (different ratios) were in the range of 88 to 103%.

From the good results of %recovery, it can be confirmed that this method can be exploited for the determination of As(III) and As(V) in natural water samples.

Table 3-22 Analytical recoveries of As(III) added to some natural water samples

Samples (village/site)	As(III) concentration ($\mu g\ L^{-1}$)		% Recovery	% RSD
	Added values	Measured values*		
Village 1/3	0.0	ND	-	-
	10.0	9.69 ± 0.49	96.9	5.1
	20.0	17.41 ± 0.23	87.0	1.3
	30.0	30.87 ± 0.54	102.9	1.8
Village 2/2	0.0	0.27 ± 0.03	-	-
	10.0	10.39 ± 0.24	103.9	2.4
	20.0	20.82 ± 0.17	104.1	0.8
	30.0	30.42 ± 0.89	101.4	2.9
Village 12/3	0.0	ND	-	-
	10.0	9.60 ± 0.21	96.0	2.2
	20.0	19.83 ± 0.38	99.1	1.9
	30.0	25.77 ± 0.40	85.9	1.6
Village 13/3	0.0	ND	-	-
	10.0	9.75 ± 0.60	97.5	6.1
	20.0	18.82 ± 0.52	94.1	2.8
	30.0	27.28 ± 0.37	90.9	1.4

* Values given are the means (n = 2), ND = Non detectable (< 0.42 μgL^{-1})

Table 3-23 Analytical recoveries of As(V) added to some natural water samples

Samples (village/site)	As(V) concentration ($\mu g\ L^{-1}$)		% Recovery	% RSD
	Added values	Measured values*		
Village 1/3	0.0	ND	-	-
	10.0	9.64 ± 0.20	96.4	2.1
	20.0	19.14 ± 1.25	95.7	6.6
	30.0	29.47 ± 0.98	98.2	3.3
	40.0	39.53 ± 0.75	98.8	1.9
Village 2/2	0.0	1.42 ± 0.00	-	-
	10.0	8.56 ± 0.56	85.6	6.5
	20.0	20.18 ± 0.02	100.9	0.1
	30.0	28.07 ± 0.28	93.6	1.0
	40.0	39.26 ± 0.14	98.1	0.4
Village 12/3	0.0	ND	-	-
	10.0	9.45 ± 0.46	94.5	4.9
	20.0	21.10 ± 0.19	105.5	0.9
	30.0	29.43 ± 1.94	98.1	6.6
	40.0	40.01 ± 2.14	100.0	5.4
Village 13/3	0.0	ND	-	-
	10.0	9.43 ± 0.31	94.3	3.3
	20.0	21.03 ± 0.24	105.1	1.2
	30.0	29.65 ± 0.80	98.8	2.7
	40.0	39.88 ± 0.10	99.7	0.3

* Values given are the means (n = 2), ND = Non detectable (<1.47 μgL^{-1})

Table 3-24 Analytical recoveries of As(total) added to some natural water samples

Samples (village/site)	Total concentration of As (μg L^{-1})		% Recovery	%RSD
	Added values [Ratios of As(III):As(V)]	Measured values*		
Village 1/3	30.0 [1 : 1]	30.85 ± 1.47	102.8	4.8
	30.0 [1 : 2]	28.92 ± 0.74	96.4	2.6
	30.0 [1 : 5]	28.20 ± 0.68	94.0	2.4
Village 2/2	30.0 [1 : 1]	26.93 ± 0.77	89.8	2.9
	30.0 [1 : 2]	26.46 ± 1.84	88.2	6.9
	30.0 [1 : 5]	27.12 ± 0.83	90.4	3.1
Village 12/3	30.0 [1 : 1]	28.47 ± 0.93	94.9	3.3
	30.0 [1 : 2]	27.37 ± 0.46	91.2	1.7
	30.0 [1 : 5]	26.96 ± 0.67	89.9	2.5
Village 13/3	30.0 [1 : 1]	29.42 ± 0.72	98.1	2.5
	30.0 [1 : 2]	30.62 ± 0.36	102.1	1.2
	30.0 [1 : 5]	29.24 ± 0.85	97.5	2.9

* Values given are the means (n = 2)

3.4 The effect of interferences

Potential interferences caused by ions commonly found in environmental water, especially of antimony, cadmium, copper, iron, lead, mercury, nickel and zinc were investigated. Interfering ions at various concentrations were added to standard solution of As(III) and As(total). 5% higher deviation of peak signal (by adding interfering ion) was assumed that the ion interfered the system.

3.4.1 Antimony; Sb(III)

Antimony presented is the most serious interference cation to As(III) determination by ASV when gold film electrode was used (Sun *et al*., 1997). An observation as shown in **Figure 3-26** indicates that Sb(III) which its chemical properties is similar to As(III) and it could interfere the As(III) determination by producing a peak at the same potential of As(III). The increase in the signal of As(III) was observed when Sb(III) presented in the system. Influence of Sb(III) concentration on As(III) determination was investigated by adding various concentrations of Sb(III) into a standard solution containing 30 μg L^{-1} As(III). The results are presented in **Table 3-25**. As the results, increase in As(III) response was observed by increasing Sb(III) concentration. So it is likely to cause an interference if higher Sb(III) concentration of 2 μg L^{-1} is present in the system.

In addition, the effect of Sb(III) on As(total) was also studied as shown in **Figure 3-27**. Although the signal of Sb(III) presented in reducing agent disappears in the window potential, it is possible to interfere the As(total) determination due to the competition of Sb(III) deposited on gold film electrode. The various concentrations of Sb(III) on As(total) determination were investigated by adding Sb(III) into 30 μg L^{-1} As(total) standard solution. Decreased signal on As(total) determination was observed as shown in **Table 3-26**. It can be concluded that higher Sb(III) concentration of 25 μg L^{-1} were able to interfere the As(total) determination.

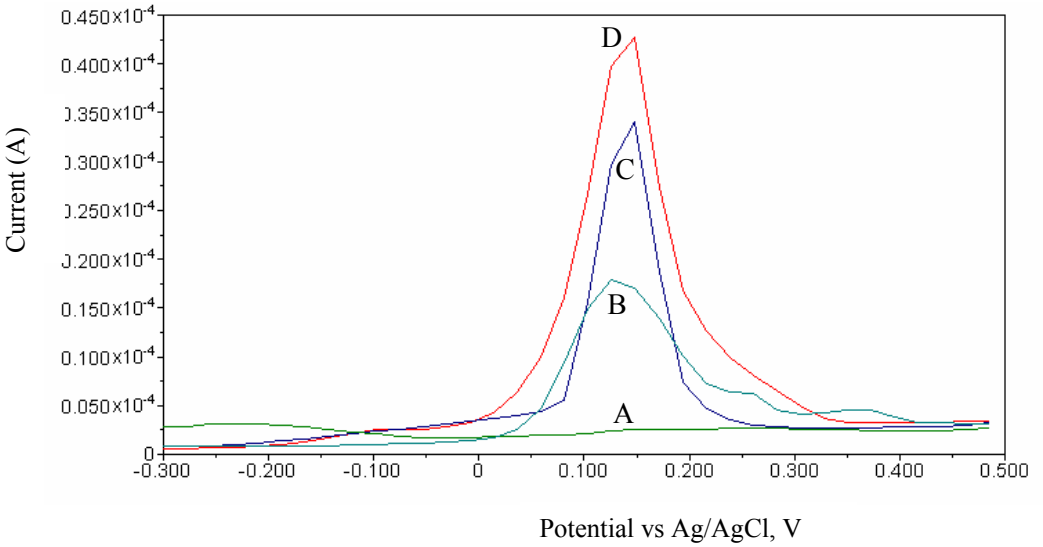

Figure 3-26 Voltammograms of Sb(III) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Sb(III) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Sb(III) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-25 Effect of the Sb(III) concentration on 30 μg L^{-1} As(III) determination

Sb(III) concentration (μg L^{-1})	**Current* (μA)**	**% Increase of As(III) signal**
0	14.577 ± 0.115	-
1	14.976 ± 0.099	2.74
2	15.274 ± 0.020	4.78
3	16.298 ± 0.193	11.8
4	17.106 ± 0.086	17.4

* Values given are the means (n = 2), % RSD < 2

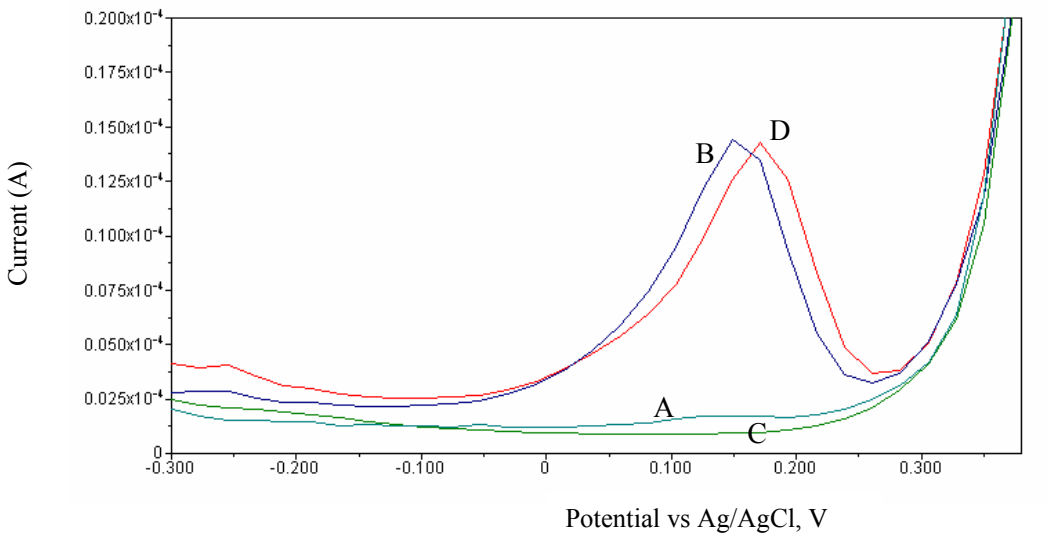

Figure 3-27 Voltammograms of Sb(III) on As(total) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Sb(III) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Sb(III) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-26 Effect of the Sb(III) concentration on 30 μg L^{-1} As(total) determination

Sb(III) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(total) signal**
0	11.211 ± 0.422	-
20	10.880 ± 0.722	2.95
25	10.694 ± 0.460	4.61
30	10.595 ± 0.458	5.49
40	10.160 ± 0.404	9.37
50	9.921 ± 0.067	11.5
60	9.412 ± 0.345	16.0

* Values given are the means (n = 2), % RSD < 7

3.4.2 Cadmium; Cd(II)

Cadmium presented in natural water was able to possible interfere. The voltammogram of Cd(II) on As(III) determination was shown in **Figure 3-28**. The stripping peak of Cd(II) appeared closes to the stripping peak of As(III), therefore it is possible to interfere the As(III) determination. The effect of cadmium concentration on As(III) determination was studied. The various concentrations of Cd(II) were added into 30 µg L^{-1} As(III) standard solution. The stripping peak current of As(III) decreased higher than 5% when concentrations of Cd(II) were higher than 60 µg L^{-1}. This is because Cd(II) was co-deposited on gold film electrode.

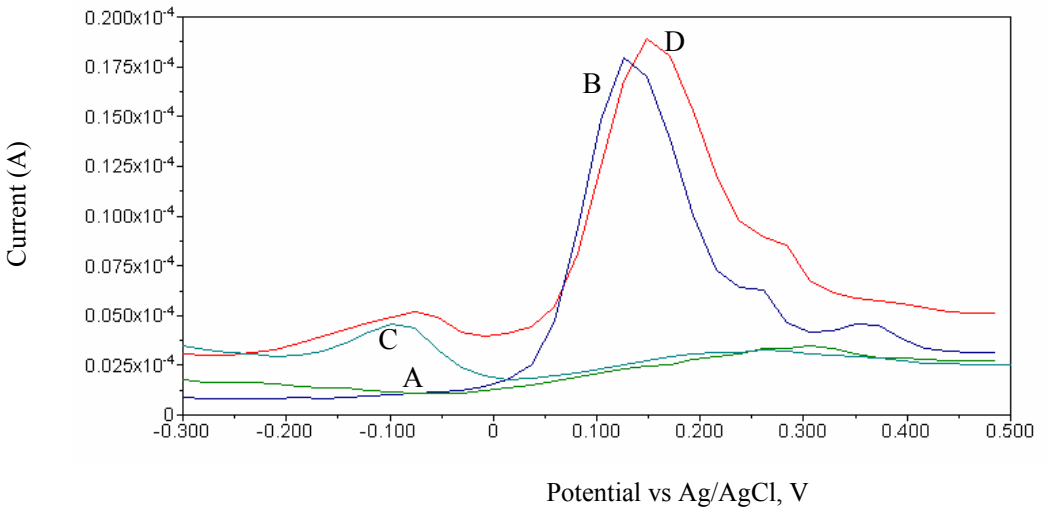

Figure 3-28 Voltammograms of Cd(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 µg L^{-1}; (C) Cd(II) 30 µg L^{-1}; (D) As(III) 30 µg L^{-1}+ Cd(II) 30 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-27 Effect of the Cd(II) concentration on 30 µg L^{-1} As(III) determination

Cd(II) concentration (µg L^{-1})	Current* (µA)	% Decrease of As(III) signal
0	14.307 ± 0.131	-
30	14.136 ± 0.279	1.20
60	13.814 ± 0.041	3.45
70	13.447 ± 0.745	6.01
80	11.811 ± 0.476	17.4

* Values given are the means (n = 2), % RSD < 6

For the determination of As(total), the effect of Cd(II) on As(total) determination was studied. The voltammograms of Cd(II) presented in reducing agent were examined and were compared with voltammogram of As(total) as shown in **Figure 3-29**. To investigate the extent of its effect on As(total) determination, various concentration of Cd(II) was spiked into 30 µg L^{-1} As(total). The results are shown in **Table 3-28**. It can be concluded that higher Cd(II) concentrations of 40 µg L^{-1} were able to interfere As(total) determination due to Cd(II) was co-deposited on gold film electrode.

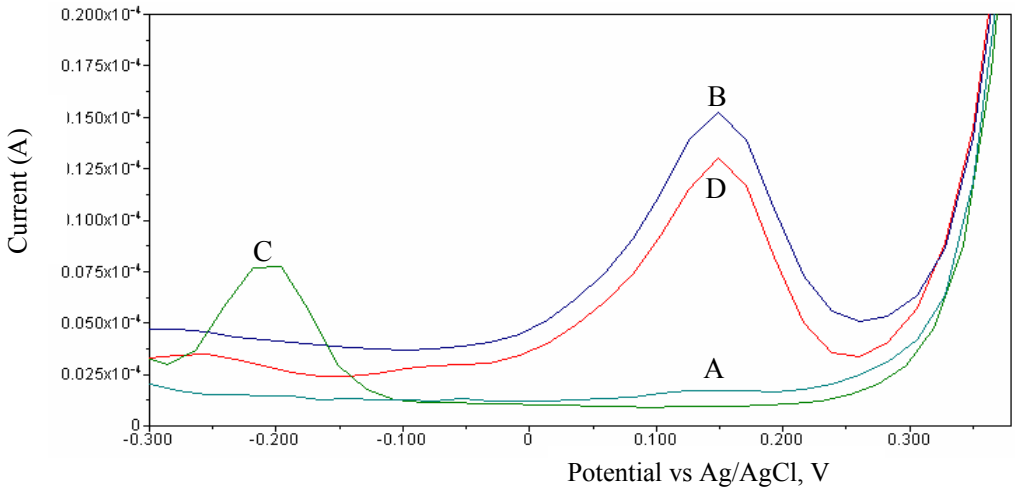

Figure 3-29 Voltammograms of Cd(II) on As(total) determination; (A) Blank (0.2 M HCl); (B) As(total) 30 µg L^{-1}; (C) Cd(II) 30 µg L^{-1}; (D) As(total) 30 µg L^{-1} + Cd(II) 30 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-28 Effect of the Cd(II) concentration on 30 μg L^{-1} As(total) determination

Cd(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(total) signal**
0	10.493 ± 0.161	-
30	10.114 ± 0.351	3.61
40	10.126 ± 0.157	3.50
50	9.902 ± 0.413	5.64
60	9.698 ± 0.521	7.57

* Values given are the means (n = 2), % RSD < 6

3.4.3 Copper; Cu(II)

Copper presented is the most serious interference cation to determine As(III) by ASV when gold film electrode was used (Sun *et al*., 1997). It is able to form intermetalic compounds with arsenic on the electrode surface in the deposition step (Dugo *et al*., 2005). The voltammograms of Cu(II) compared with As(III) voltammograms are shown in **Figure 3-30**. The stripping peak of Cu(II) appeared closes to the stripping peak of As(III), therefore, it is possible to interfere the As(III) determination. In order to study the influence of copper concentration on the analytical response, it was examined by adding Cu(II) at various concentrations into 30 μg L^{-1} As(III) standard solution. Higher Cu(II) concentration resulted in decrease in As(III) signal as shown in **Table 3-29**. It can be concluded that higher Cu(II) concentrations of 90 μg L^{-1} were able to interfere As(III) determination due to Cu(II) was co-deposited on gold film electrode.

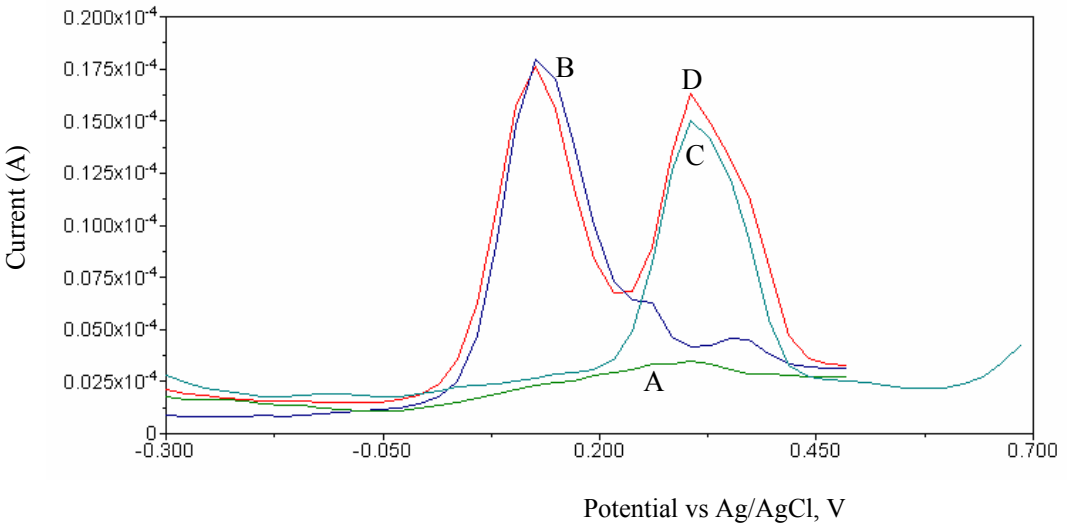

Figure 3-30 Voltammograms of Cu(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Cu(II) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Cu(II) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-29 Effect of the Cu(II) concentration on 30 μg L^{-1} As(III) determination

Cu(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(III) signal**
0	14.406 ± 0.306	-
30	14.228 ± 0.362	1.23
60	14.132 ± 0.552	1.90
90	13.930 ± 0.116	3.30
100	13.508 ± 0.192	6.23
110	12.233 ± 0.026	15.3
120	9.031 ± 0.622	37.3

* Values given are the means (n = 2), % RSD < 7

For the determination of As(total), The influence of Cu(II) presented in reducing agent was studied. The voltammograms of Cu(II) presented in reducing agent are shown in **Figure 3-31**. The stripping peak of As(total) presented in Cu(II) was shifted to more positive potential and broader peak shape were obtained. The effect of copper concentration on As(total) determination was studied by spiking Cu(II) at various concentrations into 30 μg L^{-1} As(total) standard solution. The results are as shown in **Table 3-30**. It can be concluded that higher Cu(II) concentrations of 7 μg L^{-1} were able to interfere As(total) determination.

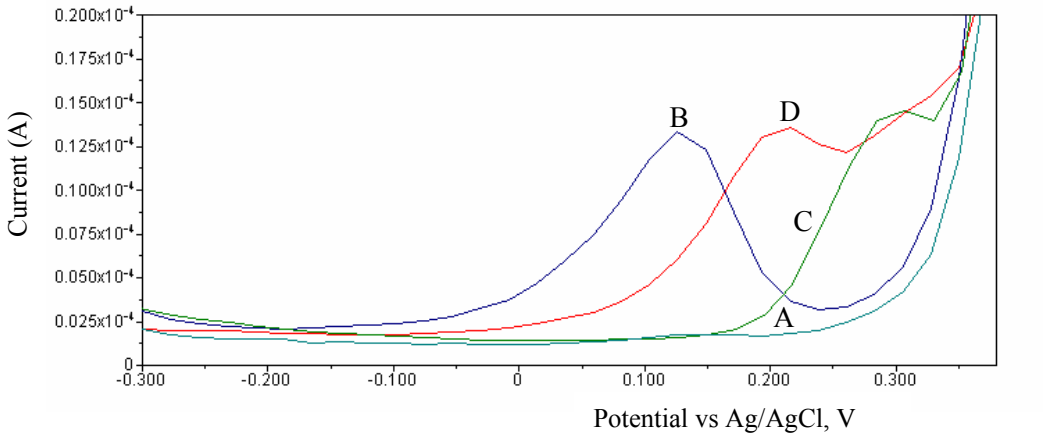

Figure 3-31 Voltammograms of Cu(II) on As(total) determination**;** (A) Blank (0.2 M HCl); (B) As(total) 30 μg L^{-1}; (C) Cu(II) 30 μg L^{-1}; (D) As(total) 30 μg L^{-1} + Cu(II) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-30 Effect of the Cu(II) concentration on 30 μg L^{-1} As(total) determination

Cu(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(total) signal**
0	9.315 ± 0.148	-
5	9.066 ± 0.015	2.68
6	9.037 ± 0.080	2.98
7	9.032 ± 0.079	3.04
8	8.724 ± 0.220	6.35
9	8.508 ± 0.514	8.67

* Values given are the means (n = 2), % RSD < 7

3.4.4 Iron; Fe(II)

Iron has been commonly presented in natural water, it is possible to interfere the As(III) determination. The voltammograms of Fe(II) and As(III) are shown in **Figure 3-32**. The stripping peak of Fe(II) presented closes to the stripping peak of As(III), it is possible to interfere As(III) determination. Influence of Fe(II) concentration on As(III) determination was investigated by spiking Fe(II) at various concentrations into 30 μg L^{-1} As(III) standard solution, as shown in **Table 3-31**. It can be concluded that higher Fe(II) concentrations of 30 μg L^{-1} were able to interfere As(III) determination by co-depositing on gold film electrode.

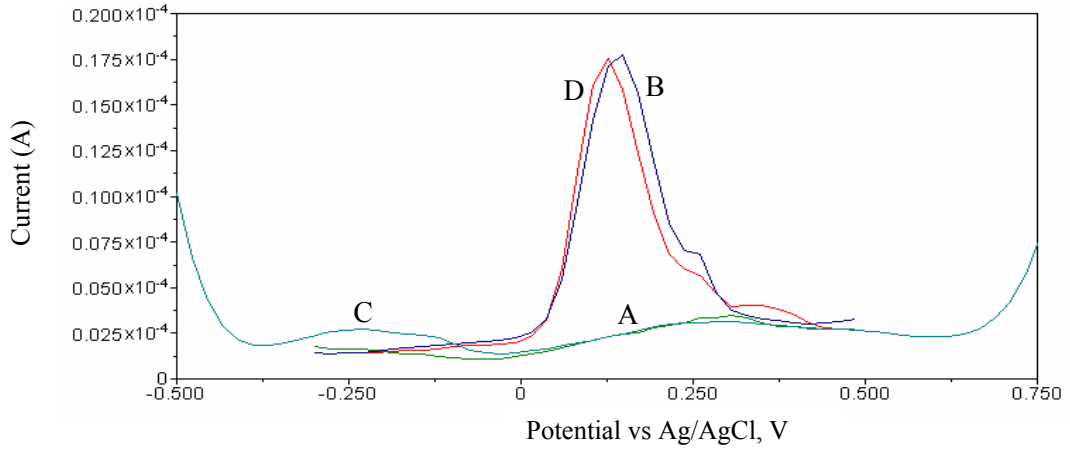

Figure 3-32 Voltammograms of Fe(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Fe(II) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Fe(II) 30 μgL^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-31 Effect of the Fe(II) concentration on 30 μg L^{-1} As(III) determination

Fe(II) concentration (μg L^{-1})	Current* (μA)	% Decrease of As(III) signal
0	15.009 ± 0.394	-
30	14.906 ± 0.127	0.69
40	14.031 ± 0.250	6.52
50	13.740 ± 0.264	8.46
60	13.577 ± 0.262	9.54

* Values given are the means (n = 2), % RSD < 3

For As(total) determination, the effect of Fe(II) concentration on As(total) determination was studied. The voltammograms of Fe(II) presented in reducing agent were evaluated and were compared with As(total) voltammograms, as shown in **Figure 3-33**. Although, Fe(II) peak position appeared far from As(total) peak, it is possible to interfere the As(III) determination due to the competition of Fe(II) deposited on gold film electrode. The various concentrations of Fe(II) were added into 30 µg L^{-1} of As(total) standard solution. The results are shown in **Table 3-32**. It can be concluded that higher Fe(II) concentrations of 40 µg L^{-1} were able to interfere As(total) determination by co-depositing on gold film electrode.

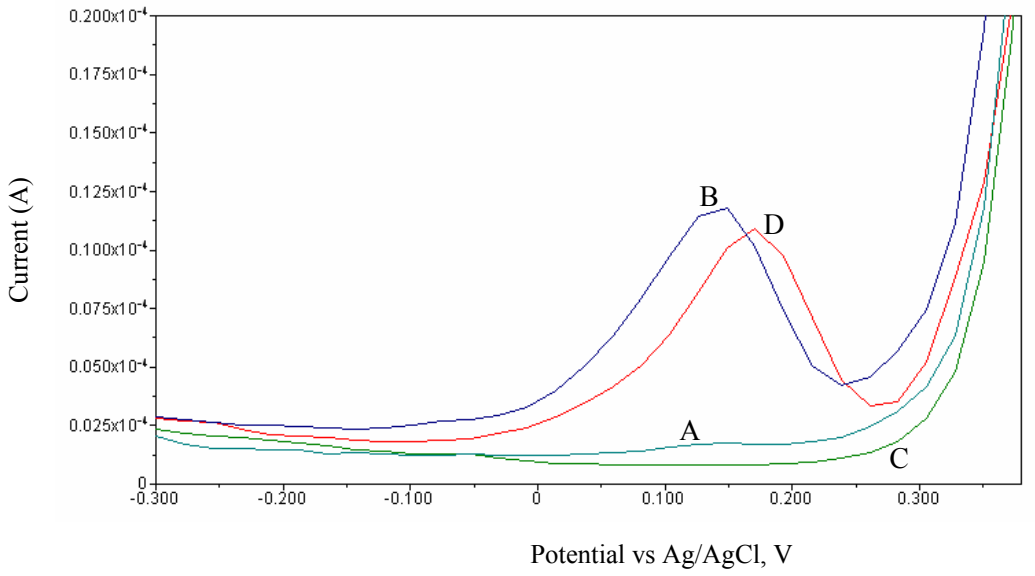

Figure 3-33 Voltammograms of Fe(II) on As(total) determination**;** (A) Blank (0.2 M HCl); (B) As(total) 30 µg L^{-1}; (C) Fe(II) 30 µg L^{-1}; (D) As(total) 30 µg L^{-1} + Fe(II) 30 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-32 Effect of the Fe(II) concentration on 30 μg L^{-1} As(total) determination

Fe(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(total) signal**
0	7.866 ± 0.158	-
30	7.729 ± 0.277	1.74
40	7.462 ± 0.352	5.14
50	7.001 ± 0.158	11.0
60	6.867 ± 0.270	12.7

* Values given are the means (n = 2), % RSD < 5

3.4.5 Lead; Pb(II)

The effect of lead as possible interference was studied. The voltammograms of Pb(II) compared to As(III) are shown in **Figure 3-34**. The stripping peak of Pb(II) presented closes to the stripping peak of As(III), it is possible to interfere As(III) determination by co-depositing on gold film electrode. The effect of Pb(II) concentration on As(III) determination was studied by spiking Pb(II) at various concentrations into 30 μg L^{-1} of As(III) standard solution. The results are shown in **Table 3-33**. It can be concluded that higher Pb(II) concentration of 40 μg L^{-1} were able to interfere As(III) determination.

For investigation the effect of Pb(II) on As(total) determination, the voltammograms of Pb(II) in reducing agent are shown in **Figure 3-35**. The effect of Pb(II) concentration on As(total) determination was studied by spiking Pb(II) at various concentrations into 30 μg L^{-1} As(total) standard solution, as shown in **Table 3-34**. It was found that higher Pb(II) concentrations of 60 μg L^{-1} were able to interfere As(total) determination.

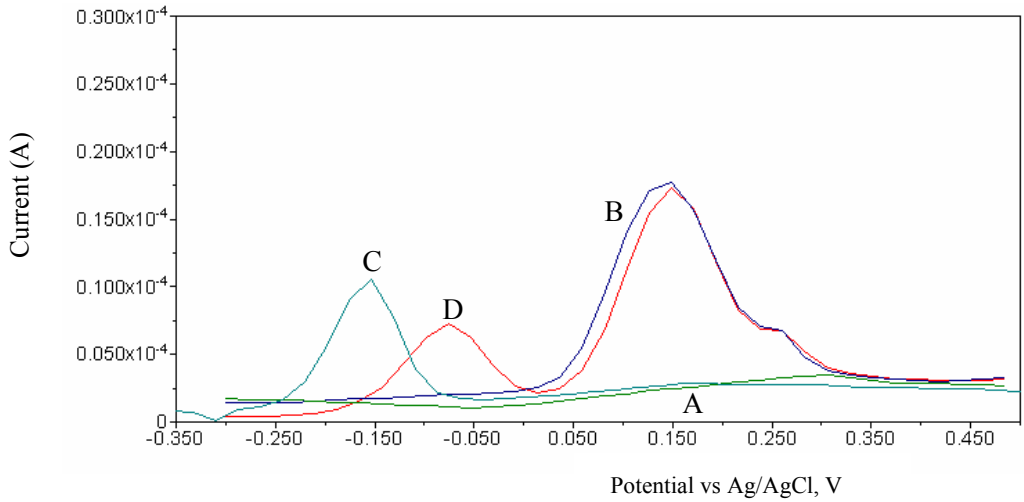

Figure 3-34 Voltammograms of Pb(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Pb(II) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Pb(II) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-33 Effect of the Pb(II) concentration on 30 μg L^{-1} As(III) determination

Pb(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(III) signal**
0	14.110 ± 0.059	-
30	13.900 ± 0.320	1.50
40	13.896 ± 0.222	1.52
50	13.260 ± 0.188	6.03
60	12.972 ± 0.183	8.06
70	12.420 ± 0.056	12.0
80	11.314± 0.264	19.8

* Values given are the means (n = 2), % RSD < 3

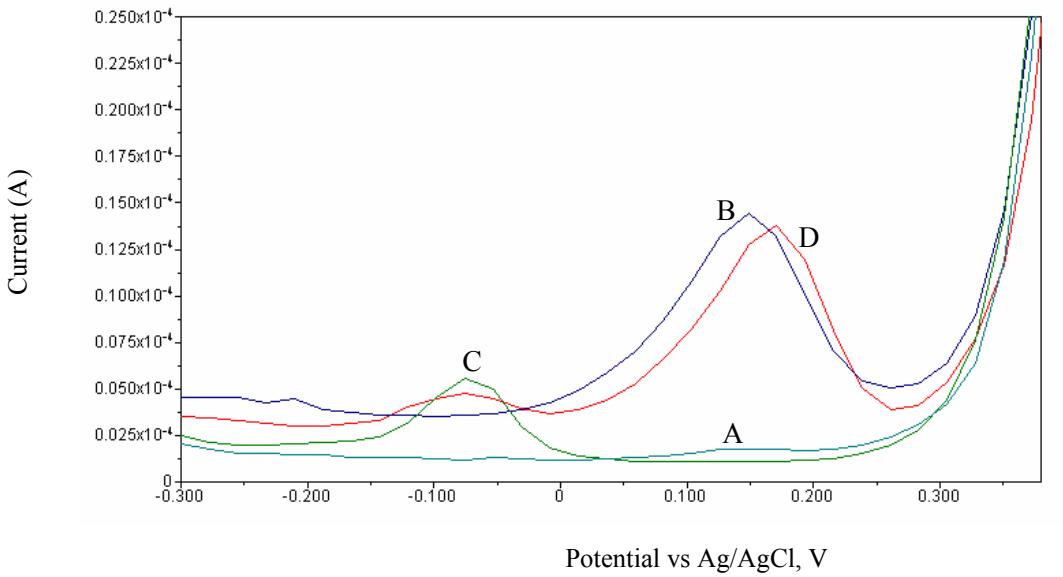

Figure 3-35 Voltammograms of Pb(II) on As(total) determination**;** (A) Blank (0.2 M HCl); (B) As(total) 30 µg L^{-1}; (C) Pb(II) 30 µg L^{-1}; (D) As(total) 30 µg L^{-1} + Pb(II) 30 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-34 Effect of the Pb(II) concentration on 30 µg L^{-1} As(total) determination

Pb(II) concentration (µg L^{-1})	**Current* (µA)**	**% Decrease of As(total) signal**
0	10.385 ± 0.463	-
30	10.205 ± 0.625	1.73
40	10.255 ± 0.540	1.24
50	9.972 ± 0.063	3.98
60	9.948 ± 0.097	4.21
70	9.489 ± 0.338	8.62
80	9.051 ± 0.315	12.8

* Values given are the means (n = 2), % RSD < 7

3.4.6 Mercury; Hg(II)

Hg(II) is one of the possible interference ions affected As(III) determination (Adeloju *et al*., 1999). It was found that the stripping peak of Hg(II) presented at more positive potential compared to As(III) as shown in **Figure 3-36**. The effect of Hg(II) concentration on As(III) signal was studied by spiking Hg(II) at various concentrations into 30 µg L^{-1} As(III) standard solutions. As the results from **Table 3-35**, higher concentration of Hg(II), greater decrease in signal were observed. This is possible due to the co-deposition of Hg(II) on the electrode surface. It can be concluded that higher Hg(II) concentrations of 30 µg L^{-1} were able to interfere As(III) determination.

On the other hand, Hg(II) is the substantial influence to As(total) by increasing the analytical response as shown in **Table 3-36**. It was found that stripping peak of Hg(II) in the presence of reducing agent is appeared at the same position of As(total) peak as shown in **Figure 3-37**. Degree of signal increment was dependent on the concentration of Hg(II). It can be concluded that higher Hg(II) concentrations of 2 µg L^{-1} were able to interfere As(total) determination.

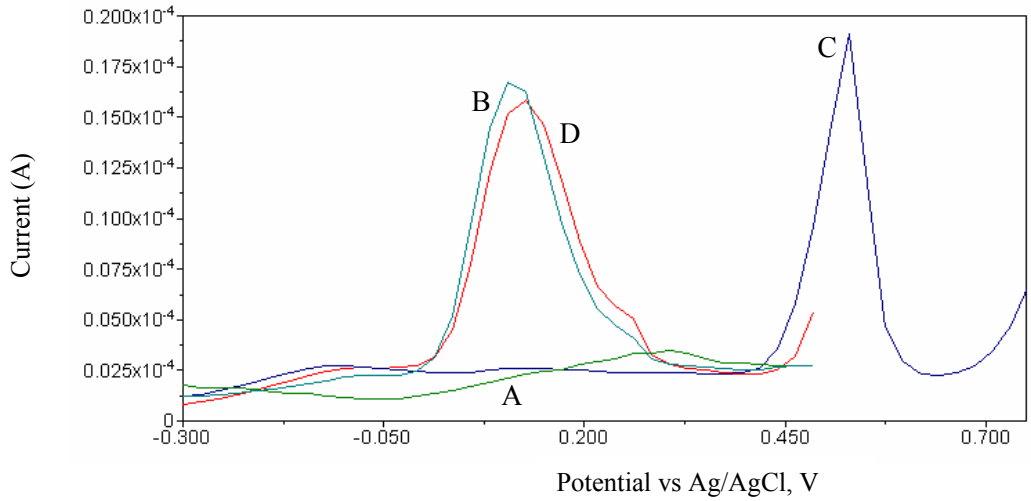

Figure 3-36 Voltammograms of Hg(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 µg L^{-1}; (C) Hg(II) 30 µg L^{-1}; (D) As(III) 30 µg L^{-1} + Hg(II) 30 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-35 Effect of the Hg(II) concentration on 30 µg L^{-1} As(III) determination

Hg(II) concentration (µg L^{-1})	**Current* (µA)**	**% Decrease of As(III) signal**
0	14.588 ± 0.189	-
30	14.531 ± 0.297	0.39
40	13.380 ± 0.704	8.28
50	11.571 ± 0.741	20.7
60	10.540 ± 0.726	27.7

* Values given are the means (n = 2), % RSD < 7

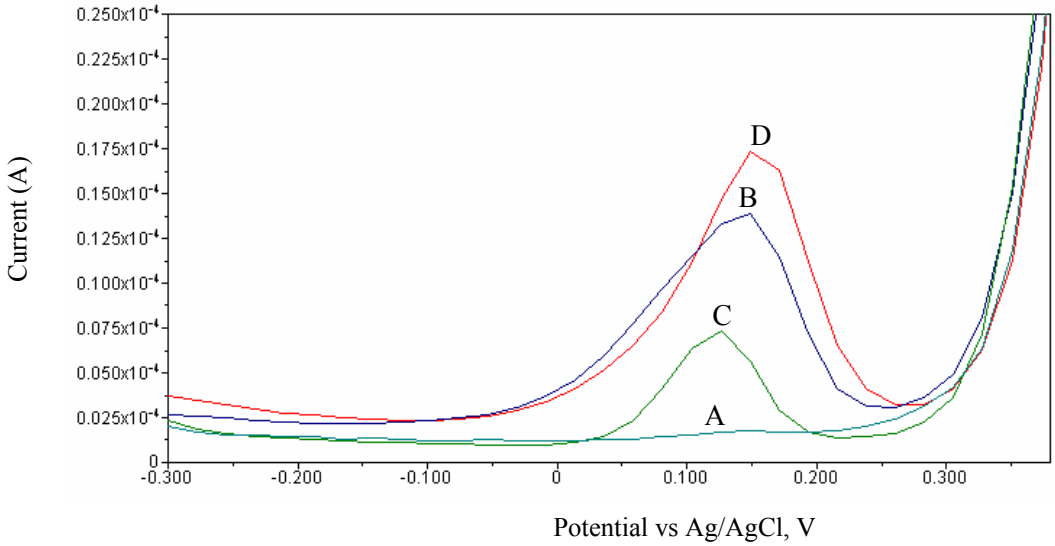

Figure 3-37 Voltammograms of Hg(II) on As(total) determination; (A) Blank (0.2 M HCl); (B) As(total) 30 µg L^{-1}; (C) Hg(II) 30 µg L^{-1}; (D) As(total) 30 µg L^{-1} + Hg(II) 30 µg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-36 Effect of the Hg(II) concentration on 30 μg L^{-1} As(total) determination

Hg(II) concentration (μg L^{-1})	Current* (μA)	% Increase of As(total) signal
0	8.629 ± 0.012	-
1	9.988 ± 0.139	4.15
2	9.336 ± 0.205	8.19
3	9.536 ± 0.375	10.5
4	10.260 ± 0.219	18.9

* Values given are the means (n = 2), % RSD < 4

3.4.7 Nickel; Ni(II)

The effect of Ni(II) as foreigner ion on As(III) determination was investigated. Stripping peak of Ni(II) did not disturb to As(III) peak as can be seen in **Figure 3-38**. However, Ni(II) may be co-deposited on the gold film electrode, then led to suppress As(III) deposition. As the results in **Table 3-37**, it can be concluded that higher Ni(II) concentrations of 90 μg L^{-1} were able to interfere As(III) determination.

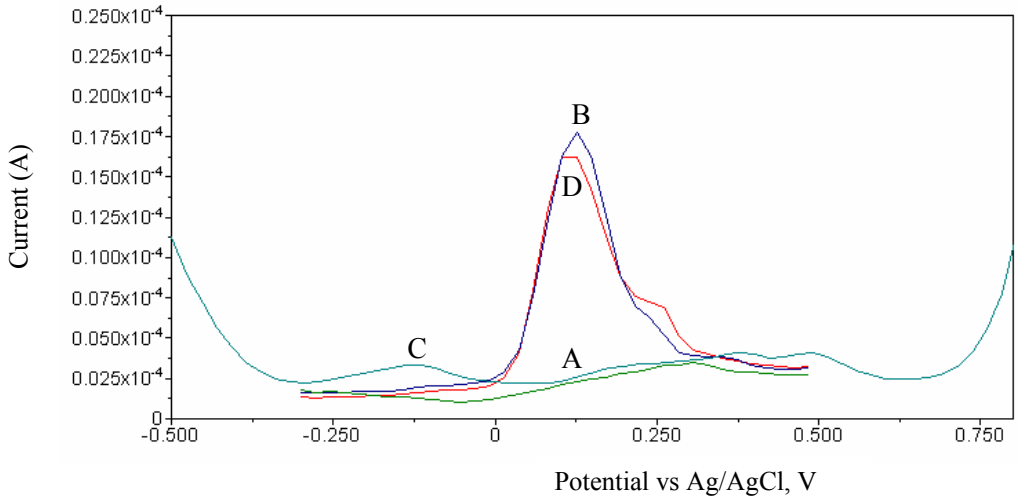

Figure 3-38 Voltammograms of Ni(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Ni(II) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Ni(II) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-37 Effect of the Ni(II) concentration on 30 μg L^{-1} As(III) determination

Ni(II) concentration (μg L^{-1})	Current* (μA)	% Decrease of As(III) signal
0	14.968 ± 0.356	-
30	14.794 ± 0.166	1.17
60	14.678 ± 0.056	1.94
90	14.233 ± 0.193	4.91
100	13.957 ± 0.409	6.76

* Values given are the means (n = 2), % RSD < 3

The effect of Ni(II) on As(total) determination was also tested. The results are shown in **Figure 3-39**. Ni(II) had affected the As(total) determination as the same way of As(III) determination. Ni(II) can be presented up to 50 μg L^{-1} without any serious effects to As(total) determination.

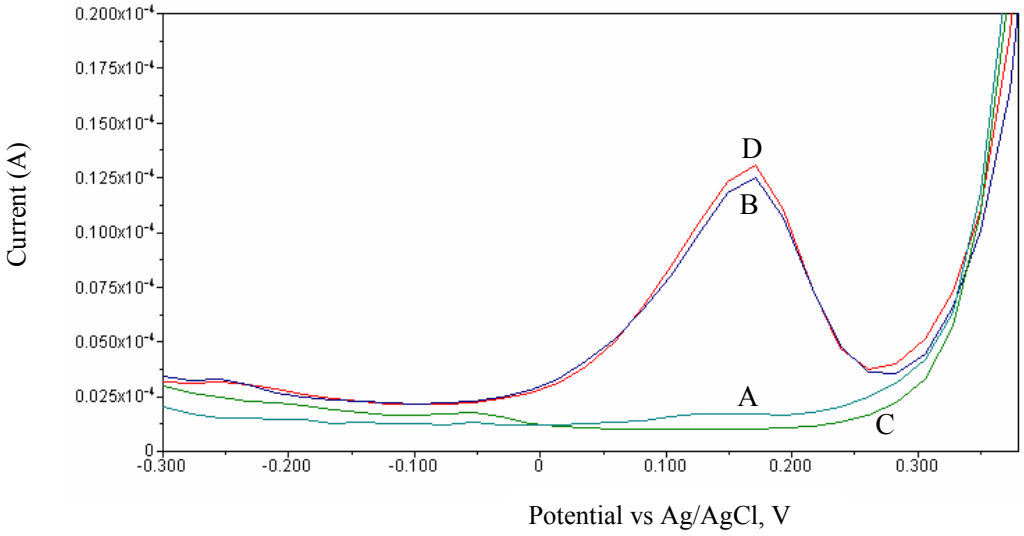

Figure 3-39 Voltammograms of Ni(II) on As(total) determination; (A) Blank (0.2 M HCl); (B) As(total) 30 μg L^{-1}; (C) Ni(II) 30 μg L^{-1}; (D) As(total) 30 μg L^{-1} + Ni(II) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-38 Effect of the Ni(II) concentration on 30 μg L^{-1} As(total) determination

Ni(II) concentration (μg L^{-1})	Current* (μA)	% Decrease of As(total) signal
0	9.583 ± 0.269	-
30	9.499 ± 0.384	0.88
40	9.365 ± 0.328	2.27
50	9.194 ± 0.267	4.06
60	8.331 ± 0.392	13.1

* Values given are the means (n = 2), % RSD < 5

3.4.8 Zinc; Zn(II)

Potential interference caused by Zn(II) commonly found in natural water was investigated. The voltammogram of Zn(II) on As(III) determination is shown in **Figure 3-40**. Although, the stripping peak of Zn(II) non-appeared in the range of these window potentials, it is able to interfere As(III) determination by co-depositing on gold film. The effect of Zn(II) concentration on As(III) determination was studied by spiking Zn(II) at various concentrations into 30 μg L^{-1} As(III) standard solution, as shown in **Table 3-39**. It can be noted that the concentration of Zn(II) up to 150 μg L^{-1} did not disturb to As(III) determination.

The effect of Zn(II) on As(total) determination was studied. Stripping peak of Zn(II) presented in reducing agent did not overlap the As(total) stripping peak. The effect of Zn(II) concentration on As(total) determination was studied by spiking Zn(II) at various concentrations into 30 μg L^{-1} As(total) standard solution, as shown in **Table 3-40**. It can be concluded that the concentration of Zn(II) up to 90 μg L^{-1} had no significant effect on As(total) determination.

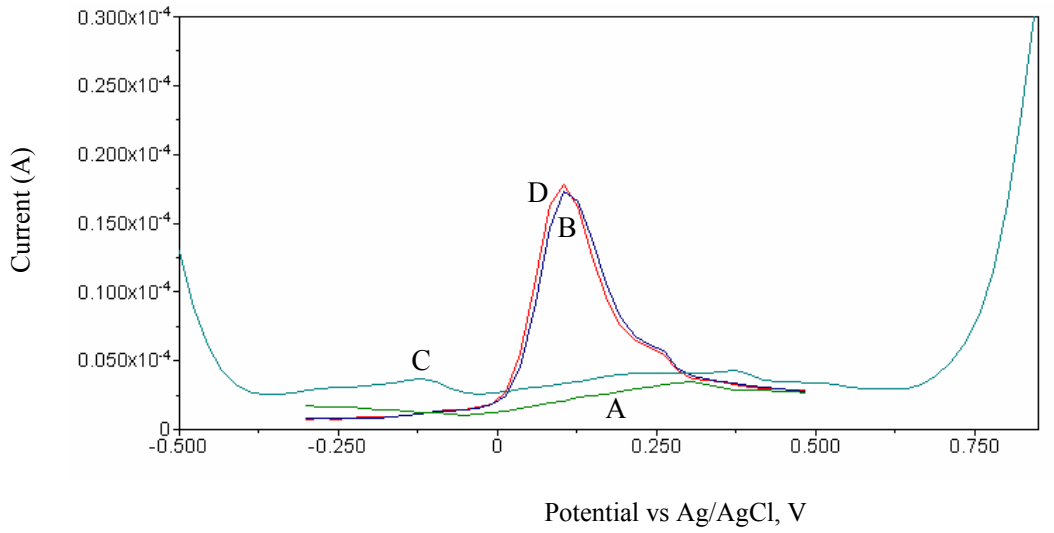

Figure 3-40 Voltammograms of Zn(II) on As(III) determination; (A) Blank (0.2 M HCl); (B) As(III) 30 μg L^{-1}; (C) Zn(II) 30 μg L^{-1}; (D) As(III) 30 μg L^{-1} + Zn(II) 30 μgL^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-39 Effect of the Zn(II) concentration on 30 μg L^{-1} As(III) determination

Zn(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(III) signal**
0	15.600 ± 0.195	-
30	15.397 ± 0.668	1.30
60	15.236 ± 0.002	2.34
90	15.493 ± 0.213	0.69
120	15.095 ± 0.800	3.24
140	14.932 ± 0.595	4.28
150	14.807 ± 0.274	5.09
180	14.305 ± 0.463	8.30

* Values given are the means (n = 2), % RSD < 6

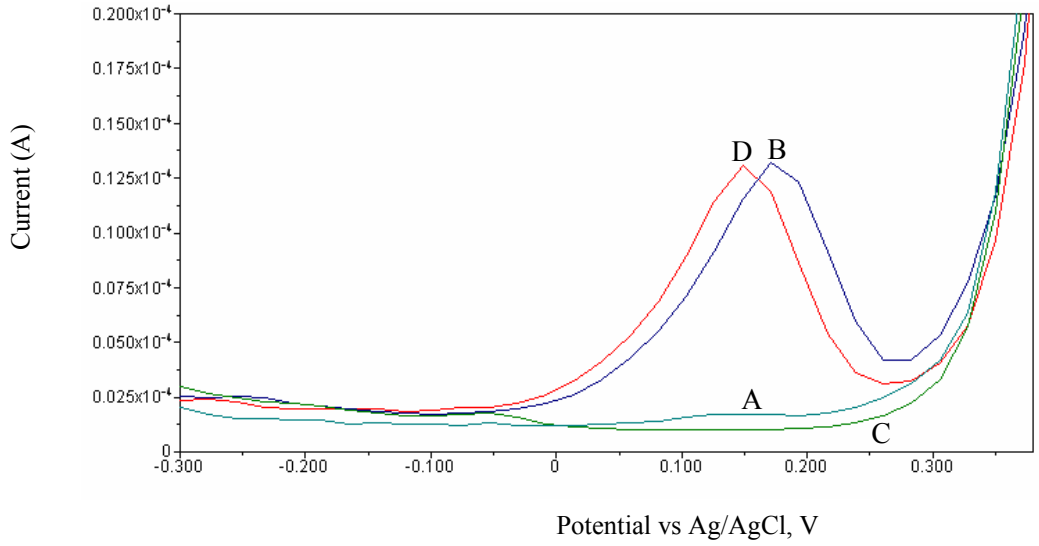

Figure 3-41 Voltammograms of Zn(II) on As(total) determination; (A) Blank (0.2 M HCl); (B) As(total) 30 μg L^{-1}; (C) Zn(II) 30 μg L^{-1}; (D) As(total) 30 μg L^{-1} + Zn(II) 30 μg L^{-1}; deposition potential, -0.30 V; deposition time, 240 sec; scan rate, 90 mV sec^{-1}

Table 3-40 Effect of the Zn(II) concentration on 30 μg L^{-1} As(total) determination

Zn(II) concentration (μg L^{-1})	**Current* (μA)**	**% Decrease of As(total) signal**
0	10.471 ± 0.018	-
30	10.495 ± 0.256	0.23
60	10.449 ± 0.645	0.21
90	10.151 ± 0.233	3.05
100	9.830 ± 0.494	6.12
110	9.222 ± 0.473	12.0
120	8.090 ± 0.598	22.7

* Values given are the means (n = 2), % RSD < 8

In conclusion, the effect of various interferences caused by cations commonly found in natural water, especially of Sb(III), Cd(II), Cu(II), Fe(II), Pb(II), Hg(II), Ni(II) and Zn(II), was investigated. The interfering level of each ion on As(III) and As(total) determination is presented in **Table 3-41.**

Table 3-41 The interfering levels of interference ions on As(III) and As(total) determinations

Interferences	**Interfering levels (μg L^{-1})**	
	As(III)	**As(total)**
Sb(III)	3	30
Cd(II)	70	50
Cu(II)	100	8
Fe(II)	40	40
Pb(II)	50	70
Hg(II)	40	2
Ni(II)	100	60
Zn(II)	150	100

3.5 Application of the method

3.5.1 Comparison of the calibration and standard addition methods

To investigate the effect of sample matrix, the experiment to compare the standard method between calibration curve and standard addition curve for the determination of As(III) and As(V) in natural waters was performed. The standard addition method was carried out with 2 different water samples, shallow well water (collected from village 12 site 3) and stream water (collected from village 2 site 2). The results are shown in **Figure 3-42** and **Figure 3-43.** The slopes of calibration curve and standard addition curve were test using two-way ANOVA by R software (see **Appendix D-1**)

As the result in **Figure 3-42**, it was found that there was no significant difference (P<0.001) in slope between calibration curve and standard addition curve for As(III) determination. It can be concluded that the matrix effects in natural water samples were not affected on As(III) determination. Therefore, calibration curve method was chosen for As(III) quantification.

On the other hand, the significant difference (P<0.001) of slope from both methods for As(total) determination was found (as shown in **Figure 3-43**). It can be concluded that the matrix effects in natural water samples had affected on As(total) determination. Hence, standard addition method was chosen for As(total) quantification.

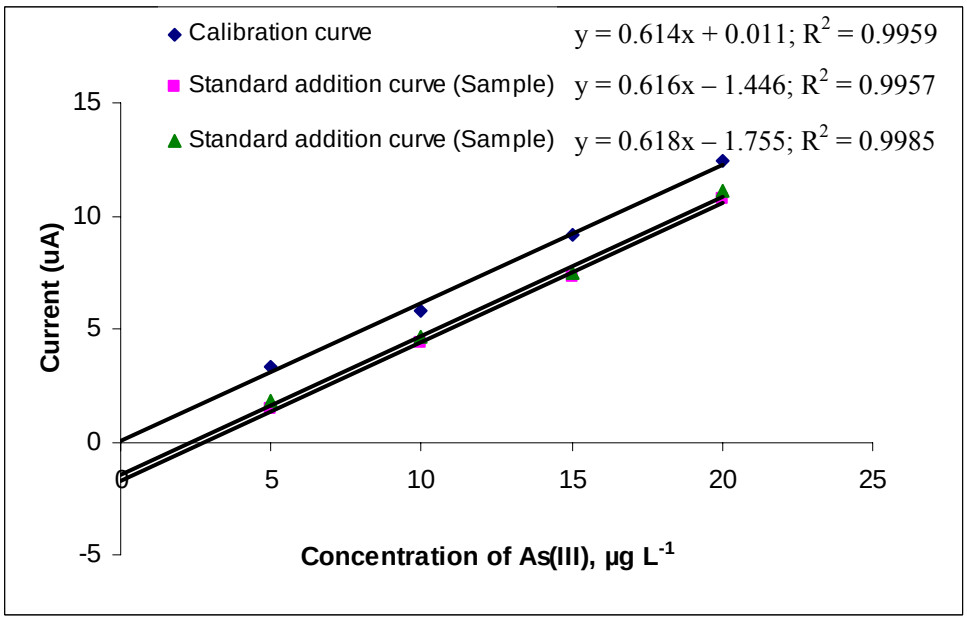

Figure 3-42 The comparison of calibration curve and standard addition curves for As(III) determination

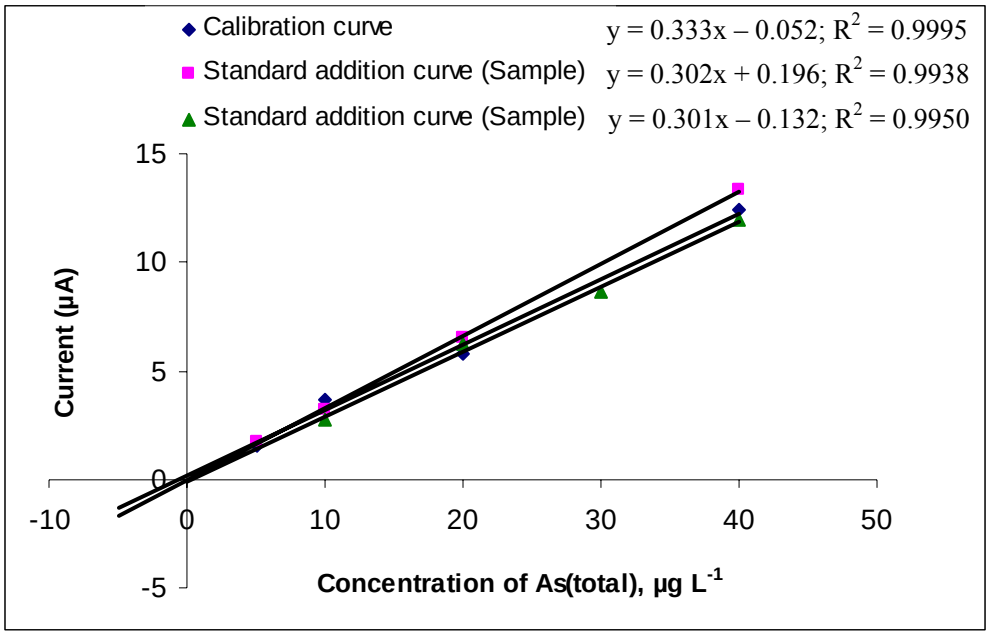

Figure 3-43 The comparison of calibration curve and standard addition curves for As(total) determination

3.5.2 Determination of As(III) and As(V) in natural water samples

The proposed method was applied to determine As(III) and As(V) in natural water samples discharged from arsenic contaminated area in Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province as described in section **2.9.1**.

As(III) and As(total) in water samples were analyzed using the developed method under optimum conditions (as see in **Table 3-12**). As(III) and As(total) contents were calculated using calibration method and standard addition method, respectively. As(V) concentrations were calculated from the difference in amount of As(total) and As(III). The results are shown in **Table 3-42**.

In order to validate the results obtained with the proposed method, As(total) was also determined by ICP-OES. As(total) concentrations by developed method are in good agreement with those obtained by ICP-OES (t-test, $P< 0.01$) as shown in **Appendix D-2**.

Table 3-42 Concentrations of As(III), As(V) and As(total) by FI-ASV method compared with As(total) by ICP-OES method in natural water samples

Sample site	Concentration*, μg L^{-1}			
	FI-ASV			ICP-OES
	As(III)[1]	As(V)[2]	As(total)[2]	As(total)[3]
Village 1/1	ND	ND	ND	ND
Village 1/2	7.75 ± 0.11	16.8 ± 1.3	24.5 ± 1.4	ND
Village 1/3	ND	11.9 ± 0.3	11.9 ± 0.3	ND
Village 1/4	20.5 ± 0.1	11.9 ± 0.3	32.5 ± 0.3	33.9 ± 3.8
Village 2/1	2.53 ± 0.16	118 ± 7	120 ± 7	120 ± 1
Village 2/2	0.431 ± 0.023	33.3 ± 0.3	33.7 ± 0.3	ND
Village 2/3	1.12 ± 0.07	230 ± 4	231 ± 4	231 ± 1
Village 2/4	1.46 ± 0.02	173 ± 3	175 ± 3	166 ± 2
Village 12/1	9.06 ± 0.14	311 ± 2	320 ± 2	313 ± 3
Village 12/2	2.14 ± 0.03	359 ± 19	361 ± 19	465 ± 3
Village 12/3	ND	ND	ND	ND
Village 12/4	0.804 ± 0.018	180 ± 4	181 ± 4	173 ± 2
Village 13/1	4.19 ± 0.02	14.4 ± 1.1	18.6 ± 1.1	ND
Village 13/2	ND	1577 ± 33	1577 ± 33	1587 ± 7
Village 13/3	ND	9.01 ± 0.33	9.01 ± 0.33	ND
Village 13/4	ND	387 ± 7	387 ± 7	404 ± 8

* Values given are the means (n = 2), % RSD < 8

[1] ND = Non detectable (<0.42 μg L^{-1})

[2] ND = Non detectable (<1.47 μg L^{-1})

[3] ND = Non detectable (<30.0 μg L^{-1})

CHAPTER 4

CONCLUSION

Arsenic is considered to be the toxic element and it can be found in natural waters. Different forms of arsenic have significant impacts on the toxicity. For inorganic arsenic, As(III) is much more toxic than As(V). Therefore, speciation analysis is more important than total arsenic determination in order to assess actual environmental risk.

In this research, flow injection - anodic stripping voltammetry for As(III) and As(V) determination was developed. A pre-plated gold film electrode (coated on glassy carbon electrode) was used as a working electrode due to its highly sensitivity and better reversibility of the electrode reaction. In addition, new gold film plating can minimize contamination from previous analysis. By using the optimum conditions, pre-plating gold film electrode was prepared as following procedure. A 100 mg L^{-1} Au(III) solution was aspirated to the electrochemical flow-cell with flow rate of 1.0 mL min^{-1} for 90 sec with the applied potential of -0.40 V vs Ag/AgCl. This gold film electrode can be used up to 8 consecutively analysis cycles without decline in sensitivity.

In order to achieve maximum sensitivity in the voltammetric response, electrochemical parameters were optimized. A 0.2 M HCl was used as supporting electrolyte. The deposition potential of -0.30 V, deposition time of 240 sec, flow rate of 2.5 mL min^{-1} and scan rate of 90 mV sec^{-1} were obtained as optimum conditions for As(III) determination.

For the determination of total inorganic arsenic [As(total)], As(V) was reduced to As(III) and then was analyzed in the same way as in the As(III) determination. From the studies, 6.5 mM KI with reduction time of 45 min was selected as optimum reducing agent to convert As(V) to As(III) effectively. As(V) content was calculated from the difference in amount of As(total) and As(III).

The calibration graphs were linear in the range of 1.0 to 30 μg L^{-1} and 2.0 to 100 μg L^{-1} for As(III) and As(total), respectively. The LODs (based on 3σ, n =

11) of 0.42 μg L^{-1} As(III) and 1.47 μg L^{-1} As(total) were provided. The LOQs (based on 10σ, n = 11) of 1.39 μg L^{-1} As(III) and 4.90 μg L^{-1} As(total) were obtained. Precisions within 5% (at 2 and 20 μg L^{-1}, n = 11) for As(III) and within 8% (at 10 and 100 μg L^{-1}, n = 11) for As(total) were established. Effect of interferences caused by cations commonly found in natural waters, especially, Sb(III), Cd(II), Cu(II), Fe(II), Pb(II), Hg(II), Ni(II), Zn(II) and their interference levels were investigated.

The developed method was applied to real natural water samples which were collected from arsenic contaminated area at Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province. The calibration curve method and standard addition method were used for As(III) and As(total) determinations, respectively. Concentrations of As(III) in natural water samples were in the range of ND to 20.5 μg L^{-1} and for As(V) in the range of ND to 1577 μg L^{-1}.

All natural water samples contained arsenic concentrations exceeding the WHO drinking water guidelines of 10 μg L^{-1} (WHO, 1996) and mostly found in As(V) species.

To validate the method, natural water samples were spiked with As(III) and As(V). The recovery percentages in the range of 85 to 105% for As(III), and 85 to 106% for As(V) were obtained. The accuracy of the method was evaluated by analysing SRM 1640 natural fresh water. The results obtained were in good agreement with the certified value. Concentrations of As(total) in natural water samples using the proposed method were also compared with those obtained by ICP-OES. It was found that there was no significant difference for both methods at 99% confidence level (t-test).

The proposed method was successfully applied to natural water samples. It could be alternative method for As(III) and As(V) determinations. The system provides widely used benefits, i.e., semi-automation, less amount of reagent consumption, short analysis time and suitability for routine analysis.

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APPENDICES

APPENDIX – A

A-1 Procedure for polishing the glassy carbon and stainless block

The glassy carbon electrode was used as substrate to plate gold film. Before deposition of a gold film, the gassy carbon electrode was polished as follow:
(a) Rinse the electrode surface with water to flush away any encrusted material on the surface. Follow this with a methanol rinse. Wipe dry with a fresh lab tissue.
(b) Wet the disk surface with de-ionized water. Shake the alumina suspension and add several drops of alumina polish, spacing them evenly around the pad surface
(c) Place the electrode face down on the pad. Using a smooth, circular motion, and even pressure, move the electrode all over the pad. After 1-2 minutes, remove the electrode and rinse it well with de-ionized water.
(d) Rinse the electrode briefly with ethanol and wipe it dry. The electrode is now ready to use.

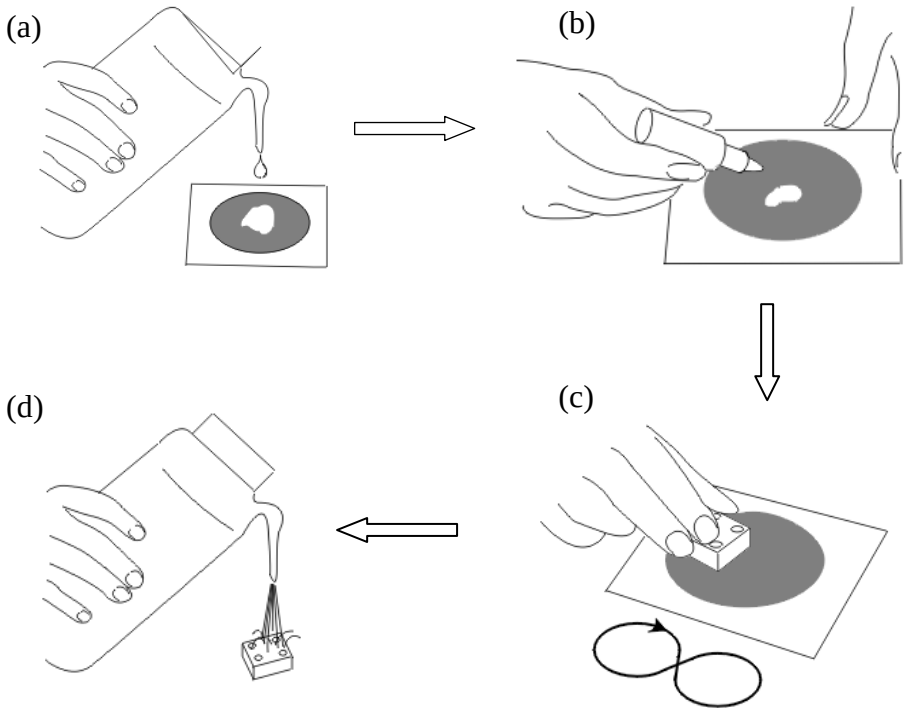

Figure A-1 Polishing glassy carbon

The stainless steel used as counter electrode was polished as follows: first, the electrode was polished with stains remover cream for iron and stainless steel surface and washed it with detergent and tap water. Second, it was polished with diamond powder and washed it with ethanol and de-ionized water, respectively. Finally, it was polished with alumina powder (0.05 μm diameter) and then washed with de-ionized water.

APPENDIX – B

Table B-1 Comparison of current using calibration curve and standard addition curve for As(III) determination

Concentration of As(III), μg L^{-1}	Calibration curve	Standard addition curve	
		Shallow well water	Stream water
5	3.318	1.428	1.790
10	5.807	4.378	4.648
15	9.178	7.315	7.455
20	12.420	10.744	11.122

Values given are the means (n = 2)

Table B-2 Comparison of current using calibration curve and standard addition curve for As(total) determination

Concentration of As(III), μg L^{-1}	Calibration curve	Standard addition curve	
		Shallow well water	Stream water
5	1.565	1.750	-
10	3.654	3.166	2.736
20	5.804	6.529	6.232
30	-	-	8.606
40	12.376	13.311	11.969

Values given are the means (n = 2)

APPENDIX – C

Table C-1 Details of the natural water samples collected from various sites in Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province. (Sampling on February 9, 2008)

Sites	Latitude Longitude	Temperature of air	Temperature of water	pH	Resources of water samples	
Village 1/1	8° 17′ 826″ 99° 86′ 586″	32.0	27.0	5.74 ± 0.05	Shallow well water	453/2 Village 1
Village 1/2	8° 18′ 203″ 99° 86′ 779″	31.2	27.5	6.08 ± 0.03	Shallow well water	783 Village 1
Village 1/3	8° 18′ 122″ 99° 87′ 053″	32.5	27.5	7.18 ± 0.01	Shallow well water	754 Village 1
Village 1/4	8° 17′ 549″ 99° 87′ 150″	31.5	30.5	6.84 ± 0.09	Shallow well water	474/3 Village 1
Village 2/1	8° 18′ 460″ 99° 84′ 576″	28.5	27.3	7.70 ± 0.11	Stream water	Klong Ronna Bridge, Village 2
Village 2/2	8° 17′ 943″ 99° 82′971″	30.0	26.0	8.34 ± 0.21	Stream water	Klong Wang Tea-wada , Village 2
Village 2/3	8° 19′ 211″ 99° 83′ 337″	31.2	26.0	7.58 ± 0.32	Stream water	Hua Mueng Bridge, Village 2
Village 2/4	8° 19′ 267″ 99°83′170″	31.0	26.0	6.40 ± 0.08	Stream water	Hua Mueng mine, Village 2

Table C-1 (Continued)

Sites	Latitude Longitude	Temperature of air (°C)	Temperature of water (°C)	pH	Resources of water samples	
Village 12/1	8° 19′ 349″ 99° 85′ 196″	34.0	29.0	8.11 ± 0.04	Stream water	Klong Num Khun, Village 12
Village 12/2	8° 18′ 342″ 99° 84′ 984″	31.0	28.5	7.00 ± 0.02	Shallow well water	197/2 Moo.12
Village 12/3	8° 18′ 209″ 99° 85′ 026″	31.0	27.0	5.89 ± 0.10	Shallow well water	Cheep Pradit Temple, Village 12
Village 12/4	8° 18′ 443″ 99° 84′ 803″	30.0	27.0	7.12 ± 0.04	Shallow well water	223 Village 12
Village 13/1	8° 17′ 368″ 99° 87′ 145″	28.0	28.0	6.28 ± 0.04	Shallow well water	515/1 Village 13
Village 13/2	8° 17′ 431″ 99° 86′ 105″	27.0	27.0	7.24 ± 0.01	Shallow well water	418/1 Village 13
Village 13/3	8° 16′ 802″ 98° 86′ 623″	28.0	28.0	7.18 ± 0.01	Shallow well water	512/8 Village 13
Village 13/4	8° 17′ 516″ 99° 85′ 908″	28.0	28.0	7.42 ± 0.08	Shallow well water	503/1 Village 13

C-2 Sampling sites

(a) **(b)**

(c) **(d)**

Figure C-1 Some sampling sites in Ron Phibun Sub-district, Ron Phibun District, Nakhon Si Thammarat Province, Thailand

(a) Klong Wang Tea-wada, Village 2

(b) 418/1, Village 13

(c) Cheep Pradit Temple, Village 12

(d) 223, Village 12

APPENDIX – D

D-1 Comparison between the slope of calibration curve and standard addition curve using two-way ANOVA by R software

The slopes of the calibration and the standard addition were compared using two-way ANOVA. In making a significance test, a true of hypothesis which is known as a null hypothesis, denote by H_0 that the interaction of both slope is not significant and alternative hypothesis (H_1) that the interaction of both slope is significant. If *P* value is less than α (level of significance) then the null hypothesis was rejected at that significant level (Miller and Miller, 1993).

Table D-1 Statistical values for the comparison between the slope calibration curve and standard addition curve using two-way ANOVA by R software

Matrix	***Df***	**Sum Sq**	**Mean Sq**	***F***	**P**
Natural water for As(III) determination	3	0.136	0.045	0.6378	0.6116
Natural water for As(total) determination	3	1.212	0.404	30.960	$9.418\times10^{-5***}$

Significant codes: '***' 0.001

Where: *Df* is the degree of freedom (*Df* = n-1, n is the number of concentration)

Sum Sq is the sum of squares, it refers to an interim quantity used in the calculation of an estimate of the population variance

Mean Sq is mean square, it refers to a sum of squared terms divided by the number of degrees of freedom

F is the ratio of two sample variances

P is probability

D-2 Comparison between the results of FI-ASV and ICP-OES method using t-test

Table D-2 The results of statistical test using t-test

Villages / Sites	FI-ASV method	ICP-OES method	X_d	$X_d - \overline{X}_d$	$(X_d - \overline{X}_d)^2$
Village 1/4	32.5	33.2	-0.7	11.27	127.01
Village 2/1	120	120	0	11.97	143.28
Village 2/3	231	231	0	11.97	143.28
Village 2/4	175	166	9	20.97	439.74
Village 12/1	320	313	7	18.97	359.86
Village 12/2	361	465	-104	-92.03	8469.52
Village 12/4	181	173	8	19.97	398.80
Village 13/2	1577	1587	-10	1.97	3.88
Village 13/4	387	404	-17	-5.03	25.30
		$\sum X_d$	-107.7	$\sum (X_d - \overline{X_d})^2$	= 10110.68
		$\overline{X_d} = \frac{\sum X_d}{n}$	-11.97		

$$S_d = \sqrt{\frac{\sum (X_d - \overline{X_d})^2}{n-1}} = \sqrt{\frac{10110.68}{9-1}} = 35.55$$

$$t = \frac{\overline{X_d}\sqrt{n}}{S_d} = \frac{-11.97\sqrt{9}}{35.55} = |-1.01| = 1.01$$

t-value from table at 99% (d.f. = 8) = 3.36

t-value from calculation < t-value from table

Therefore, the results for both methods were non-significant.

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List of Publication and Proceeding

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