OPTIMIZATION STUDIES FOR THE INORGANIC ARSENIC [As(III) AND As(V)] DETERMINATION USING SQUARE WAVE CATHODIC STRIPPING VOLTAMMETRY

ANGELINE LIMOK ANAK MONG

A thesis submitted in fulfillment of the requirements for the degree of Master of Science (Analytical Chemistry)

> Faculty of Resource Science and Technology UNIVERSITI MALAYSIA SARAWAK 2013

Acknowledgement

First and foremost, I would like to thank my supervisor, Professor Dr Lau Seng, for accepting me as his graduate student. A heartfelt appreciation for giving me the permission to use the IBEC laboratory. Without his support and guidance, I probably could not conduct my research and manage my thesis. Although he needs to juggle between work and being a supervisor, a big thank to him for sharing his time, expertise and constructive ideas.

Secondly, a big thanks to my family especially my parents for giving me the determination and support to undertake a Master Degree. All of these could never happen without their encouragements and sacrifices.

Thirdly, to my postgraduate friends in IBEC especially Pui and Pang, a big appreciation for updating me with the current situation in the postgraduate world. Thank you for making this journey a fun and meaningful one. To all the IBEC staff, without your help, it would be a challenge for me to conduct my laboratory analysis.

Abstract

Optimization studies for the Inorganic Arsenic [As(III) and As(V)] determination using Square Wave Cathodic Stripping Voltammetry (SWCSV)

The presence of arsenic in the environment has received worldwide attention. The primary exposure of arsenic poisoning is through the ingestion of the contaminated drinking water which seriously effects human's health such as the cardiovascular system. Thus, a fast, simple and reliable technique with inexpensive equipment such as voltammetry is required for the monitoring of arsenic contamination in the environment. In this study, the determination of inorganic As(III), total inorganic arsenic [As(III) + As(V)] and arsenic total were conducted using square wave cathodic stripping voltammetry at a hanging mercury drop electrode . Parameters that affect the performance of the technique such as Cu(II) concentration, deposition time, deposition potential, pulse amplitude and frequency were studied and optimized. The preconcentration was carried out in 1M HCl in the presence of Cu(II). Due to the limited maximum frequency allowed by the voltammetry instrumentation, the determination of arsenic was conducted under the frequency 149 Hz. In As(III) determination, it was found that 10 ppm Cu(II) under 90 s deposition time gave the highest peak currents. The optimum deposition potential and pulse amplitude was at -0.35 V and 60 mV respectively. The linearity range up to 450 µg/L offers a great advantage in quantifying the environmental samples without dilution. The detection limit obtained was 0.076 μ g/L. In the determination of total inorganic arsenic, the reducing effect of thiosulfate and its efficiency was investigated. The concentration of thiosulfate and Cu(II) and instrumental parameters such as deposition time and deposition potential was also optimized. It was

observed that thiosulfate at concentration of 1.5 mM was effective for the reduction of As(V) to As(III). At higher thiosulfate concentrations, split peaks were detected which could be contributed by the presence of colloid of sulfur at the electrode surface. For total inorganic arsenic determination, the optimum condition for the preconcentration of arsenic was in 1 M HCl in the presence of 10 ppm Cu(II) and 1.5 mM thiosulfate at a deposition potential of -0.45 V and deposition time of 90 s. The detection limit was 0.648 μ g/L. The efficiency of the reduction of As(V) by thiosulfate was studied by comparing the results obtained in the analysis of three sample solution with different ratio of As(III)/As(V) concentration. The mean peak current of 8 ppb As(V) and 2 ppb As(III) + 6 ppb As(V) were not significantly different from 8 ppb As(III) which shows that the reduction of As(V) by thiosulfate is quantitative. Significant interferences were observed for ions Fe^{3+} and Cd^{2+} that caused more than 50% peak suppression. No significant interference was observed in the presence of Zn^{2+} ion. The optimized method was applied in the determination of arsenic in environmental samples collected from Tasik Biru, Bau. It was observed that the concentration of arsenic in the environmental samples were more than 10-48 times higher than the maximum permissible value of 10 µg/L arsenic in the drinking water recommended by WHO. For verification purposes, the determination of arsenic total in the digested environmental samples were also conducted using Atomic Absorption Spectometry instrument and no significant differences in the concentration was obtained. Thus, square wave cathodic stripping voltammetry method is suitable for the routine analysis of arsenic in the environmental samples.

Abstrak

Kajian pengoptimuman untuk penentuan Arsenik Bukan Organik [As(III) dan As(V)] menggunakan voltametri gelombang segiempat sama secara katod

Kehadiran arsenik dalam persekitaran telah mendapat perhatian di seluruh dunia. Pendedahan utama keracunan arsenik adalah melalui pengambilan air minuman yang tercemar yang boleh menyebabkan kesan yang serius terhadap kesihatan manusia seperti sistem kardiovaskular dalam badan manusia. Oleh itu, teknik yang cepat, mudah dan tepat dengan peralatan yang murah seperti voltametri diperlukan untuk menguji pencemaran arsenik di dalam alam sekitar. Dalam kajian ini, penentuan As(III), arsenik bukan organik [As(III) + As(V)] dan jumlah arsenik telah dijalankan menggunakan voltametri gelombang segiempat sama secara katod pada elektrod merkuri titis tergantung. Parameter yang memberi kesan kepada prestasi teknik seperti kepekatan Cu(II), masa pemendapan, potensi pemendapan, amplitud denyutan dan frekuensi telah dikaji dan dioptimumkan. Pemekatan peringkat awal telah dijalankan di dalam 1M HCl dengan kehadiran Cu(II). Disebabkan oleh frekuensi maksimum adalah terhad yang dibenarkan oleh instrumentasi voltammetri, penentuan arsenik telah dijalankan di bawah frekuensi 149 Hz. Dalam penentuan As(III), didapati bahawa kepekatan Cu(II) 10ppm di bawah masa pemendapan 90 s telah memberikan arus puncak yang tertinggi. Potensi pemendapan yang optimum dan amplitud denyutan adalah pada – 0.35 V dan 60 mV. Julat linear sehingga 450 µg/L menawarkan kelebihan besar dalam mengukur sampel alam sekitar tanpa pencairan. Had pengesanan yang diperolehi ialah 0.076 µg/L. Dalam penentuan jumlah arsenik bukan organik, kesan tiosulfat dan keberkesanan penurunannya telah dikaji. Kepekatan tiosulfat dan Cu(II) dan parameter peralatan seperti masa pemendapan dan potensi

pemendapan juga dioptimumkan.Diperhatikan bahawa tiosulfat pada kepekatan 1.5 mM berkesan untuk menurunkan As(V) kepada As(III). Pada kepekatan tiosulfat yang lebih tinggi, puncak perpecahan telah dikesan yang boleh disumbangkan oleh pembentukan kompleks di antara Cu(II) dan ion-ion tiosulfat di permukaan elektrod. Bagi penentuan jumlah arsenik bukan organik, keadaan optimum untuk pemekatan arsenik adalah dalam 1 M HCl dengan kehadiran 10 ppm Cu (II) dan 1.5 mM thiosulfate pada potensi pemendapan – 0.45 V dan pemendapan masa 90 s. Had pengesanan adalah 0.648 µg/L. Keberkesanan penurunan oleh tiosulfat terhadap As(V) telah dikaji dengan membandingkan keputusan yang diperolehi daripada analisis yang dilakukan terhadap tiga sampel dengan nisbah kepekatan As(III)/As(V) yang berbeza. Didapati bahawa min puncak bagi 8 ppb As(V) dan 2 ppb As(III) + 6 ppb As(V) tidak menunjukkan perbezaan yang ketara daripada 8 ppb As(III) dan ini menunjukkan bahawa penurunan As(V) oleh tiosulfat adalah kuantitatif. Gangguan yang ketara telah diperhatikan untuk ion Fe^{3+} dan Cd^{2+} vang menyebabkan lebih daripada 50% penindasan puncak. Tiada gangguan yang ketara dari segi kehadiran ion Zn^{2+} . Kaedah yang optimal telah digunakan bagi penentuan arsenik di dalam sampel alam sekitar dari Tasik Biru, Bau. Ia telah diperhatikan bahawa kepekatan arsenik dalam sampel alam sekitar adalah 10-48 kali ganda lebih tinggi daripada nilai maksimum yang dibenarkan, 10 µg/L, dalam air minuman yang disyorkan oleh WHO. Sebagai tujuan pengesahan, penentuan jumlah arsenik dalam sampel alam sekitar telah juga dijalankan dengan menggunakan instrumen Penyerapan Spektometri Atom dan tiada perbezaan kepekatan yang ketara telah diperolehi. Oleh itu, kaedah voltametri gelombang segiempat sama secara katod adalah sesuai untuk analisis rutin arsenik di dalam sampel alam sekitar.

Table of Contents

Page

Title of Page	i
Acknowledgement	ii
Abstract	iii
Abstrak	v
Table of Contents	vii
List of Tables	xiv
List of Figures	XV
List of Abbreviations	xxi
List of Symbols	xxii

Chapter 1	Introduction	
1.1	Arsenic in the environment	1
1.2	Introduction on Tasik Biru	2
1.3	Arsenic toxicity	4
1.4	Arsenic determination	6
1.5	Fundamental principles of voltammetric techniques	8
1.5.1	Introduction on voltammetry	8
1.5.2	Instrumentation on voltammetry	9
1.5.3	Cyclic Voltammetry (CV) technique	12
1.5.4	Square-wave Voltammetry	15
1.5.5	Stripping analysis	17

	(a) Anodic Stripping Voltammetry (ASV)	18
	(b) Cathodic Stripping Voltammetry (CSV)	19
1.6	Limitations of arsenic determination	20
1.7	Research objectives	23
1.8	Scope of study	24

Chapter 2 Literature Review

2.1	Theory	v on Arsenic	26
	2.1.1	Chemical properties	26
2.2	Distrib	ution of arsenic and its species	28
	2.2.1	Soils	28
	2.2.2	Natural waters	29
2.3	Analyt	ical procedures for arsenic speciation and determination	31
2.4	Other of	determination method of As	32
	2.4.1	Atomic Absorption Spectrometry (AAS)	32
	2.4.2	Inductive Coupled Plasma-Mass Spectrometry	
		(ICP-MS)	34
2.5	Electro	ochemical techniques	36
	2.5.1	Stripping analysis	37
		(a) Anodic Stripping Voltammetry (ASV)	37
		(b) Cathodic Stripping Voltammetry (CSV)	40

Chapter 3	Metho	dology	
3.1	Experi	ments	45
3.2	Chemi	cals	46
3.3	Prepara	ation of reagants and solutions	46
	(a)	Arsenic(III) stock solution	46
	(b)	Arsenic(III) intermediate standard solution	47
	(c)	Arsenic(III) working standards solutions	47
	(d)	Arsenic(V) stock solution	47
	(e)	Cu(II) solutions	47
		(i) Cu(II) solution (20 000 mg/L)	47
		(ii) Cu(II) solution (1000 mg/L)	48
	(f)	Hydrochloric acid (HCl) solutions	48
		(i) Hydrochloric acid (4M)	48
		(ii) Hydrochloric acid (2M)	48
	(g)	Sodium thiosulfate	48
3.4	Instrumentation and apparatus		
	3.4.1	Glassware and samples bottles	48
	3.4.2	Instrumentation for electrochemical measurement	49
		(a) Working electrode (WE)	49
		(b) Reference electrode (RE)	50
		(c) Auxiliary electrode (AE)	50
3.5	Determination of Arsenic(III) concentration		
	3.5.1	Procedures for As(III) determination	50

3.5.2	Genera	l investigation using Cyclic	
		Voltammetry (CV)	52
	3.5.3	Factors affecting the peak currents	52
		(a) Deposition potential (E _d)	52
		(b) Cu(II) concentration	53
		(c) Deposition time (t _d)	53
		(d) Pulse amplitude (E _{sw})	54
		(e) Frequency	54
	3.5.4	Quality assurance	55
		(a) Calibration curves and linearity range	55
		(b) Limit of detection (LOD)	56
		(c) Precision	56
		(i) Within-run (repeatability)	56
		(ii) Between-run (reproducibility)	57
		(d) Accuracy of the method	57
3.6	Determ	58	
	3.6.1	Procedures for As(V) determination	58
	3.6.2	Chemical reduction of As(V)	58
	3.6.3	Optimization	60
		(a) Deposition potential (E _d)	61
		(b) Cu(II) concentration	61
		(c) Thiosulfate concentration	61
		(d) Deposition time (t _d)	62
	3.6.4	Efficiency of reduction	62

	3.6.5	Quality assurance	63
		(a) Calibration curves and linearity range	63
		(b) Limit of detection (LOD)	64
		(c) Within-run precision (repeatability)	65
		(d) Between-run precision (reproducibility)	65
		(e) Accuracy and recovery of the reduction of As(V)	65
to As(III)			
3.7	Interfe	rence studies	66
3.8	Applic	ation on environmental samples	66
	3.8.1	Sampling and water pretreatment	66
	3.8.2	Procedure	67
		(a) Determination of As(III) and	67
		total inorganic arsenic	
(b) Determin	ation of a	arsenic total	68
Chapter 4	Result	s and discussion	
4.1	Determ	nination of As(III)	71
	4.1.1	General investigation using Cyclic	
		Voltammetry (CV)	71
	4.1.2	Factors affecting the peak currents	72
		(a) Deposition potential	72
		(b) Copper(II) concentration	74

(d) Pulse amplitude	79
· · / ·	

77

(c) Deposition time

		(e) Frequency	81
	4.1.3	Quality assurance	83
		(a) Calibration curves and linearity range	83
		(b) Limit of detection (LOD)	86
		(c) Within-run precision	89
		(d) Between-run precision	89
		(e) Accuracy of the method	92
4.2	Determ	ination of As(V)	94
		4.2.1 Chemical reduction of As(V) by thiosulfate	94
		4.2.2 Optimization	98
		(a) Deposition potential	98
		(b) Cu(II) concentration	99
		(c) Thiosulfate concentration	102
		(d) Deposition time	104
		4.2.3 Efficiency of reduction	105
		4.2.4 Quality assurance	108
		(a) Calibration curves and linearity range	108
		(b) Limit of detection	111
		(c) Within-run precision	112
		(d) Between-run precision	114
		(e) Accuracy and recovery of the reduction	114
of As(V) to	As(III)		
4.3	Effect of	of interferences	117
4.4	Applica	ation on environmental samples	120

	4.4.1 Water pretreatment	120
4.4.2	Determination of As(III) and	121
total inorganic	e arsenic	
4.4.3	Determination of Arsenic total	126
Chapter 5	Conclusion	131
Chapter 6	Recommendations for future works	134
References	1	136

List of Tables

Table No	Title	Page
3.1	The list of chemical reagents used in the experiment	46
3.2	The optimized conditions for the determination of As(III) and $[As(III) + As(V)]$	68
3.3	Spectrometer parameters for arsenic total determination	70
4.1	Summarized statistical determination of limit of detection of As(III)	88
4.2	Within-run Precision calculated from the mean RSD of 10 replicates measurements of 5 ppb As(III) peak currents	90
4.3	Between-run Precision calculated from the mean RSD of 10 replicates measurements of 5 ppb As(III) peak currents.	91
4.4	Results obtained for As(III) spiked purified water samples	93
4.5	Statistical comparisons of solutions with the same total arsenic concentration (8 ppb) but with different ratios of As(III)/As(V)	107
4.6	Within-run Precision calculated from the mean RSD of 10 replicates measurements of 2 ppb As(V) peak currents	113
4.7	Between-run Precision calculated from the mean RSD of 10 replicates measurements of 5 ppb As(V) peak currents	115
4.8	Results obtained for As(V) spiked purified water samples	116
4.9	Percentage increase or decrease of the peak currents of three ions towards As(III)	118
4.10	Determination of inorganic As(III) and total inorganic arsenic in environmental samples collected from Tasik Biru, Bau.	125
4.11	Determination of arsenic total in digested environmental samples collected from Tasik Biru, Bau.	129
4.12	Comparison between the results obtained by SWCSV and by AAS for the determination of arsenic total (digested samples)	130

List of Figures

Figure No	Title	Page
1.1	The map of Tasik Biru in Bau. Tasik Biru is labelled in red dot.	3
1.2	Schematic diagram of a three-electrode cell (Bard & Faulkner, 1980)	10
1.3	Voltammetric electrochemical cell with three electrode system : WE- working electrode; RE- reference electrode; CE- counter electrode (Wang, 2006)	11
1.4	Typical cyclic voltammogram for a reversible $O_x + n\bar{e} \longrightarrow R_{ed}$ redox process.(E_{pc} = cathodic peak potential, E_{pa} = anodic peak potential, i_{pc} = cathodic peak current, i_{pa} = anodic peak current) (Heineman & Kissinger, 1984)	13
1.5	Square-wave waveform showing the amplitude (Esw), step height (Δ E), square-wave period (τ), delay time (Td) and current measurements times (1 and 2). (Kissinger, 1984)	15
1.6	Square-wave voltammogram for reversible electron transfer ; (A) forward current ; (B) reverse current ; (C) net current (Kissinger, 1984)	16
4.1	Cyclic voltammograms of 5 ppb As(III) in 2 M HCl with 20 ppm Cu(II). Scan 1 was preceded by 55 s stirred deposition at -0.35 V and followed by scan 2 and 3. The scan rate was 0.1 V/s	72
4.2	Effect of varying the deposition potential on SWCS peak height and peak potential of 5 ppb As(III) in 2 M HCl and 20 ppm Cu(II). Deposition potential, Ed, was altered from -0.25 V to -0.55 V. (t _d = 55 s, E _{sw} = 60 mV, f = 149 Hz)	73
4.3	SWCS voltammograms of 5 ppb As(III) in 2 M HCl. The Cu(II) concentration: 0 ppm, 1 ppm, 2 ppm, 5 ppm, 7 ppm and 9 ppm. ($E_d = -0.35$ V, $t_d = 55$ s, $E_{sw} = 60$ mV, $f = 149$ Hz)	75
4.4	SWCS voltammograms of 5 ppb As(III) in 2 M HCl. The Cu(II) concentration: 10 ppm, 20 ppm, 30 ppm and 40 ppm. The voltammogram showed triplicate scans of each Cu(II) concentration. $(E_d = -0.35 \text{ V}, t_d = 55 \text{ s}, E_{sw} = 60 \text{ mV}, f = 149 \text{ Hz})$	76

4.5	Effect of varying Copper(II) concentration on SWCS peak current and peak potential of 5 ppb As(III) in 1 M HCl. Copper(II) concentration ranged from $0 - 40$ ppm were investigated. Values shown were the average of three determinations each. (E _d = -0.35 V, t _d = 55 s, E _{sw} = 60 mV, f = 149 Hz)	76
4.6	Effect of varying the deposition time at four copper concentration levels (10 ppm, 20 ppm, 30 ppm, 40 ppm) on the SWCS peak current of 5 ppb As(III) in 2 M HCl. Values shown were the average of three determinations each. ($E_d = -0.35$ V; f = 149 Hz; $\Delta E = 5$ mV; $E_{sw} = 60$ mV)	78
4.7	Effect of varying the pulse amplitude on the SWCS peak current and peak potential of 5 ppb As(III) in 2 M HCl and 10 ppm Cu(II). Pulse amplitude, E_{sw} was altered from 10 - 250 mV. Values shown were average of three determinations each. ($E_d = -0.35$ V; $t_d = 90$ s; $f = 149$ Hz; $\Delta E = 5$ mV)	79
4.8	SWCS voltammograms of 5ppb As(III) in 2 M HCl and 10 ppm Cu(II). Pulse amplitude in : $a = 10 \text{ mV}$, $b = 20 \text{ mV}$, c = 30 mV, $d = 40 mV$, $e = 50 mV$, $f = 60 mV$, $g = 70 mV$, h = 80 mV, $i = 90 mV$, $j = 100 mV$, $k = 150 mV$, $1 = 200 mV$, $m = 250 \text{ mV}$. ($E_d = -0.35 \text{ V}$; $t_d = 90 \text{ s}$; $f = 149 \text{ Hz}$; $\Delta E = 5 \text{ mV}$)	80
4.9	SWCS voltammograms of 5 ppb As(III) in 2 M HCl and 10 ppm Cu(II). Pulse amplitude is in the range of 100 mV to 250 mV. The scans were done in triplicate. $(E_d = -0.35 \text{ V}; t_d = 90 \text{ s}; f = 149 \text{ Hz}; \Delta E = 5 \text{ mV})$	81
4.10	Effect of varying the frequency on the SWCS peak current of 5 ppb As(III) in 2 M HCl and 10 ppm Cu(II). Values shown were average of three determinations each. $(E_d = -0.35 \text{ V}; t_d = 90 \text{ s}; \Delta E = 5 \text{ mV})$	82
4.11	Calibration curve of As(III) in the range of $0 - 5$ mg/L in 2 M HCl and 10 ppm Cu(II). (E _d = -0.35 V, t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	83
4.12	Calibration curve of As(III) in the range of $0 - 1.05$ mg/L in 2 M HCl and 10 ppm Cu(II). (E _d = -0.35 V, t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	84
4.13	Calibration curves of As(III) in 2 M HCl and 10 ppm Cu(II). ($E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz)	86

4.14	Calibration curve of As(III) in 2 M HCl and 10 ppm Cu(II). Error bars represent one standard deviation at 95% confidence level. ($E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz)	87
4.15	SWCS voltammograms of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II). The Na ₂ S ₂ O ₄ concentration and deposition potential in : (a) 0 mM, $E_d = -0.35$ V; (b) 0.5 mM, $E_d = -0.35$ V; (c) 0.5 mM, $E_d = -0.45$ V; (d) 1.0 mM, $E_d = -0.45$ V; (e) 1.5 mM, $E_d = -0.45$ V; (f) 2.0 mM, $E_d = -0.45$ V; (g) another 10ppm Cu(II) was added to (f). (t _d = 90 s, $E_{sw} = 60$ mV, f = 149 Hz)	95
4.16	SWCS voltammograms of 5 ppb As(V) in 2 M HCl and 3.2 mM Na ₂ S ₂ O ₄ .The Cu(II) concentration in ; (a) 10 ppm; (b) 20 ppm; (c) 30 ppm. $(E_d = -0.45V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz)$	96
4.17	Voltammograms of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II) in the presence of 3.2 mM Na ₂ S ₂ O ₄ . (a) Cyclic linear scan voltammetry at $E_d = -0.45$ V, $t_d = 90$ s, scan rate = 0.75 V/s. (b) SWCSV at $E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz	97
4.18	SWCS voltammograms (2 scans) of 5 ppb As(V) in 2 M HCl, 10 ppm Cu(II) and 3.2 mM Na ₂ S ₂ O ₄ . Na ₂ S ₂ O ₄ was added first into the solution followed by As(V). $(E_d = -0.45 \text{ V}, t_d = 90 \text{ s}, E_{sw} = 60 \text{ mV}, f = 149 \text{ Hz})$	98
4.19	Effect of deposition potential on the SWCSV determination of 5 ppb As(V) in 2 M HCl, 10 ppm Cu(II) and 3.2 mM Na ₂ S ₂ O ₄ . The deposition potential used in : (a) -0.25 V; (b) -0.30 V; (c) -0.35 V; (d) -0.40 V; (e) -0.45 V; (f) -0.50 V; (g) -0.55 V. (t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	99
4.20	Effect of Cu(II) concentration on the SWCSV determination of 5 ppb As(V) in 2 M HCl and 3.2 mM $Na_2S_2O_4$. The Cu(II) concentration used in : (a) 0 ppm; (b) 1 ppm; (c) 3 ppm; (d) 5 ppm; (e) 7 ppm; (f) 10 ppm. From (g) to (h), another 5 and 10 ppb As(V) was added respectively to (f)	100
4.21	Effect of varying Cu(II) concentration on 5 ppb As(V) in 2 M HCl and 3.2 mM Na ₂ S ₂ O ₄ . The Cu(II) concentration was altered from 10 ppm to 110 ppm. $(E_d = -0.45 \text{ V}, t_d = 90 \text{ s}, E_{sw} = 60 \text{ mV}, f = 149 \text{ Hz})$	101

4.22	SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 3.2 mM Na ₂ S ₂ O ₄ . The As(V) concentration used in : (a) 0.1 ppb; (b) 0.5 ppb; (c) 1 ppb; (d) 2 ppb; (e) 4 ppb; (f) 6 ppb; (g) 8 ppb; (h) 10 ppb; (i) 12 ppb; (j) 15 ppb. ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz)	102
4.23	Effect of $Na_2S_2O_4$ concentration on the SWCSV determination of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II). The $Na_2S_2O_4$ used in : (a) 0.5 mM; (b) 1.0 mM; (c) 1.5 mM; (d) 2.0 mM; (e) 2.5 mM; (f) 3.0 mM; (g) 3.5 mM. (E _d = -0.45 V, t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	104
4.24	SWCS voltammograms of 2 M HCl containing 5 ppb As(V), 10 ppm Cu(II) and 1.5 mM Na ₂ S ₂ O ₄ . The deposition time use in : (a) 30 s; (b) 40 s; (c) 50 s; (d) 60 s; (e) 90 s; (f) 120 s; (g) 150 s; (h) 180 s; (i) 210 s; (j) 240 s; (k) 270 s;(l) 300 s. (E _d = -0.45 V, E _{sw} = 60 mV, f = 149 Hz)	105
4.25	SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 1.5 mM Na ₂ S ₂ O ₄ . The arsenic concentration in : (a) 8 ppb As(III): (b) 8 ppb As(V); (c) 2 ppb As(III) + 6 ppb As(V). ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz)	106
4.26	The calibration curve obtained with 9 aliquots of As(V) added into 2 M HCl solution containing 2 ppb As(V). 10 ppm Cu(II) and 1.5 mM Na ₂ S ₂ O ₄ . Each point represents the mean of three determinations. $(E_d = -0.45 \text{ V}, t_d = 90 \text{ s}, E_{sw} = 60 \text{ mV}, f = 149 \text{ Hz})$	108
4.27	The calibration curve obtained with 9 aliquots of As(III) added into 2 M HCl solution containing 2 ppb As(V), 10 ppm Cu(II) and 1.5 Mm Na ₂ S ₂ O ₄ . Each point represents the mean of three determinations. ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz)	109
4.28	Comparison of two analytical methods using regression line. The results were obtained from SWCSV peak currents of 9 aliquots of 2 ppb arsenic added into solution containing 2 M HCl, 10 ppm Cu(II) 1.5 mM Na ₂ S ₂ O ₄ and 2 ppb As(V). The results on x-axis was spiked with 9 x 2 ppb As(III); y-axis was spiked with 9 x 2 ppb As(V). $(E_d = -0.45 \text{ V } [\text{As}(\text{V})]; E_d = -0.35 \text{ V } [\text{As}(\text{III})], t_d = 90 \text{ s},$ $E_{sw} = 60 \text{ mV}, f = 149 \text{ Hz})$	110

4.29	Calibration curve of As(V) in the range of $0 - 200 \ \mu g/L$ in 2 M HCl containing 10 ppm Cu(II) and 1.5 Mm Na ₂ S ₂ O ₄ . (E _d = -0.45 V, t _d = 90 s, E _{sw} = 60 mV, f= 149 Hz)	111
4.30	Calibration curve of As(V) in the range of $0 - 14 \mu g/L$ in 2 M HCl solution containing 10 ppm Cu(II) and 1.5 Mm Na ₂ S ₂ O ₄ . Error bars represent one standard deviation at 95 % confidence level. (E _d = -0.45 V, t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	112
4.31	SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 10 ppb As(III). The concentration of Fe ³⁺ added : (a) 0 μ g/L; (b) 500 μ g/L; (c) 1000 μ g/L. (E _d = -0.35 V, t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	119
4.32	SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 10 ppb As(III). The concentration of Zn^{2+} added : (a) 0 µg/L; (b) 500 µg/L; (c) 1000 µg/L. (E _d = -0.35 V, t _d = 90 s, E _{sw} = 60 mV, f = 149 Hz)	120
4.33	SWCS voltammograms of determination of As(III) in water samples from Tasik Biru (6 m depth) in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz. (Each scan was repeated three times)	123
4.34	SWCS voltammograms of determination of total inorganic arsenic in water samples from Tasik Biru (6 m depth) in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz. (Each scan was repeated three times)	124
4.35	Calibration graph obtained from the determination of total inorganic arsenic (11 m depth) of water sample from Tasik Biru in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, $f = 149$ Hz. (Each determination of the water samples was repeated three times and the mean concentration is the average of three determinations. Voltammetry software automatically generated the calibration graph and concentration)	124

- 4.36 Calibration graph obtained from the determination of arsenic total (6 m depth) of digested water sample collected 128 from Tasik Biru in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz. (Each determination of the water samples was repeated three times and the mean concentration is the average of three determinations)
- 4.37 Calibration graph obtained from the detection of 128 arsenic standard solution (blank, 10 mg/L, 20 mg/L and 30 mg/L) by AAS instrument. The calibration graph exhibited a linearity with $r^2 = 1.000$.

List of Abbreviations

AAS	atomic absorption spectrometry
AE	auxiliary or counter electrode
AES	atomic emission spectrometry
AFS	atomic fluorescent spectrometry
ASV	anodic stripping voltammetry
AB	arsenobetaine
AC	arsenocholine
BFD	blackfoot disease
CL	confidence limit
CSV	cathodic stripping voltammetry
CV	cyclic voltammetry
df	degree of freedom
DMA	dimethylarsenic
DMAA	dimethylarsinic acid
DME	dropping mercury electrode
DPASV	differential pulse anodic stripping voltammetry
DPP	differential pulse polarography
DPV	differential pulse voltammetry
ETAAS	electrochemical atomic absorption spectrometry
GFAAS	graphite furnace atomic absorption spectrometry
HG	hydride generation
HGAAS	hydride generation atomic absorption spectrometry
HMDE	hanging mercury drop electrode
HPLC	high performance liquid chromatography
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectometry
LOD	limit of detection
MAA	methylarsonic acid
MCL	maximum contaminant level
MFE	mercury film electrode
MMA	monomethylarsinate
MMAA	monomethylarsenic acid
MME	multi-mode electrode
NAA	neutron activation analysis
PTFE	polytetrafluoroethylene
RE	reference electrode
RSD	relative standard deviation
SWCSV	square wave cathodic stripping voltammetry
SWV	square wave voltammetry
TMAO	trimethylarsine oxide
USEPA	United States Environmental Protection Agency
WE	working electrode
WHO	World Health Organization

List of Symbols

atm	atmospheric
°C	Celsius
g/mol	gram/molecular
Hz	hertz
Ei	initial potential
μg	microgram
μg/L	microgram/litre
µg/mL	microgram/millilitre
μm	micrometre
m	metre
mg	milligram
mg/kg	milligram/kilogramme
mg/L	milligram/litre
mL	millilitre
mm^2	millimetre square
mM	millimolar
mV	millivolt
min	minutes
Μ	molarity
ng/mL	nanogram/millilitre
nm	nanomolar
i _{net}	net current
Ω	ohm
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
E _p	peak potential
i _p	peak current
mV	potential step
S	second
E_{sw}	square-wave amplitude
ΔE	square-wave potential step
τ	square-wave pulses
E°	standard potential
w/v	weight/volume
sub-ppb	fractional parts per billion
V	volt

Chapter 1

INTRODUCTION

1.1 Arsenic in the environment

Arsenic is a trace element that can be found in most geological materials. It has been widely used in few sectors such as in agriculture as pesticides, livestock as feed additives, electronics as solar cells and in industry as glassware. The presence of arsenic in the environment can be hazardous to living organisms. Living organisms are exposed to toxic arsenic primarily from food and water. Arsenic exists in several chemical forms in nature, such as inorganic arsenic and organic arsenic. Inorganic arsenic, namely arsenite [As(III)] and arsenate [As(V)], are the dominant species in natural waters although minority organic arsenic Organic arsenics include arsenobetaine (AB), arsenocholine does exist. (AC), monomethylarsonate (MMA), dimethylarsinate (DMA) and many more can be found in marine plants and animals. The distribution of inorganic arsenic species in water varies with the geochemical conditions, especially the redox conditions of the water (He et al., 2004). Inorganic trivalent arsenite, As(III), is more toxic to plants and animals than pentavalent arsenate, As(V), and organic arsenic.

Arsenic is released to water from weathering of the rocks and soils. In groundwater, the erosion of soils and minerals may contribute to the availability of arsenic. Higher levels of arsenic tend to be found more in groundwater rather than in surface water and the naturally occurrence of arsenic in groundwater is a major public threat that is affecting million of people worldwide which use groundwater as a source of drinking water. Arsenic along with fluoride, are now recognized as the most serious inorganic contaminants in drinking water on a worldwide basis (Smedley & Kinniburgh, 2001). Arsenic concentrations in rocks depend on the rock type, with sedimentary rocks containing much higher concentrations. It has been estimated that more than 99% of the total arsenic contained in various natural reservoirs is present in rocks and minerals (Bhumbla & Keefer, 1994). Arsenic in natural environment occurs in soils at an average concentration of about 5-6 mg/kg, but this varies among geographic regions (Peterson *et al.*, 1981). Arsenic that is brought to the surface of the earth during mining of arsenic containing coal increases the potential for contamination of soil with arsenic. Besides that, arsenic can also be released to water from anthropogenic sources that include modern industry, mining operations, agriculture, forestry, manufacturing processes, wastewaters, coal burning power plant, chemical wastes and disposal of industrial and animal wastes. Arsenic is also a natural contaminant in lead, zinc, gold and copper ores and can be released during mining process (Crecelius *et al.*, 1974; Ragaini *et al.*, 1977).

1.2 Introduction on Tasik Biru

Tasik Biru is located at Bau area, about 35 km from Kuching, Sarawak. Being an interior district in Kuching division, its geographical features are composed of rugged terrain and limestone hills. Tasik Biru is not a natural lake, having been formed as a result of open cast mining. The gold mine, known as Tai Parit Mine was operated by Borneo Company from 1896, was flooded in 1921 and became a lake (The New Straits Times, 1999). In the 1970s, the name was changed to Tasik Biru. Tasik Biru was closed to the public in 1990 to enable a private company to extract gold ore on the bed and sides of the lake (The new Straits Times,

1999). After the extraction stopped in 1997, the open cast mining was rehabilitated and redeveloped into an eight hectare lake which is 2 hectare bigger before the mining was conducted in 1990. The lake has been used for recreational and tourism activities. Tourism Ministry has also constructed a canteen, a pontoon and a gangway at Tasik Biru.

However, it was declare unsafe for any recreational activities due to the presence of high arsenic in the water (The New Straits Times, 28 October 1999). It was reported that the concentration of arsenic in Tasik Biru is 0.87 ppm which is 40 times higher than the permissible level of 0.02 ppm recommended by the World Health Organisation (New Straits Times, 1999). It was believed that the arsenic originates from arsenopyrite, a mineral found in the mineral rock.



Figure 1.1: The map of Tasik Biru in Bau. Tasik Biru is labeled with a red dot.

1.3 Arsenic toxicity

The toxicity of arsenic is species dependent. The trivalent form of arsenic, As(III), both in inorganic and organic form, are generally more toxic than the pentavalent forms of arsenic, As(V) (Ferguson & Garis, 1972). Inorganic arsenic compounds are about 100 times more toxic than organic arsenic compounds (Jain & Ali, 2000). Humans may be exposed to arsenic poisoning through inhalation, dermal absorption and ingestion of food, water and soil contaminated with arsenic.

There are two types of toxicity from arsenic poisoning which are acute and sub-acute. The acute arsenic poisoning usually occur through ingestion of contaminated food or drink and requires immediate medical attention. The major early manifestations due to acute arsenic poisoning include burning and dryness of the mouth and throat, dysphasia, colicky abnormal pain, projectile vomiting, profuse diarrhea and hematuria (Done & Peart, 1971). Sub-acute arsenic toxicity mainly involves the respiratory, gastro-intestinal, cardiovascular, nervous and haematopoietic systems. It may cause loss of appetite, nausea and some vomiting, dry throat, shooting pains, diarrhea, nervous weakness, tingling of the hands and feet. Longer exposure resulted in dry, falling hair, brittle loose nails, eczema, darken skin exfoliation and a horny conditions of the palms and soles (Pinto & Mcgill, 1953).

The ingestion of arsenic through food or water may have serious effects on the human cardiovascular system. Many epidemiological studies have shown that there is a doseresponse relationship between arsenic exposure and the development of diseases that affect the cardiovascular system. Subclinical disorders that have been associated with long term drinking water exposure to arsenic are carotid atherosclerosis and electrodiagram abnormalities (Wang *et al.*, 2007). Low-level exposure of arsenic in humans may also cause vascular system damage. A classical example is Blackfoot disease (BFD) which is endemic in an area of Taiwan where most drinking water contains 0.17 - 0.8 ppm arsenic (Tseng, 1977), corresponding to dose of about 0.01-0.5 mg As/kg/day. BFD is described by a progressive loss of circulation in hands and feet, which leads to severely painful gangrene formation for the extremities, often necessitating amputation of the limbs. The effect of arsenic on the vascular system have also been reported in Chile in which ingestion of 0.6 to 0.8 ppm arsenic in drinking water (equivalent to 0.02 - 0.06 mg As/kg/day) increased the incidence of Rynaud's disease and of cyanosis of fingers and toes (Naqvi *et al.*, 1994). In Antofagasta, over 12% of the population exhibiting dermatological manifestations related to arsenic due to consumption of high arsenic containing drinking water (Borgono & Greiber, 1972).

Arsenic has been found to be carcinogenic by both inhalation and ingestion routes and it is the only known substance for which there is adequate evidence of carcinogenic risk by both inhalation and ingestion routes of exposure. Besides that, arsenic is also the only carcinogen where exposure through drinking water has been clearly demonstrated to cause human cancer. It has been reported that inorganic arsenic compounds increase the risk of lung cancer when exposure occurs through inhalation (Taylor *et al.*, 1989) and ingestion of inorganic arsenic also increases the risk of skin cancer. The United States Environmental Protection Agency (USEPA) has classified arsenic as a Group A carcinogen based on the 1986 Cancer Assessment Guidelines (USEPA, 1998).

1.4 Arsenic determination

The environmental concern about arsenic has prompted the development of various analytical methods for the determination and speciation of arsenic. Hydride generation techniques combined with an efficient separation and highly sensitive detection techniques have been widely used for arsenic analysis. Methods such as ion chromatography (IC) coupled with inductively coupled plasma mass spectrometry (ICP-MS), hydride generation atomic absorption spectrometry (HGAAS), and atomic fluorescent spectrometry (AFS) can provide extensive speciation information on both inorganic and organic arsenic (He *et al.*, 2004).

However, these methods require expensive instrumentation, elaborate analysis procedure, complicated with high operational costs, and therefore, are not easily available in poor countries that are badly hit by arsenic poisoning. Electrochemical techniques can be used to provide speciation information based on labile/inert discrimination, redox state, and half-wave potential measurements (Florence, 1989). The advantages of electrochemical techniques are that the methods are selective towards electroactive species, relatively simple, and require inexpensive instrument with high sensitivity. Besides that, the sample also requires much less handling and is less contaminated than when other speciation methods, such as solvent extraction, ultrafiltration, or dialysis are used.

Electrochemical techniques such as voltammetry have long been applied to the determination of arsenic and is a simple, fast, inexpensive and a speciation capability method to directly speciate As(III) and As(V). Voltammetric techniques are based on the

measurement of the current response of an analyte to applied potential waveforms. The current is a quantitative measure of how fast a species is being reduced or oxidized at the electrode surface (Kounaves, 1990). Voltammetry can be applied for the determination of trace metals and organic substances from sub-ppb to ppm or higher. Voltammetric techniques are suitable for speciation studies due to its sensitivity both to low metal concentrations and to the chemical forms of metals in solution. For detection of lower concentrations of solution, these voltammetric techniques are combined with a pre-concentration step prior to determination.

The voltammetric techniques preceded by electrochemical pre-concentration of the analyte from sample solution into or onto the working electrode prior to electrochemical measurement of the concentrated analyte are referred to as stripping techniques (Neeb, 1989; Wang, 1985). Interest in stripping analysis has been sparked by its ability to measure simultaneously four to six trace metals at concentration levels down to the fractional parts per billion (sub-ppb), utilizing inexpensive instrumentation (Wang, 1985). Because of its 'built in' pre-concentration step, extremely high sensitivity can be obtained in stripping analysis. Stripping analysis offers two distinctive advantages, which is its speciation capability for measuring trace metals in natural waters and its suitability for in-situ field measurements. Hence, the high sensitivity and low cost of stripping analysis have led to its useful application in large number of analytical problems such as determination of various trace metals in environmental, clinical, foodstuffs, beverages or pharmaceutical samples. Compared to element-sensitive methods such as AAS, AES or NAA, voltammetry is a species sensitive method.

7

1.5 Fundamental principles of Voltammetric techniques

1.5.1 Introduction on Voltammetry

Electroanalysis is one of the analytical methods that is used for qualitative and quantitative analyses of samples with different concentration. Electroanalytical methods in which information about the analyte is derived from the measurement of current as a function of applied potential are categorized as voltammetric methods (Skoog & Leary, 1992). Electroanalysis compromises of three classes, that are potentiometry, coulometry and polarography or known as voltammetry. They differ in the type of potential waveform impressed in the cell, the type of electrode used and the state of the solution in the cell (quiescent or flowing). Electroanalytical methods have several advantages over other analytical techniques in which its electrochemical measurements are often specific for certain oxidation state of an element, the instrumentation is relatively inexpensive and they provide information about activities rather than concentration of the chemical species.

Voltammetry is a technique in which a potential is applied to a polarizable electrode (against a suitable reference electrode) and the resulting current is measured (Palus, 1990a). Voltammetry was developed from polarography, a particular type of voltammetry that was invented by J. Heyrovsky in the early 1920s (Skoog & Leary, 1992). Polarography differs from other types of voltammetry in which the DME is used as the working electrode, where as in voltammetry techniques, it uses various kinds of working electrodes, such as, HMDE, carbon paste electrode and platinum electrode.

In voltammetry, the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it (Kounaves, 1990). Voltammetry can be divided into many different techniques such as cyclic voltammetry, pulse techniques and stripping techniques in which the distinction between them is the waveform of the exciting potential. The analytical advantages of the various voltammetric techniques include excellent sensitivity, a large number of useful solvents and electrolytes, rapid analysis time and simultaneous determination of several analytes. Voltammetry is widely applied for the quantitative determination of a variety of dissolved inorganic and organic substances, fundamental studies of oxidation and reduction processes in various media, adsorption processes on surfaces and electron-transfer mechanisms at chemically modified electrode surfaces (Kounaves, 1990: Skoog & Leary, 1992).

1.5.2 Instrumentation on Voltammetry

Voltammetry consists of an electrochemical cell that is connected to a potentiostat system that controls the potential of the working electrode with respect to the reference electrode. The basic component of voltammetry instrument is consisting of potentiostat, computer and the electrochemical cell (with a three electrode system). Figure 2.1 shows a schematic diagram of a three-electrode cell arrangement.



Figure 1.2: Schematic diagram of a three-electrode cell (Bard & Faulkner, 1980).

Three-electrode cells are commonly used in voltammetry experiments. Figure 2.2 shows the voltammetric electrochemical cell with three electrode system. The cell is usually a covered beaker of 5-50 mL volume and contains the three electrodes (working, reference and auxiliary), immersed in a solution containing the analyte and a supporting electrolyte (Skoog & Leary, 1992; Wang, 2006). The supporting electrolyte should be prepared from highly purified reagents and should not be easily oxidized or reduced (hence minimizing potential contamination or background contributions, respectively) (Wang, 2006). The three-electrode system is employed to minimize cell resistance (iR) drop effects with a suitable working electrode or microelectrode, whose potential is varied linearly with time (Bond, 1980; Skoog & Leary, 1992). The second electrode is a reference electrode whose potential remained constant throughout the experiment such as the saturated calomel electrode or the silver/silver chloride electrode (Ag/AgCl) and the third electrode is a counter (auxiliary) electrode which is often a coil of platinum wire or a pool of mercury that serves to conduct electricity from the source through the solution to the working electrode or microelectrode.

Since the reference electrode is placed as close as possible to the working electrode, and because no current passes through the reference electrode, the potential drop caused by the cell resistance (iR) is minimized.



Figure 1.3: Voltammetric electrochemical cell with three electrode system : WE- working electrode; RE- reference electrode; CE- counter electrode (Wang, 2006).

The performance of the voltammetric procedure is strongly influenced by the material of the working electrode. The working electrode should provide high signal-to-noise characteristic, as well as a reproducible response and its selection depends on two important factors which is the redox behavior of the target analyte and the background current over the potential region required for the measurement (Wang, 2006). A wide range of materials can be applied as working electrodes such as mercury, carbon or noble metals.

The microelectrodes used in voltammetry take a variety of shapes and forms. The range of potentials that can be used with these electrodes in aqueous solutions varies and depends upon the electrode material and also on the composition of the solution in which it is immersed (Skoog & Leary, 1992).

Mercury microelectrodes have been widely employed due to its relatively high hydrogen overvoltage that permits a number of ions to be reduced before solvent or proton ions are reduced and greatly extend the negative potential window compared to solid electrode materials. It also possesses a highly reproducible, readily renewable and smooth surface by forming a new drop. Furthermore, many metal ions are reversibly reduced to amalgam at the surface of the mercury electrode, which simplifies the chemistry (Skoog & Leary, 1992). The disadvantages of using mercury as electrode are that its limitation is in the anodic range, due to the oxidation of mercury and its toxicity. Thus, solid electrodes such as glassy carbon, wax-impregnated graphite, platinum or gold are also used due to its wide positive potential range than mercury (Palus, 1990a).

1.5.3 Cyclic Voltammetry (CV) technique

Cyclic voltammetry (CV) has become an important and widely used electroanalytical technique for acquiring qualitative information about electrochemical reactions. CV is perhaps the most effective electroanalytical technique for mechanistic study of redox systems (Heineman & Kissinger, 1984). The versatility of CV results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions and on coupled chemical reactions or adsorption processes (Wang, 2006). CV is often the first experiment performed in an electrochemical study and it offers a rapid location of redox potential of the electroactive species, and evaluation of the effects of media on the redox process (Heineman & Kissinger, 1984; Wang, 2006).

In CV, although the potential scan is usually terminated at the end of the first cycle, depending on the information sought, it can be continued for any number of cycles. Depending upon the composition of the analyte, the direction of the initial scan may be either positive or negative. During the potential scan, the current at the working electrode in an unstirred solution is measured and the resulting current-potential plot is called cyclic voltammogram. The CV is a time-dependent function of a large number of physical and chemical parameters (Wang, 2006). Figure 2.3 illustrates the expected cyclic voltammogram of a reversible redox couple species R_{ed} , which is capable of being oxidized to O_x at the surface of the working electrode as in the following reaction:





Figure 1.4: Typical cyclic voltammogram for a reversible $O_x + n\bar{e} \longrightarrow R_{ed}$ redox process. ($E_{pc} = cathodic peak potential, E_{pa} = anodic peak potential, i_{pc} = cathodic peak current, i_{pa} = anodic peak current)$ (Heineman & Kissinger, 1984).

Based on the literatures from Sawyer and Roberts Jr. (1974), Heineman and Kissinger (1984), Skoog and Leary (1992) and Wang (2006), it is hypothetically assumed that the solution to be 1.0 mM R_{ed} with 1.0 M H_2SO_4 as the supporting electrolyte (Figure 2.3). The initial potential, E_i is imposed at which no electrolysis reaction occurs and is scanned through the standard electrode potential(s) of the species in the solution. It is assumed that only the reduced species, R_{ed} , is present initially. Thus, a positive potential scan is chosen for the first cycle, starting from a value where no oxidation occurs. During the positive potential scan of + 0.25 V to + 0.75 V, the applied potential becomes sufficiently positive at + 0.40 V, causing the oxidation of R_{ed} to O_x occur at the electrode surface resulting in an anodic peak current. After traversing the potential region in which oxidation process occur, the direction of the potential sweep is reversed. During the reverse scan at + 0.75 V to a negative scan, oxidation continues (as is evident by the anodic current) until the applied potential becomes sufficiently negative to cause the reduction of O_x to R_{ed} , resulting in a cathodic peak current.

CV, while not used for routine quantitative analysis, has become increasingly popular for studying redox states. The method allows a wide potential range to be rapidly scanned for reducible or oxidizable species. Perhaps the most useful aspects of CV is its application to the qualitative diagnosis of electrode reactions which are coupled to homogeneous chemical reactions and its ability to generate a species during one scan and then probe its fate with subsequent scans.
1.5.4 Square-wave Voltammetry (SWV)

Square-wave polarography was invented by Barker in the early 1950s with the squarewave potential-waveform consisted of small square-wave potential amplitude superimposed on the slowly changing staircase ramp. Osyteryoungs (Wang, 1985) developed the method of working now known as square-wave voltammetry. According to Osyteryoungs, SWV is a large amplitude differential technique in which a waveform composed of a symmetric squarewave, superimposed on a base staircase potential is applied to the working electrode (Wang, 2006). Figure 2.4 shows the square-wave excitation signal. In the excitation signal, the square-wave amplitude (E_{sw}) is the square-wave pulse height, and the square-wave potential step (ΔE) is the staircase step height, both in millivolts. The duration of a potential step is identical with the period of the square-wave pulses (τ). The frequency is in cycle/time (Hz) and usually varies up to 250 Hz.



Figure 1.5: Square-wave waveform showing the amplitude (Esw), step height (ΔE), square-wave period (τ), delay time (Td) and current measurements times (1 and 2). (Kissinger, 1984).

The waveform (Figure 2.4) in SWV is composed of symmetrical square-wave pulses superimposed on a base staircase potential. The current is sampled twice, during each square-wave cycle, at the end of each pulse, once at the end of the forward pulse (1), and once at the end of the reverse pulse (2). The net current (i_{net}) is obtained by taking the differences between the forward and reverse currents. The difference between the two measured current values is plotted versus the base staircase potential, resulting in a bell-shaped current-potential curve as shown in Figure 2.5.



Figure 1.6: Square-wave voltammogram for reversible electron transfer; (A) forward current ; (B) reverse current ; (C) net current (from Kissinger, 1984).

In the resulted voltammogram, the peak current is proportional to the concentration of the electroactive species. Excellent sensitivity accrues from the fact that the net current is larger than either the forward or the reverse components (since it is the difference between them) (Wang, 2006). By sampling the current just before the square-wave changes its polarity, the charging current due to electrical charging of electrode double layer can be eliminated effectively. SWV is among the most sensitive tools for metal speciation in both qualitative and quantitative analysis. Square-wave voltammetry has several advantages such as its sensitivity is higher than differential pulse technique and the ability to eliminate the background currents. The major advantage of this technique is its speed (Wang, 2006). Due to its extremely fast potential scan rates, the analysis time is reduced and a complete voltammogram can be obtained in a few seconds, compared to differential technique. Coupled with the effective discrimination against the charging current, very low detection limit near 1 x 10^{-8} M can be attained (Wang, 2006). SWV does have high information content especially from the voltammograms of the forward and reverse currents, and it has the ability to provide information of electrode processes over a wide potential span (Bard & Faulkner, 2001).

1.5.5 Stripping analysis

An electrochemical stripping analysis is a two-step electroanalysis technique. During the first step, the analyte is deposited (preconcentrated) into or onto the surface of an electrode, where as, in the second step, the deposited analyte normally mercury is removed ("stripped") from the electrode, and the accompanying electrochemical signal is used to determine the concentration of each analyte species present in the sample (Heineman *et al.*, 1984).

The enrichment electrolysis (preconcentration process) consists of the reduction or oxidation of the metal ions to free metal that are deposited into or onto the surface of the working electrode such as mercury electrode. If the enrichment electrolysis, is carried out under suitable conditions, the enriched electrode surface will contain a higher concentration of the element under investigation than was to be found in the original aqueous sample solution (Neeb, 1989). After the pre-concentration process, the determination of the analyte may be carried out in principle with any voltammetric or polarographic techniques with different waveforms.

(a) Anodic Stripping Voltammetry (ASV)

Anodic stripping voltammetry (ASV) is the stripping variation used primarily for the determination of heavy metals (Heineman *et al.*, 1984). In this technique, the preconcentration (deposition) step is caused by reduction of the metal ions in the solution at negative potential to the elemental state and concentrated into or onto a mercury electrode.

 M^{n+} + $ne^ \longrightarrow$ $M^{o}(Hg)$ enrichment/deposition/preconcentration step

The stripping step is accomplished by scanning the potential in the positive direction linearly or in other potential waveform and when the potential reaches the standard potential (E°) , of the metal ion couple, the preconcentrated metals or amalgamated metals are reoxidized back into the solution as ionic form producing anodic current.

 $M^{o}(Hg) \longrightarrow M^{n+} + ne^{-}$ stripping step determination

The current-potential voltammogram recorded during the stripping step provides the desired analytical information of interest. The peak potential position, E_p , of each metal is a characteristic of that metal and is related to the standard potential of its redox couple (Wang, 1990). The peak current (i_p), is proportional to the metal concentration in the test solution.

Therefore, it can be used for qualitative identification. This ASV technique is applicable to metal ions that can be preconcentrated at an electrode by reduction and is effective especially for metals that dissolve in mercury (Heineman *et al.*, 1984). Besides mercury electrodes, other solid electrodes such as wax-impregnated graphite and glassy carbon also can be used.

(b) Cathodic Stripping Voltammetry (CSV)

Cathodic stripping voltammetry (CSV) is the "mirror image" of anodic stripping voltammetry (Wang, 1990). It involves preconcentration by oxidation of the analyte as an insoluble film on the electrode, followed by stripping in a negative direction potential scan in which the concentrated analyte is being reduced and measured. Unlike ASV, the analyte or anions may be determined by deposition as an insoluble film of the mercury salt on the working electrode surface according to the following general reaction (Heineman *et al.*, 1984; Wang, 1990):

Hg + X^{n-} \longrightarrow HgX + ne⁻ enrichment/deposition/preconcentration step

where the stoichiometry depends on what X^{n-} is. In the stripping step, the negative direction potential scan causes the reduction of the salt to Hg and X^{n-} , giving a cathodic current peak (Heineman *et al.*, 1984). The stripping step involves the following reaction:

HgX + ne⁻ \longrightarrow Hg + Xⁿ⁻ stripping step determination

The desired quantitative information is accomplished by measuring the height of the resulting reduction peak current and it is proportional to the concentration of the anion in the solution. Thus, anions may be determined by CSV in a similar way that metal ions are determined with ASV (Heineman *et al.*, 1984). Besides that, CSV is used for determination of a wide range of organic and inorganic compounds, capable of forming insoluble salt with mercury.

With CSV, even the determination in cathodic direction takes place in a reductive deposition process, for example, the determination of As^{3+} by enrichment electrolysis, yielding As° , which is deposited on the mercury (Hg) electrode as Cu_3As_2 in the presence of Cu(II) ions (Neeb, 1989). The cathodic determination step occurs with the formation of As^{3-} with AsH_3 :

Enrichment :
$$As^{3+} + e^- + Cu^{2+} \longrightarrow Cu_3As_2$$

Determination : $Cu_3As_2 \longrightarrow AsH_3 + Cu^{2+} + e^-$ (CSV)

1.6 Limitations of arsenic determination

Like many other speciation techniques, the determination of arsenic by cathodic stripping voltammetry is subjected to interferences which may affect its precision and accuracy. An important interference in the electrochemical speciation technique is the presence of organic compounds, particularly surface active substances during the determination of heavy metals in various environmental samples (Wang, 1985). The adsorption of the organic matter on the mercury electrode will form an organic layer which may hinder the diffusion of the metal ions and cause a non linear relationship between the peak current and deposition time. Besides that, trace elements that can exist in natural waters especially the trace element that may deposit on hanging mercury drop electrode (HMDE) or compete with arsenic in its reaction with copper or even in the formation of other compounds with arsenic can also cause interference on the determination of arsenic in natural waters.

One of the main limitations of electrochemical speciation methods is the inability to measure the concentrations of individual ionic species (Florence, 1989). Direct electrochemical speciation techniques are limited to measuring gross behavioral differences of group of species which applies to the labile/inert discrimination. It is then impossible to use cathodic stripping voltammetry (CSV) to distinguish between labile arsenic(III) species such as $H_2AsO_3^-$, $HAsO_3^-$ or monomethylarsinate (MMA(III)) which may coexist in the water sample, where as other group of species such as inorganic arsenic As(V) can only be determined after the sample underwent chemical treatment. It is important to choose the suitable chemical treatment because certain reducing step for As(V) involves heating of the solution for complete conversion of As(V) to As(III) and the results are inaccurate and not precise due to losses of volatile compounds containing arsenic (Ferreira & Barros, 2002). Not only that, certain excess reductant may interferes with the determination of As(V).

The formation of intermetallic compounds between metals deposited in the mercury electrode or between the electrode material and a deposited material may cause peak depression or peak shifting of the constituent metals (Wang, 1985). These interferences could be minimized or eliminated by reducing the amounts of metals incorporated into the mercury

electrode, use of the differential pulse waveform or other sensitive stripping modes. According to the study by He *et al.* (2004), arsenic requires a preconcentration step in the presence of Copper(II) or Selenium(IV) to form an intermetallic compound at the mercury electrode and then stripped cathodically. In this study, a suitable amount of Cu(II) and Se(IV) is required in order to obtain a sharp and symmetric arsenic peak.

The performance of the voltammetric procedure is greatly influenced by the choice of the working electrode material. The ideal working electrode should have a favourable electrochemical behaviour of the analyte(s) of interest, a reproducible surface area and low background current (Wang, 1985). Eventhough the HMDE offers simplicity, economy and reproducibility (Bond, 1980). There are two disadvantages that are associated with the HMDE.

One basic disadvantage is that it has a low surface area to volume ratio (Bond, 1980; Wang, 1985). The relatively small surface area reduces the efficiency of the preelectrolysis step, while the large volume means that a finite time is required for metals dissolved in the mercury to diffuse from the drop interior and causes broadening of the stripping peaks, thereby causing the loss of selectivity (Bond, 1980). Another disadvantage of HMDE is that only low stirring rates of the solution may be employed to avoid dislodging the drop (Bond, 1980; Wang, 1985). This again causes restriction on the efficiency of the deposition step in which longer preelectrolysis times are required if faster stirring rates could be employed. Due to the relatively large volume of the HMDE, a rest period between the deposition step and stirring steps is required to allow the amalgam concentration to become more homogeneous.

1.7 Research objectives

Since it was reported in the literatures that square wave voltage modulation is more sensitive and much faster than either linear scan or differential pulse (Li & Smart, 1996; Skoog & Leary, 1992; Barra & dos Santos, 2001), square wave pulse was used in the stripping step in this experiment. The objectives of this research is to optimize the condition for arsenic(III) and total inorganic arsenic [As(III) + As(V)] determination by square wave cathodic stripping voltammetry (SWCSV). Parameters that effect the peak current of As(III) such as Cu(II) concentration, deposition potential, deposition time, pulse amplitude and frequency were studied in detail.

In general, the determination of total inorganic arsenic [As(III) + As(V)] requires a preliminary step to reduce As(V) to As(III) and a proper reducing agent is required for its reduction prior determination. Although different types of reductant have been used for the reduction of As(V) such as mannitol, sodium dithionite, sodium metabisulfite, potassium iodide and sodium thiosulfate, the reducing step of As(V) is not simple and often there are problems associated with the reducing agent used. Ferreira and Barros (2002) found that sodium thiosulfate is a convenient reductant for As(V) which allowed fast reduction even at room temperature. Thus, the reduction of As(V) in this experiment were carried out using sodium thiosulfate. This research also investigate the optimum concentration of sodium thiosulfate required and its efficiency in the reduction of As(V). Instrumental parameters that effect the peak current such as Cu(II) concentration, deposition potential, and deposition time were also investigated and optimized.

Not only that, this research also investigate the effect of interference of trace element that may exist in natural waters such as Zn^{2+} , Fe^{3+} and Cd^{2+} on the peak current of arsenic. The linearity range, detection limits, precision and accuracy in arsenic determination were also investigated. The optimum results were then applied for the determination of arsenic in the environmental samples collected form Tasik Biru, Bau.

1.8 Scope of study

This study was based on the preconcentration of As(III) in the presence of Cu(II) to form intermetallic compound at the hanging mercury electrode (HMDE) mode by controlled potential electrolysis. As(0) was then subsequently strip by applying a cathodic potential scan, where it was reduced to -3 oxidation state. The total inorganic arsenic [As(III)+As(V)] was obtained by reducing As(V) to As(III) using thiosulfate as the reducing agent and As(V) was then obtained by the difference. The supporting electrolyte used in this study was 2 M hydrochloric acid (HCl).

The parameters and conditions that influence the efficiency of the procedure were optimized. Since arsenic has to be coprecipitated with Cu(II) to form intermetallic compound at HMDE mode, the concentration of Cu(II) that may influence the deposition of arsenic was investigated. As(V) is an inelectroactive form of arsenic and a reducing agent was required in order to reduce it to electroactive As(III). The instrumental conditions that affect the analysis of arsenic such as deposition potential, deposition time, and pulse amplitude were also investigated to optimize the condition for arsenic determination.

The procedure for measuring both As(III) and total inorganic arsenic was examined via evaluation of the linearity range, limit of detection (LOD), within-run (repeatability) and between-run (reproducibility) precisions and accuracy of the method. Then, the optimized method is applied to the determination of inorganic As(III) and As(V) and also total inorganic arsenic [As(III) & As(V)] in the environmental samples collected from Tasik Biru, Bau as this area was previously a gold mining area and was reported to be contaminated with high concentration of arsenic.

Chapter 2

LITERATURE REVIEW

2.1 Theory on Arsenic

2.1.1 Chemical Properties

Arsenic was first discovered by a German chemist, Albertus Magnus in 1193-1280 (Goessler & Kuehnelt, 2002). Arsenic is represented by the symbol As and has the atomic number and atomic weight of 33 and 74.92160 g/mol respectively. Elemental arsenic has many allotropic forms such as yellow, gray and black arsenic (Francesconi *et al.*, 1994). When heated in air, the elemental arsenic oxidizes and burns with a bluish flame and it produces an odor of garlic and dense white fumes of As_2O_3 (Francesconi *et al.*, 1994). Arsenic forms various types of inorganic and organic compounds in the trivalent and pentavalent states and the toxicity of the compounds varies widely. Arsenic occurs in the natural environment in four oxidation states namely As(V), As(III), As(0) and As(-III) (Hung *et al.*, 2004). The pentavalent arsenate [As(V)] is the most stable form/species in inorganic arsenic.

Arsenic shares many chemical properties with its nearest neighbours in the group, phosphorus and antimony. It forms colourless, odourless crystalline oxides As_2O_3 and As_2O_5 which are readily soluble in water to form acidic solutions. Like phosphoric acid, arsenic(V) acid is a weak acid. Arsenic also forms an unstable, gaseous hydride that is arsine (AsH₃) similarly like phosphorus. Both arsenite and arsenate can be easily interconvert in solution by using oxidizing or reducing agent such as potassium iodide and potassium permanganate. Arsenite readily form complexes with sulfur compounds (e.g., ammonium pyrrolidine dithiocarbamate) and this property can be used to separate arsenite from other forms of arsenic, where as arsenate forms a complex with molybdic acid that can be reduced to formed blue colour that is suitable for colorimetric analysis (Francesconi *et al.*, 1994). Besides that, both arsenite and arsenate can be coprecipitated with hydrated ferric hydroxide. Not only that, under reducing conditions, organoarsenic compounds may produce arsines which is useful in analytical works. The acidic or basic properties of arsenic compounds greatly influence their environmental behavior and analysis.

Arsine also known as arsenic trihydride is produced by the hydrolysis of metal arsenides and by the reduction of metals of arsenic compounds in acidic solutions, has been detected in landfill gases, in samples collected from hot spring environment and in gases from anaerobic wastewater treatment facilities (Feldmann *et al.*, 1994; Koch *et al.*, 1999; Michalke *et al.*, 2000). Arsenobetaine is an organic arsenic compound and is the major form of arsenic compound in marine animals (Francesconi & Kuehnelt, 2002). Although arsenobetaine is the major form of arsenic in marine animals, it has not been detected in seawater or marine algae, freshwater samples or freshwater algae and plants but it is found in low concentrations in fresh water animals such as fish and probably mussels.

The world's ocean contain about 0.5-2.0 µg As/L, although higher concentration can occur in estuaries, particularly those receiving anthropogenic discharges (Cullen & Reimer, 1989). In lake water, trivalent monomethylated and dimethylated arsenic species have also been discovered. In air, the major sources of arsenic are metal smelters, the burning of coals

and volcanoes (Léonard, 1991). The main form of arsenic compound released by these processes is arsenic trioxide (As_2O_3). Recent work has revealed the presence of arsine and methylated arsines (MeAsH₂, Me₂AsH and Me₃As) as trace compounds of air samples, especially over sites where biological activity is high (Francesconi & Kuehnelt, 2002).

2.2 Distribution of Arsenic and its species

Arsenic is a commonly occurring toxic metal in the environment. The occurrence of elemental arsenic in natural waters and soils can pose a hazard in humans and animals. Arsenic is introduced into the environment through geochemical and anthropogenic sources. The level of arsenic may be much higher in soils and waters contaminated by human activities such as the use of arsenical pesticides, application of fertilizers, irrigation, dust from the burning of fossil fuels and disposal of industrial and animal wastes (Sandberg & Alllen, 1975).

2.2.1 Soils

Uncontaminated soils typically contain 5-10 mg/kg of arsenic (Henke, 2009), but soils that are contaminated with human activities such as soils that are repeatedly treated with arsenical pesticides, herbicides and defoliants and soils that are in the vicinity of smelters contain levels of arsenic often 10-100 fold that those of untreated areas (Walsh *et al.*, 1977). The anthropogenic influence on the level of arsenic in soils depends on the intensity of the human activity, the distance from the pollution sources and the pollutant dispersion pattern.

In most soils, arsenic occurs mainly as inorganic species and is more prevalent than organoarsenicals (Henke, 2009). Inorganic arsenic may be converted to organoarsenic compounds by soil microorganisms (Chu, 1994). Takamatsu *et al.* (1982) examined the amount of arsenic compounds in soils polluted with arsenic in Japan and found that arsenate was the major compound, while lower levels of dimethylarsonate and monomethylarsonate were also detected.

Under strong reducing conditions, arsenite dominates in soils although elemental arsenic and arsine can also be present (Bhumbla & Keefer, 1994). In less reduced environments such as in flooded soils, arsenite are formed, where as, in aerated soils, arsenate predominates. In both soil and water system, arsenic species are subjected to chemically and microbiologically oxidation and reduction (Walsh *et al.*, 1977). Besides that, other arsenic reactions such as adsorption, dissolution, precipitation, methylation followed by volatilization and mineralization also commonly occur in which some reaction are associated with bacterial and fungal microorganisms. Arsenite added to a well drained soil is biologically oxidized in a few days (Quastel & Scholefield, 1953) and arsenate in reduced environments such as sediments is reduced through arsenite to volatile organic arsine which is extremely toxic.

2.2.2 Natural waters

The concentration of arsenic in natural waters is large, ranging less than 0.5 μ g/L to more than 5000 μ g/L. The typical concentrations of arsenic in fresh water are less than 10 μ g/L, frequently less than 1 μ g/L. In fresh water system, the concentrations of arsenic vary depending on the source of arsenic, the amount available and local geochemical environment (Smedley & Kinniburgh, 2002). Under natural conditions, the highest concentration of arsenic are found in groundwater as a result of the strong influence of water rock interactions and the tendency in aquifers for the physical and geochemical conditions to be favourable for arsenic mobilization and accumulation.

In river waters, the concentrations of arsenic are low usually in the region of 0.1 - 0.8 µg/L depending on many factors including the composition of any underwater sediments, rocks, and any soils, sediments and rocks surrounding the surface waters, input from geothermal sources or high arsenic groundwaters, any contamination from humans and biological activity, which may reduce and methylate arsenic (Henke, 2009). In natural waters, dissolved arsenic can occur in inorganic and organic forms. From geological sources, arsenic is found in two valency states which is salts of the arsenic acid (H₃AsO₄: As(V)) and the arsenous acid (H₃AsO₃: As(III)) (Jekel, 1994). Methylated arsenic compounds like monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) are present in natural waters due to anthropogenic sources although the organic arsenicals presence are also produced by methylation reactions of inorganic arsenic salts catalysed by microbial activity (Smedley & Kinniburgh, 2002). Arsenate is the predominant species in natural waters and is in equilibrium with the reduced form, arsenite (Andreae, 1979). Arsenic concentrations in seawater are usually 1.1 – 1.9 µg/L with an average of 1.7 µg/L (Henke, 2009).

Unless they are associated with volcanic deposits, geothermal fluids or mining sites and other area impacted by human activity, arsenic concentrations in lakes and their sediments are usually similar to uncontaminated streams that are less than 1 μ g/L and less than 10 mg/kg respectively (Smedley & Kinniburgh, 2002). In lakes and river waters, As(V) is the dominant species though significant seasonal variation in concentration have been found (Henke, 2009). The concentration and relative proportions of As(V) and As(III) in the lake water vary depending on factors such as changes in input sources, redox conditions and biological activity.

Concentrations of arsenic in groundwater are less than 10 µg/L and sometimes substantially lower under natural conditions. High concentrations of arsenic are found in groundwater in a variety of environments which includes oxidizing (under conditions of high pH) and reducing aquifers and in areas affected by mining, geothermal and industrial activity (Smedley & Kinniburgh, 2002). Besides that, most high arsenic groundwater provinces are the result of natural occurrences of arsenic.

2.3 Analytical procedures for Arsenic speciation and determination

Speciation analysis is defined as the determination of the concentrations of different physicochemical forms of an element that together make up its total concentration in the sample (Florence, 1989). All of the species can coexist and may or may not be in thermodynamic equilibrium with one another. Generally, the two main reasons for studying the speciation of elements namely to study the biological or the geochemical cycling of the elements and to measure the toxicity of the metal ion in its different physicochemical forms (Morrison, 1989; Bott, 1995). Biological cycling includes bioaccumulation, bioconcentration, bioavailability, and toxicity and geochemical cycling involves the transport, adsorption and precipitation of the element. Each different physicochemical form of an element has a different toxicity and it is important to identify those metal species which are likely to have adverse effects on biota and includes measurements both of bioavailability and toxicity.

Arsenic forms a vast range of compounds both inorganic and organic in the trivalent and pentavalent states (Francesconi *et al.*, 1994). It is important to determine these individual arsenic species because these species exhibit different toxicity, biochemical and environmental behaviours. Various analytical methods have been developed for the determination of total arsenic and its species in different matrices. The most widely used speciation techniques involve a combination of chromatographic with spectrometric detection although electrochemical techniques have been increasingly used in a number of researches.

2.4 Other Determination Methods of As

2.4.1 Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrometry (AAS) for analysis of arsenic using flame has never seriously been considered as a viable technique for the determination of arsenic in environment samples. This is because this technique suffers from flame interferences and low sensitivity which causes high detection limit (~1 mg As/L). However, the combination of atomic absorption spectrometry and hydride generation technique is widely used and a reliable method for then analysis of arsenic in environmental samples. The use of electrothermal atomic absorption spectrometry (ETAAS) or graphite furnace atomic absorption spectrometry (GFAAS) in the determination of arsenic in the environmental samples was hampered by loss of arsenic during the ashing stage where arsenic sublimes at 613°C. It also showed that different arsenic compounds show large differences in the signal intensities. These problems were overcome by the use of matrix modifiers which prevented loss of arsenic by volatilization during ashing stage and give uniform signal intensities irrespective of the arsenic compounds present in the sample.

Methods have been developed to overcome matrix problems inherent in the determination of arsenic by GFAAS that is to clean up the sample prior to analysis. With careful control of resin and elution conditions, the separation of As(III), As(V), MAA and DMAA in a form suitable for GFAAS analysis is allow. GFAAS or ETAAS technique is based on the absorption of free atoms produced from the sample deposited in a small graphite tube which can be heated at high temperatures. Zhang *et al.* (2004) proposed a method for the differential determination of trace amount of As(III) and As(V) in water samples based on the coprecipitation with Ni-ammonium pyrrolidine dithiocarbamate (APDC) complex at pH 2-3 and measured by ETAAS using solid sampling technique. It was discovered that As(III) was quantitatively coprecipitated with Ni-PDC complex but As(V) was hardly coprecipitated under the same condition. The proposed method was successfully applied for the determination of arsenic in seawater with detection limit of 0.02 ng/mL. It was reported that a successful ashing and atomization conditions should be established in order to obtain the reproducible and accurate results by ETAAS using solid sampling technique.

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 ETAAS. In this method, the analyte is converted into hydrophobic species, which are retained on a membrane filter and further material collected with the membrane filter is dissolved in

organic solvent or sulphuric acid. Then, the sample is pre-concentrated and analyzed with ETAAS. This simple and rapid method had been successfully applied in river water sample analysis. Hence, electrothermal atomic absorption spectrometry is certainly a method for determination of arsenic at trace concentration levels but caution must be taken as arsenic might be lost during ashing and matrix interferences may occur.

2.4.2 Inductive Coupled Plasma-Mass Spectrometry (ICP-MS)

Inductively coupled mass spectrometry (ICP-MS) is a new and very sensitive method of elemental determination. It uses high temperature plasma as an ionization source. The technique has been applied to the analysis of wide range of elements, including arsenic in marine samples. In the plasma, usually at argon plasma, at temperature 7500 K, arsenic species are completely decomposed, atomized and partially ionized to ⁷⁵As⁺ ion which can then be detected by quadrapole mass spectrometry. Quadrapole mass filters are the most common mass analyzers because it is rather inexpensive. In pure water, the detection limit of arsenic may be as low as several mg/L.

However, there are difficulties in determining arsenic in marine waters because the ⁷⁵As⁺ ion suffers from interference by the molecular ion ⁴⁰As³⁵Cl⁺ formed by combination of the plasma gas and chloride ion overlapping the arsenic signal. Thus, pretreatment methods may be required to separate the arsenic species from the salt matrix before ICP-MS. Few strategies have been developed to overcome this problem such as mathematical correction, addition of nitrogen to the carrier gas, addition of ethanol to the samples, addition of tertiary

amines and chromatographic separation. When nitrogen gas is added to carrier argon gas, nitrogen gas reduces the formation of interfering ArCl⁺ ion to a negligible level.

ICP-MS technique is one of the most widely applied techniques for arsenic detection. However, the determination of low concentrations of arsenic in real samples using ICP-MS suffers from low sensitivity due to the poor ionization efficiency in ICP-MS. In order to overcome this problem, ICP-MS applications are coupled with several other systems which include hydride generation, high performance liquid chromatography (HPLC) and ion exchange chromatography. Feng *et al.* (1998) have developed a method by using hydride generation high resolution ICP-MS for direct determination of arsenic species in environmental water. High resolution ICP-MS can separate two very near peaks such as ⁷⁵As and ³⁸Ar³⁷Cl or ⁴⁰Ar³⁵Cl effectively.

A coupled system including HPLC and ICP-MS gives a suitable method for the determination of non-volatile species such as arsenic. Although ICP-MS has multi-element capability, this coupled method has mainly been used for speciation of single elements. Demesmay *et al.* (1994) used an ICP-MS detector coupled with an HPLC system to determine arsenate, arsenite, monomethylarsonate, arsenobetaine, dimethylarsinate and arsenocholine. The interface is established through PTFE capillary tubing and an anion-exchange column with a mobile phase of phosphate buffer with 2% acetonitrile was used. An ionic-strength step gradient is necessary to reach an optimum separation of the six arsenic species.

2.5 Electrochemical techniques

There are several types of electrochemical methods that can be used for the determination of arsenic at trace levels. The difference in the electrochemical behavior of As(III) and As(V) where As(III) is electroactive and As(V) is electroinactive enables electrochemical techniques to be used for the analysis of the two inorganic forms of arsenic (Francesconi *et al.*, 1994). Electrochemical techniques that are commonly used are differential pulse polarography and voltammetric methods, ASV and CSV. The polarographic methods are not sensitive for the determination of arsenic at extremely small concentration, where as, the stripping voltammetry are more suitable for trace determination in real samples because the arsenic is pre-concentrated on the working electrode (Kumaresan & Riyazuddin, 2001).

Cathodic stripping analysis of arsenic using HMDE is based on arsenic preconcentration in highly acidic media and after that scanning in cathodic direction to obtain peak due to the formation of arsine (Li & Smart, 1996). To increase the sensitivity, the intermetallic complexes of arsenic, where by, As(III) reacts with copper or selenium to form intermetallic complexes, which can be stripped cathodically or anodically from the working electrode. The detection limit for arsenite with differential pulse polarography is about 20 μ g/l where the detection limit for ASV is the lowest that is at 0.02 μ g/l. It is impossible that such low detection limits would be achieved with environmental samples because the electrochemical methods suffer from interferences (Francesconi *et al.*, 1994). Simple measurements are only possible if the matrix is completely eliminated either by chromatographic separation or by complete mineralization (Greschonig *et al.*, 1992).

2.5.1 Stripping analysis

In the past, arsenic has been determined by several electrochemical techniques such as differential pulse polarography technique (DPP), stripping voltammetry such as ASV and CSV. Besides that, voltammetric stripping analysis has been found to have the lowest detection limit of any commonly used electroanalytical techniques (Heineman *et al.*, 1984). The high sensitivity and low cost of stripping analysis have led to its application in number of analytical problems, for instance in the determination of various trace metals in environmental and clinical samples, as well as for analysis of foodstuffs, gunshot residues, beverages or pharmaceutical products (Wang, 1985).

(a) Anodic Stripping Voltammetry (ASV)

ASV is the most widely used electroanalytical method for trace element speciation in natural waters (Bond, 1980). Forsberg *et al.* (1975) investigated in detail the determination of arsenic by ASV and DPASV at various electrodes such as HMDE, Pt and gold. In this investigation, it was found that the arsenic oxidation peak appeared as a shoulder on the mercury oxidation wave on a HMDE. Gold was found to be superior to platinum as a working electrode material due to higher hydrogen overvoltage. Anodic stripping voltammetry is suitable for the determination of arsenic especially when gold or gold plated carbon electrodes are used as working electrodes (Kopanica & Novotonỳ, 1998). This is because problems such as 'memory' effects, limited sensitivity and poor precision are often associated with ASV at solid electrodes which make them inconvenient for routine analysis.

Forsberg *et al.* (1975) investigated the determination of arsenic using ASV and DPASV. In this research, gold and platinum working electrodes were compared in a variety of electrolyte concentration. It is indicated that the sensitivity increased with increasing acid concentration and 1M solutions of either hydrochloric or perchloric acids were most suitable as supporting electrolytes. Gold electrode was found to be superior to platinum as a working electrode material. In the determination of total arsenic in water samples, As(V) was reduced to As(III) by heating As(V) with sodium sulphite in concentrated acid solutions. The calculated detection limit for both ASV and DPASV were the same at 0.02 ng/mL in 1M HClO₄.

The determination of arsenic(III) by differential pulse anodic stripping voltammetry (DPASV) in aqueous solutions using disc gold electrode were reported by Kopanica and Novotnỳ (1998). In this research, 1 M H₂SO₄ and 0.1 M HCl are used as the supporting electrolyte. Though the presence of HCl is found necessary for arsenic determination, however, its concentration should not exceed 0.15 M. The linearity between As(III) concentration and the anodic peak current was obtained in a wide concentration range (between 0.2 and 250 μ g/L), when varied electrolysis times are applied. The detection limit of 0.15 μ g/L was obtained for electrolysis time of 300 s.

Conventionally, As(III) is electrochemically reducible to the element while As(V) is not reducible electrochemically. Most approaches reduced As(V) to As(III) by a chemical reductant such as iodide, mannitol and sulfur dioxide prior to the electrochemical detection of total arsenic. Huang and Dasgupta (1999) published a paper regarding inexpensive voltammetric equipment that utilizes anodic stripping voltammetry with renewable gold film electrode for the measurement of As(III) and As(V) in water. HCl is used because arsenic oxidation and reduction is reversible in higher concentration of HCl. As(III) and As(V) can be deposited onto a gold film electrode at potentials of -0.2 V and -1.6 V respectively then subsequently stripped using DPASV. The limit of detection for As(III) or As(V) is calculated to be 0.5 µg/L. While the direct determination of As(V) at sufficiently negative potentials is possible without a prior chemical reduction to As(III), there are kinetic barriers to the reduction step which makes it a less efficient than the reduction of As(III). This warrants the oxidation step where all of As(III) must be converted to As(V) by an oxidant before the total As determination step.

Kamenev *et al.* (2001) studied As(III) at graphite electrodes modified with gold and copper. The surface of the graphite electrode was modified with copper and gold to significantly enhanced the efficiency of arsenic electrodeposition. Intermetallic compound was formed at the electrode modified with copper. The addition of As(III) in solution containing 0.1 M H₂SO₄ and Cu(II) resulted in a gradual decrease and distorted As(III) and Cu(II) peaks, where as, As(III) peaks were higher in solution containing Cu(II) and EDTA. The complexing agent was used to mask the effect of Cu(II) dissolution on arsenic stripping. Arsenic is best deposited at a potential of -0.7 and -0.8V using graphite electrodes modified with copper and gold in 0.1 M HCl and 0.05 M EDTA supporting electrolytes, respectively. The detection limits for arsenic(III) determined with graphite electrodes modified with copper and gold were 47 µg/L and 17µg/L, respectively.

(b) Cathodic Stripping Voltammetry (CSV)

The problems that are associated with the determination of arsenic using ASV method can be overcome by using CSV method that allows the determination of arsenic at parts-perbillion levels. According to Myers and Osteryoung (1973), the electrochemical reduction of As(III) at mercury electrodes proceeds in two steps in acidic medium which is the reduction of As(+3) to arsenic(0) and to arsine, As(-3). Although HMDE is not analytically useful for the determination of arsenic using ASV due to interference from the oxidation of mercury, most cathodic stripping techniques are carried out by using a HMDE, as this electrode does not suffer from the disadvantages of the solid electrodes (Hung *et al.*, 2004).

CSV procedures have been developed for the determination of As(III). Since As(III) does not form amalgam with HMDE due to its insolubility towards mercury, the accumulation prior to the scan is effected with the formation of intermetallic compound with copper or selenium or by adsorption of an arsenic-complex on the surface of the HMDE and then stripped cathodically (Greulach & Henze, 1995; Ferreira & Barros, 2002). When both oxidation states, As(III) and As(V) are to be determined, the method proposed for the determination of both oxidation states consists on the determination of As(III), followed by the reduction of As(V) to As(III) and determination of total arsenic. Arsenic(V) is obtained by difference.

Sadana (1983) proposed differential pulse CSV for the determination of arsenic in drinking water in the presence of Cu(II) using HMDE. The optimized conditions for arsenic determination are 0.75 M HCl, $5 \pm 1 \mu g/mL$ Cu(II) concentration and -0.6 V deposition

potential. For the determination of total arsenic, As(V) is first reduced to As(III) by heating the sample with concentrated HCL and 48 % HBr in a steam bath set at 95 – 100 °C for 45 minutes. The mixture is then cooled to room temperature and diluted with 0.25 % (w/v) hydrazinium chloride solution before determination with CSV. This reduction process is found to be tedious and inconvenient. Not only that, some of the volatile compounds of arsenic may be lost due to long period of heating procedure. The detection limit of this method is 1 ng/mL.

Greulach and Henze (1995) developed a procedure for the analysis of arsenic(V) by DPCSV. This procedure is based on the reduction of As(V) in a mannitol and perchlorate containing solution, the coprecipitation with copper and the voltammetric determination by further reduction to AsH₃ at the HMDE. Preliminary investigations showed that mannitol is suitable for As(V) reduction instead of phenols because it does not have the strong adsorption tendencies on the surface of the mercury drop. Supporting electrolyte containing 2 M NaClO₄, 0.5 M mannitol, 0.3 M NaCl and 2 mM CuSO₄ and accumulation potential of - 0.55 V is chosen for the determination of As(V). During the accumulation process, As(III) is produced by the electrochemical reduction of As(V) which will form an intermetallic compound with copper on the HMDE. Since significant interference occurred in solution with mixture of As(III) and As(V), traces of As(III) in natural waters can be quantitatively oxidized by using UV irradiation. The detection limit has been found to be 4.4 µg/L for 60 s accumulation period.

Applying the same method, Henze *et al.* (1997) published a paper on the speciation of As(III) and As(V) in fresh water samples by DPCSV using HMDE and glassy carbon as the

working and auxiliary electrode respectively. All of the experiments were carried out in a mannitol-sulphuric acid medium. As(III) is determined first in the acidic supporting electrolyte without the addition of mannitol by coprecipitating with Cu(II) and Se(IV) and reduced to arsine at the HMDE. For the total arsenic determination, both As(III) and As(V) are accumulated onto the surface of the mercury drop electrode resulting in the peak current that corresponds to both species. The limitation for the total arsenic determination is that the response of As(III) is higher than that of As(V), thus necessitates the oxidation of As(III) to As(V) prior accumulation step. As(III) is oxidized to As(V) by UV-irradiation during pretreatment of the water sample which also serves to destroy organic substances that may cause interferences. The detection limit has found to be 0.52 μ g/L at accumulation time of 240 s.

A simple, fast and sensitive speciation method for inorganic arsenic in water at the μ g/L based on DPCSV at HMDE was developed by He *et al.* (2004). In this procedure, As(III) is deposited as Cu_xSe_yAs_z on HMDE in 1 M HCl supporting electrolyte containing Cu(II) and Se(IV). In the presence of Cu(II), Se(IV) was reported to interfere with the arsenic signal and thus only traces of Se(IV) was added to improve the peak current. Determination of total As was performed by reducing As(V) to As(III) using sodium meta-bisulfite/sodium thiosulfate reagent. As(V) is quantified by difference. The major drawback of this reducing agent is that it deteriorates fast in voltammetric applications thus require the addition of ascorbic acid which serve as an anti-oxidant which stabilizes the reducing agent up to 100 hours. The detection limit for both As(III) and As(V) is 0.5 µg/L with linearity range from 4.5 µg/L to 180 µg/L at deposition time of 60 s.

Li and Smart (1996) have published a paper on the determination of As(III) in natural waters using SWCSV at HMDE. Preconcentration was carried out in 2 M HCl in the presence of 0.8 M CuCl₂. During deposition time, the solution was stirred and purged with nitrogen gas. Hydrazine was added into the solution to reduce or combine with mercuric ions in order to prevent them from reacting with As(III) and to stabilizes the peak current. The intermetallic compound was reduced at negative potentials and the detection limit is approximately 0.06 nM (5 ppt). Besides that, the relative standard deviation was calculated at 8 % (n = 11) at 1 x 10⁻⁹ M for 1 min accumulation time.

Ferreira and Barros (2002) and Profumo *et al.* (2005) presented a method for the speciation of inorganic arsenic in natural waters using SWCSV at HMDE. The preconcentration of both As(III) and As(V) was carried out in 1 M HCl in the presence of Cu(II). Chloride plays an important role in the process of deposition of an intermetallic compound, Cu_xAs_y , probably through stabilization of the Cu(I) formed at the HMDE by complex formation with chloride. The reduction of As(V) to As(III) was carried out in the solution in room temperature using sodium thiosulfate. The total arsenic was determined as As(III) + As(V), while As(III) was measured without the addition of reductant. The detection limit for As(III) and As(V) is 0.06 ppb and 0.7 ppb respectively.

According to the paper published by Profumo *et al.* (2005), the preconcentration of both As(III) and As(V) was carried out in 0.45 M HBr in the presence of Cu(II). This method is based on the formation of a copper-arsenic intermetallic at HMDE during the preconcentration step. With respect to HCl, using HBr as supporting electrolyte shifted the As(III) peak to potential that is more positive. Instead of using thiosulfate as the reducing

agent, sodium dithionite was used for the reduction of As(V) to As(III). The total arsenic determination was done with the standard addition of As(III), where As(V) is calculated by difference from total inorganic arsenic [As(III) + As(V)] and As(III). The quantification limit of As(III) is 0.01 ppb and for As(V) is 0.02 ppb for deposition time of 300 s.

Lim (2004) studied inorganic arsenic [As(III) and As(V)] using SWCSV method. The pre concentration of As(III) was carried out in 1 M HCl in the presence of 20 ppm Cu(II) with the optimized deposition potential and accumulation time of -0.35 V and 55 s respectively. In the determination of total inorganic arsenic, the preconcentration was conducted in 1 M HCl and 40 ppm Cu(II) in the presence of both thiosulfate and thiocyanate as the reducing agent prior to determination. As(V) was obtained by differences between total inorganic arsenic and As(III). In this study, although thiosulfate was found to be a faster and more effective reducing agent as compared to thiocyanate, the combination of both as a reducing agent produced the highest peak current and the best defined peak shape. The detection limit for As(III) and As(V) was 0.05 µg/L and 0.2 µg/L respectively. The optimized method was applied in the analysis of environmental samples collected from Sungai Juhara and Tasik Biru, Bau. All of the samples contained total inorganic arsenic about 3 – 30 times higher than the maximum 10 ppb As(V) in the drinking water recommended by WHO.

CHAPTER 3

METHODOLOGY

3.1 Experiments

This study was divided into three parts. The first part was to study and optimize the experimental parameters for the determination of As(III) by square wave cathodic stripping voltammetry (SWCSV). The effect of instrumental parameters on As(III) peak current such as deposition potential, deposition time, pulse amplitude and frequency were optimized in order to develop an optimum condition for arsenic determination. Besides that, the effect of Cu(II) concentration was also optimized.

Since As(V) is electrochemically inactive, the second part was to investigate the optimum concentration and the efficiency of sodium thiosulfate for the reduction of As(V) to As(III) prior determination. According to Ferreira & Barros (2002), sodium thiosulfate was found to be the more convenient reductant. Conditions such as Cu(II) concentration, deposition potential, deposition time were also optimized which allowed the reduction to be fast and complete at room temperature.

The third part of the experiment was to apply optimized method to measure inorganic As(III) and As(V) in environmental water samples collected from Tasik Biru, Bau.

3.2 Chemicals

The chemicals reagents which were used throughout this analysis are analytical reagent grade unless stated otherwise. Table 3.1 is the list of the chemical reagents used.

Chemical Reagent	Manufacturer
Arsenic(III) Oxide (As ₂ O ₃)	Acros Organic
Arsenic standard solution (H ₃ AsO ₄)	Merck
Sodium Thiosulfate (Na ₂ S ₂ O ₃ .5H ₂ O)	Mallinckrodt
Cupric Chloride Dihydrate (CuCl ₂ .2H ₂ O)	J.T Baker
Concentrated Hydrochloric Acid (HCl) 37% w/w	Merck/ Hamburg
Concentrated Nitric Acid (HNO ₃) 69.0-70.0%	J.T Baker
Sodium Hydroxide (NaOH) 25% w/w	J.T Baker
Electrolyte solution Potassium Chloride (KCl) 3 mol/L	Metrohm Ion Analysis
Mercury	Merck
Nitrogen gas	

Table 3.1: The list of chemical reagents used in the experiment

3.3 Preparation of reagents and solutions

Ultrapure water from a water purification system Thermo Scientific Barnstead NANO pure Diamond Life Science (UV/Uf) Ultrapure Water System or Prima Reverse Osmosis, model Elga was used in the preparation of all solutions and in all of the experiments.

(a) Arsenic(III) stock solution (1000 mg/L)

Arsenic(III) stock solution (1000 mg/L) was prepared by dissolving 0.132 g of arsenic trioxide (As_2O_3) in 1 mL of NaOH (25%), and immediately acidified with 2 mL concentrated HCl and diluted to 100 mL with ultrapure water (Ferreira & Barros,

2002). The solution was stored at 4 °C and was reported to be stable for a month.

(b) Arsenic(III) intermediate standard solution (1000 µg/L)

As(III) intermediate standard solution (1000 μ g/L) was prepared by diluting 100 μ L of the stock solution to 100 mL with ultrapure water and was prepared freshly prior to use.

(c) Arsenic(III) working standards solutions

Arsenic(III) working standards solutions was prepared from arsenic intermediate standard and was used as calibration standards.

(d) Arsenic(V) stock solution (1000 mg/L)

Arsenic(V) stock solution (1000 mg/L) was bought readily Merck and was stored at room temperature. The required standard solutions were prepared daily by dilution of the stock solutions (Ferreira & Barros, 2002).

(e) Cu(II) solutions

(i) Cu(II) solution (20000 mg/L)

Cu(II) solution (20000 mg/L) was prepared by diluting 5.366 g of cupric chloride dihydrate (CuCl₂.5H₂O) in a minimum amount of 0.1 M HCl and diluted to 100 mL with ultrapure water.

(ii) Cu(II) solution (1000 mg/L)

Cu(II) solution (1000 mg/L) was prepared by diluting 5 mL of 20000 mg/L Cu(II) to 100 mL with ultrapure water.

(f) Hydrochloric acid (HCl) solutions

(i) Hydrochloric acid (HCl) (4 M)

Hydrochloric acid (4 M) was prepared by diluting 331.13 mL of concentrated HCl (37%) to 1 L in ultrapure water.

(ii) Hydrochloric acid (HCl) (2 M)

Hydrochloric acid (2 M) was prepared by diluting 500 mL of HCl (4 M) to 1 L with ultrapure water. This solution is used as a supporting electrolyte for all determinations.

(g) Sodium thiosulfate $(Na_2S_2O_3 \cdot 5H_2O)$ (1 M)

Sodium Thiosulfate (1 M) was prepared by dissolving 6.203 g of $Na_2S_2O_3 \cdot 5H_2O$ in ultrapure water and was diluted to 25 mL.

3.4 Instrument and apparatus

3.4.1 Glasswares and sample bottles

All the glasswares and sample bottles which were used for the analysis was soaked in 3 M nitric acid (HNO₃) for 72 hours and rinsed with copious amount of deionised water.

3.4.2 Instrumentation for electrochemical measurement

The voltammetric measurements were carried out with 757 VA Computrace, a PCcontrolled voltammetry system incorporated with the hardware of Metrohm's VA Stand (Metrohm, Switzerland). The software used was VA Computrace 2.0. All of the voltammetric measurements were performed with a standard three-electrode cell which is hanging mercury drop electrode (HMDE, drop size 5), a double junction silver/silver chloride and a platinum rod as the working electrode (WE), reference electrode (RE) and auxiliary electrode (AE) respectively. The solution in the voltammetric cell was stirred by a built-in rotor and a PTFE tube for purging with nitrogen gas during the deposition step.

(a) Working electrode (WE)

The working electrode is the electrode at which the reaction of interest occurs. Hanging Mercury Drop Electrode (HMDE) is a mercury electrode mode of the Multi-Mode Electrode (MME) which was used throughout the measurements. In HMDE mode, four mercury drops of defined size are formed in succession at the MME. The last drop remained suspended and the entire voltage sweep is performed on this stationary drop with preceding deposition. In order to prevent any blockage, the MME is always immersed in deionised or ultrapure water.

(b) Reference electrode (RE)

The reference electrode is a non-polar electrode. The purpose of reference electrode is to produce a stable potential that does not change against solution concentration or time. The reference electrode that was used throughout the measurement is a silver/silver chloride reference system (Ag/AgCl/3 M KCl) supplied in a holder filled with 3 M KCl as standard, thus formed a double junction system. The electrode is immersed in the solution of 3 M KCl after used in order to maintain the potential of the electrode.

(c) Auxiliary electrode (AE)

The function of auxiliary electrode is to conduct electricity from the current source into the supporting electrolyte and directly to the working electrode, maintaining the correct current. The platinum counter electrode was used throughout the measurements.

3.5 Determination of Arsenic(III) concentration

3.5.1 Procedures of As(III) determination

The general procedures outlined below are applied for all As(III) determinations, unless stated otherwise. All of the determinations were carried out in 2 M HCl as a supporting electrolyte. Before determinations, the voltammetric cell was first washed with tap water, followed by rinsing with deionised water a few times. The cell was then dried in order to
remove the excess water droplets. The three electrodes were rinsed with deionised water and any visible water droplets were removed with filter paper.

The VA was set to either 'exploratory' or 'determination' mode. The square wave mode in the direction of cathodic scan was used in all of the As(III) measurements, except for cyclic voltammetric (CV) investigation. The pulse height or voltage step (ΔE), the initial potential (E_i) and final potential (E_f) were set at a pre-determined values. The five parameters that had been identified for optimization which are copper concentration, deposition time (t_d), deposition potential (E_d), square wave amplitude (E_{sw}) and frequency (f) was set to the desired operating values.

The blank solution was prepared by transferring with a pipette 20 mL of 2 M HCl into the voltammetric cell, followed by spiking a known amount of Cu(II) concentration with a micropipette. The voltammetric cell was slide in the holder. Then, the three electrodes were immersed into the cell. The solution was purged with nitrogen for 300 s. The purpose of purging the solution with nitrogen gas was to eliminate the dissolves oxygen in the solution. The background current was obtained after a 300 s purge. Then, the voltammetric analysis was performed. A preconcentration step (enrichment) was carried out at a selected deposition time for every scan. Unless stated otherwise, each scan was repeated three times to ensure the peak current was constant. Besides that, every determination was also repeated three times with freshly prepared purged solution to ensure the reliability of the data. A purge of 10 s was applied after every subsequent addition of reagents or standards or change of the instrumental parameters. An equilibrium time of 5 s was applied before the voltammograms were recorded. The peak current at a certain parameter was calculated as the average peak current of all the determinations and scans performed at that certain parameter. Microsoft Excel was employed to produced calibration curves and compute statistical data. Further voltammetric measurements was performed in order to determine the optimum condition of each parameter that affects the peak current of As(III).

3.5.2 General investigation using Cyclic Voltammetry (CV)

In cyclic voltammetry (CV) investigation, the VA was set in the 'exploratory' mode. The cyclic voltammetry mode was chosen with the scan rate 0.1 V/s and voltage step 0.005 V. 20 mL of 2 M HCl was pipetted into the voltammetric cell followed by spiking 20 ppm (400 μ L) of Cu(II) into the cell (Lim, 2009). After purging the solution with nitrogen gas for 300 s, the background voltammogram was obtained at deposition potential of – 0.35 V and deposition time of 55 s. After that, 100 μ L of As(III) was spiked into the solution and it was swept 3 cycles using cyclic voltammetry (CV).

3.5.3 Factors affecting the peak currents

(a) **Deposition potential** (E_d)

The VA was set in the 'exploratory' mode. An aliquot of 100 μ L As(III) (1000 μ g/L) was spiked into the blank solution. The voltammograms for every deposition potential in the range of – 0.25 V to – 0.55 V were recorded. The procedure was repeated three times with

freshly prepared purged solutions. A graph of peak current (μA) versus deposition potential (V) was plotted.

(b) Cu(II) concentration

The VA was set in the 'exploratory' mode. By using the optimum deposition potential that has been determined previously, voltammograms of Cu(II) concentration were recorded in the range of 0 to 40 ppm. The Cu(II) concentrations ranged from 0 to 9 ppm were spiked into 20 mL HCl (2 M) in the voltammetric cell. After that, the experiment was continued with the addition of 10 ppm to 40 ppm of Cu(II) concentrations with 10 ppm interval. After the background was determined, an aliquot of 100 μ L As(III) (1000 μ g/L) was spiked into the blank solution. The voltammograms were recorded. Every Cu(II) concentration was determined three times with freshly prepared purged solutions. A graph of the peak current (μ A) versus Cu(II) concentration (ppm) was plotted.

(c) **Deposition time** (t_d)

The VA was set in the 'exploratory' mode. The blank solution was prepared by spiking the optimum concentration of Cu(II) that has been determined previously into 20 mL HCl (2 M). The background current was determined, and an aliquot of 100 μ L As(III) (1000 μ g/L) was spiked into the blank solution. The voltammograms were recorded in the range of 30 to 180 s with 30 s intervals. Every scan was repeated twice in order to avoid decaying of the arsenic peak at long deposition times as a result of saturation of the electrode surface (Li

& Smart, 1996). Every determination was repeated three times with freshly prepared purged solutions at the same Cu(II) concentration.

Besides that, the effect of deposition time towards SWCSV peak current at different Cu(II) concentrations was also investigated by repeating the whole procedure stated as above using 20, 30 and 40 ppm Cu(II). A graph of peak current (μ A) versus deposition time (s) was plotted for all of the Cu(II) concentrations.

(d) Pulse amplitude (E_{sw})

By using the optimum chemical and instrumental parameters that have been determined previously, the effect of pulse amplitude towards SWCSV peak current of As(III) were investigated by setting the VA in the 'exploratory' mode. An aliquot of 100 μ L As(III) (1000 μ g/L) was spiked into the blank solution. The voltammograms were recorded in the range of 10 to 100 mV with 10 mV intervals and thereafter up to 250 mV with 50 mV intervals. The procedure was repeated three times with freshly prepared purged solutions. A graph of peak current (μ A) versus pulse amplitude (mV) was plotted.

(e) Frequency

In the investigation of the effect of frequency on SWCSV peak current of As(III), the VA was set in the 'exploratory' mode with the optimized chemical and instrumental parameters. An aliquot of 100 μ L As(III) (1000 μ g/L) was spiked into the blank solution. The voltammograms were recorded in the range of 10 Hz to 149 Hz (instrument limit). The

procedure was repeated three times with freshly prepared purged solutions. A graph of peak current (μ A) versus frequency (Hz) was plotted.

3.5.4 Quality assurance

(a) Calibration curves and linearity range

In quality assurance investigation, the VA was set in the 'determination' mode with 'batch calibration' selected. With the optimum chemical and instrumental conditions, the background current was determined. After the determination of the background current, by applying *'in situ'* calibration method, known aliquots of As(III) were then spiked sequentially into the blank solution. The voltammograms were recorded after every addition of As(III) standards. Calibration curves were then constructed and the linearity range of As(III) was determined.

The procedure above was then repeated to construct calibration curves at different As(III) concentrations within the linearity range produced. Besides that, the correlation coefficient, r, was computed in order to estimate how well the experimental points fit a straight line (Miller & Miller, 1993). Quantification was accomplished either by using standard additions method or referring to the calibration curve produced.

(b) Limit of detection (LOD)

Limit of detection (LOD) is the analyte concentration giving a signal equal to the blank signal, y_B , plus three standard deviations of the blank, S_B (Miller & Miller, 1993):

$$y - y_B = 3S_B$$

The As(III) calibration curve in the range of 0 to 1 μ g/L was constructed with 3 independent determinations. The limit of detection (LOD) of the curve was calculated from the pooled standard deviations of the y-residuals from the calibration curve as a + 3S_{y/x}, where a, used as an estimate of y_B, is the calculated intercept, and S_{y/x}, used as an estimate of S_B is the pooled standard deviation of each point on the plot of regression line (Miller & Miller, 1993).

(c) Precision

Precision is composed of within-run and between-run precision. The within-run (repeatability) and between-run (reproducibility) precision of an analytical method is described as:

Relative Standard Deviation (RSD) = <u>Standard Deviation</u> $_{x 100 \%}$ Mean

(i) Within-run (repeatability)

Within-run (repeatability) precision is defined as standard deviation obtained using a given operating procedure in connection with a particular sample and repeatedly measuring a parameter in the same condition and hardware and by the same person in a short period of

time (Meier & Zünd, 1993). The measurements of 10 replicates of 5 ppb of As(III) in 20 mL of HCl and optimum concentration of Cu(II) at optimum instrumental conditions were obtained in the same day in which each measurement was done in three scans. The within-run RSD was the mean RSD of 10 measurements.

(ii) Between-run (reproducibility)

Between-run (reproducibility) precision is defined as the standard obtained for the same operating procedure over a longer period of time (Meier & Zünd, 1993). The measurements of 10 replicates of 5 ppb of As(III) in 20 mL of HCl and optimum Cu(II) concentration at optimum instrumental conditions were conducted in 10 days over a period of 2 weeks. Each measurement was done in three scans. The between-run RSD was the mean RSD of 10 measurements.

(d) Accuracy of the method

Accuracy of a method is a closeness of the agreement between the results of a measured value to the true value for the sample. Accuracy was determined by spiking known amount of As(III) in blank matrices. Recovery is a term used to describe accuracy and the percentage recovery of the spiked analyte is determined as in the following equation:

Recovery rate (%) =
$$\frac{\text{Measured Concentration}}{\text{Added Concentration}} \times 100 \%$$

Spiked samples at three different concentrations were prepared. Sample A to C were spiked with known amount of As(III) and were quantified using standard addition method. Each sample was determined at least three times with each determination scanned three times. The recovered spiked As(III) concentration for each sample was determined and the recovery rate (%) was calculated.

3.6 Determination of As(V)

3.6.1 Procedures for As(V) determination

Unless stated otherwise, the procedures for As(III) determination method in section 3.5.1 were applied for all determination of [As(III) + As(V)] except that three instrumental parameters that were optimized earlier, $E_{sw} = 60 \text{ mV}$, $\Delta E = 5 \text{ mV}$ and f = 149 Hz were kept constant throughout all [As(III) + As(V)] determinations.

3.6.2 Chemical reduction of As(V)

In this initial study, the reducing effect of sodium thiosulfate on As(V) was investigated using SWCSV at the newly modified optimal chemical and instrumental conditions for As(III). The value of Esw = 60mV, $\Delta E = 5mV$ and f = 149 Hz that were optimized earlier were kept constant, where as, the value for deposition potential, Cu(II) concentration, deposition time and the concentration of sodium thiosulfate were varied in the experiment. At the early stage of investigation, the voltammograms were recorded at $E_d = -0.35$ V before and after the addition of 0.5 mM thiosulfate into the solution containing 20 mL HCl, 10 ppm Cu(II) and 5 ppb As(V). Depending on the voltammograms produced, the deposition potential was altered during the course of investigation. In order to investigate the effect of Cu(II) and thiosulfate toward the peak heights, the concentration of thiosulfate was increased from 0.5 to 2.0 mM. Another 10 ppm pf Cu(II) was then added at thiosulfate concentration of 2.0 mM.

The effect of Cu(II) concentration on the peak current was investigated further using the same procedure as above with freshly prepared solution in the presence of sodium thiosulfate. Since the sodium thiosulfate concentration has not been optimized, in this experiment, the sodium thiosulfate concentration used was 3.2 mM (Ferreira & Barros, 2002) and the deposition potential was set at -0.45 V which is the value obtained in the initial stage of this investigation. The voltammograms were recorded with every addition of Cu(II) in the range of 10 to 30 ppm.

Deposition potential at -0.45 V was investigated further using cyclic and square wave voltammetry. The voltammograms of 20 mL HCl with the presence of 5 ppb As(V), 10 ppm Cu(II) and 3.2 mM thiosulfate were recorded at $E_d = -0.45$ V, $t_d = 90$ s, $\Delta E = 5$ mV, scan rate = 0.75 V/s and at $E_d = -0.45$ V and $t_d = 90$ s for cyclic and square wave voltammetry respectively.

It was found that in the reduction process of As(V), the sequence of adding in sodium thiosulfate to the solution is important. In previous experiments, it was found that no peak

was detected when thiosulfate was added prior to As(V). To verify this, 3.2 mM sodium thiosulfate was added to a solution containing 20 mL HCl and 10 ppm Cu(II), followed by the addition of 5 ppb As(V). The voltammograms were then compared with the voltammograms of the initial experiments, where thiosulfate was always the last solution to be added before the voltammograms were obtained.

3.6.3 Optimization

In report by Ferreira & Barros (2002), Cu(II) was less efficient in promoting the accumulation of intermetallic compound at the surface of the mercury electrode in the presence of thiosulfate, a situation that require the use of a much higher concentration of Cu(II). Due to the presence of reductant, the voltammetric conditions optimized previously for the determination of As(III) had to be modified. Thus, the effect of deposition potential (E_d), Cu(II) concentration and deposition time (t_d) were re-examined to ensure an optimal condition in determining [As(III) + As(V)]. Since the optimization of thiosulfate concentration has not been conducted prior to the optimization of deposition potential and Cu(II) concentration, the thiosulfate value of 3.2 mM (Ferreira & Barros, 2002) was adopted in the optimization of deposition potential and Cu(II) concentration, potential and Cu(II) concentration.

(a) **Deposition potential** (E_d)

The voltammograms of a solution containing 20 mL HCl, 5 ppb As(V), 10 ppm Cu(II) and 3.2 mM thiosulfate were recorded in the range of deposition potential from -0.25 V to -0.55 V with every interval of 0.05 V. Deposition potential that gave the best sensitivity was adopted as the optimal deposition potential.

(b) Cu(II) concentration

The effect of Cu(II) on the peak current in the presence of thiosulfate was investigated. Cu(II) concentrations in the range of 1 to 10 ppm were added sequentially to a solution containing 20 mL HCl, 5 ppb As(V) and 3.2 mM thiosulfate. The voltammograms were recorded at optimum E_d determined earlier. At 10 ppm Cu(II), additional of 5 and 10 ppb As(V) were added sequentially to the solution and the voltammograms were recorded. The effect of Cu(II) was investigated further by adding sequentially Cu(II) in the range of 10 to 110 ppm to a solution containing 20 mL HCl, 5 ppb As(V) and 3.2 mM thiosulfate. The voltammograms were recorded and the Cu(II) concentration that gave the best sensitivity was adopted as the optimal Cu(II) concentration.

(c) Thiosulfate concentration

Since sodium thiosulfate is a reducing agent, it must be in excess to ensure all As(V) in the solution is reduced to As(III). However, too much excess of thiosulfate in the acidic supporting electrolyte may produce large quantity of sulfur. In order to investigate the optimal

amount of thiosulfate required, thiosulfate concentrations ranged from 0.5 to 3.5 mM were added sequentially to a solution containing 20 mL HCl, 10 ppm Cu(II) and 5 ppb As(V). The voltammograms were recorded and the thiosulfate concentration that gave the best sensitivity was adopted as the optimal concentration.

(d) **Deposition time** (t_d)

An aliquot of 100 μ L As(V) and the optimized concentration of 1.5 mM thiosulfate and 10 ppm Cu(II) were added to the cell containing 20 mL HCl. The voltammograms were recorded in the range of 30 to 300 s. Every scan was repeated twice as long standing solution may affect the quality of the solution. Deposition time that gave the best sensitivity was adopted as the optimal deposition time.

3.6.4 Efficiency of reduction

The efficiency of the reduction of As(V) to As(III) was conducted by comparing the results obtained in the analysis of three different solutions with the same [As(III) + As(V)] concentration but different As(III)/As(V) ratio. The optimized concentration of Cu(II) (10 ppm) and thiosulfate (1.5 mM) was used in this experiment onwards. The solution with 20 mL HCl, 8 ppb As(V), 10 ppm Cu(II) and 1.5 mM thiosulfate were scanned three times and was repeated five times with freshly prepared purged solution at the same As(V) concentration. The same whole procedure was repeated with 8 ppb As(III) and 2 ppb As(III) + 6 ppb As(V) which corresponds to average values found in many water system (Kopanica &

Novotný, 1998). The means of the peak currents obtained were compared by using the student's t-test.

3.6.5 Quality assurance

(a) Calibration curves and linearity range

Since As(V) is an electroinactive species, it could only be detected electrochemically after it is reduced to electroactive species, As(III). Although the reduction of As(V) to As(III)using sodium thiosulfate was fast and quantifiable, the fact remains that it is As(III) being quantified in all the measurements. Thus, it is important to conduct experiments to study if the determination of As(V) could be done through multiple standard additions of As(III) or As(V). The experiments below were constructed to compare the two analytical procedure, which is the determination of As(V) by multiple standard additions of As(III) or As(V).

The solution containing 20 mL HCl, 2 ppb As(V), 10 ppm Cu(II) and 1.5 mM thiosulfate was scanned at $E_d = -0.45$ V, preceded by 90 s of preconcentration. By applying *'in situ'* calibration method, another 9 aliquots of 2 ppb As(V) were spiked sequentially to the solution in which each addition was scanned twice. This determination was repeated three times and the regression line of the calibration curve was plotted using the mean currents obtained in all three determinations. The same procedure was repeated with 2 ppb As(V) in the solution in which the addition was done with 9 aliquots of 2 ppb As(III) instead of As(V).

The two analytical procedures at different analyte concentrations were compared using a regression line in which the more precise method is plotted on the x-axis (Miller & Miller, 1993). F-test was first carried out to compare the ration variances of both procedures where the more precise method would be plotted on the x-axis and the less precise method would be plotted on the y-axis of the regression graph. Then, the significant test was carried out to test if the calculated slope and intercept differ significantly from 1 and 0 at 95 % significance level.

The linearity range was determined as described for As(III) in section 3.5.4(a) except that it was conducted in the presence of As(V) and 1.5 mM thiosulfate. A calibration curve of As(V) was plotted and the linearity range was determined.

(b) Limit of detection (LOD)

The limit of detection was defined in section 3.5.4(b). The calibration curve was established as following. Using 'in situ' calibration method, 7 aliquots of 2 ppb As(V) were spiked sequentially to the solution containing 20 mL HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate. The voltammograms were recorded for each addition. This procedure was repeated three times with freshly prepared purged solutions. The regression line of the calibration curve in the range of 0 to 14 ppb was plotted using the mean currents of all the scans in three determinations. The LOD was calculated as described in section 3.5.4(b).

(c) Within-run precision (repeatability)

Precision was defined in section 3.5.4(c). The measurement of 10 replicates of 2 ppb As(V) in 20 mL HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate at optimum instrumental conditions were obtained in the same day in which each measurement was scanned three times. The within-run RSD was the mean RSD of 10 measurements.

(d) Between-run precision (reproducibility)

The measurement of 10 replicates of 5 ppb As(V) in 20 mL HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate were conducted in 5 days, over a period of two weeks. Each measurement was scanned three times. The between-run RSD was the mean RSD of 10 measurements.

(e) Accuracy and recovery of the reduction of As(V) to As(III)

Since As(V) is electroinactive, it needs to be reduced to As(III) prior determination. In accuracy and recovery of the reduction of As(V) to As(III), the same method described in section 3.5.4(d) was applied here. Sample A was spiked with 5 ppb As(III) and 5 ppb As(V). Sample B and C were spiked with known amounts of As(V). All samples were quantified using standard addition method. Each sample was determined at least three times with each determination scanned three times. The recovered spike As(V) concentration was determined and the recovery rate (%) was calculated.

3.7 Interference studies

The effect of certain ions on As(III) quantification was investigated. Known concentrations of Zn^{2+} , Fe^{3+} and Cd^{2+} were added separately into 20 mL HCl solution containing 10 ppm Cu(II) and 10 ppb As(III). Each voltammogram was scanned three times at the optimal conditions. The effects of interference in the increase or decrease (%) of the peak current of As(III) were calculated using equation as below:

% increase/decrease of peak current = $\frac{\text{Peak Current [10 ppb As(III) + tested ion(s)]}}{\text{Peak current of 10 ppb As(III)}} \times 100 \%$

3.8 Application on environmental samples

3.8.1 Sampling and water pretreatment

Water sampling was carried out on 21^{st} May 2011. The sampling site, Tasik Biru, is located at Bau area which is about 35 km from Kuching, Sarawak. Polyethylene bottles were used for sampling and sample storage. Samples from three locations in Tasik Biru were collected. At the sampling site, the water samples were acidified with concentrated HNO₃ (65%). In the laboratory, the water samples were filtered through 0.45 µm membrane filter and then stored at 4 °C until further analysis.

3.8.2 Procedure

The water samples collected were divided into two parts which is for the determination of total inorganic arsenic, As(III) and the determination of arsenic total. The determination of total inorganic arsenic is the determination of As(III) and As(V), where as, the determination of arsenic total is the determination of organoarsenic compound in the water samples.

(a) Determination of As(III) and total inorganic arsenic

In the first part, the water samples were analyzed for inorganic arsenic using standard addition method. A known amount of the water sample was added into the measuring vessel containing the medium and was analyzed according to the optimized conditions summarized in Table 3.2. In As(III) determination, 0.5 mL of the water samples were added in the medium. Where as in total inorganic arsenic determination, 0.05 mL of the water samples were added in the medium. Each sample was determined at least three times in which every determination was scanned three times. The calibration graph was computed based on the result of the determination. As(V) was determined by the difference between As(III) and total inorganic arsenic.

Determination of total inorganic	
As(III)	arsenic
2 M HCl + 10 ppm Cu(II)	$2\ M\ HCl + 10\ ppm\ Cu(II) + 1.5\ mM \\ Na_2S_2O_3\cdot 5H_2O$
-0.35 V	– 0.45 V
90 s	90 s
60 mV	60 mV
149Hz	149 Hz
	As(III) 2 M HCl + 10 ppm Cu(II) - 0.35 V 90 s 60 mV 149Hz

Table 3.2:The optimized conditions for the determination of As(III) and total inorganic
arsenic.

(b) Determination of Arsenic total

In the second part, the water samples were analyzed for arsenic total using standard addition method. In this determination, oxidative acid digestion was used for the decomposition of dissolved organoarsenic compounds. Besides that, the digestion procedure also converted As(III) as well as organoarsenic compounds to free arsenate. The digestion procedure was done using Kjeldahl digestion system (Gerhardt).

In the digestion vials, 5 mL of HNO_3 (65%) was added to 50 mL of the water samples. Then, the water samples were digested at the temperature of 200 °C for 2 hours. After digestion, the water samples were cooled to room temperature and stored at 4 °C until further analysis. In this determination, a known amount of the digested sample was added into the measuring vessel containing the medium and was analyzed using standard addition method. The solution was analyzed using the same conditions for the determination of total inorganic arsenic described in Table 3.2. Each digested water sample was determined at least three times.

The digested water samples were also analyzed using GFAAS. The spectrometer parameters for arsenic total determination are described in Table 3.3. Since GFAAS is prone to interferences, few steps must be taken to control it. The sample container for the digested water samples must be thoroughly clean because it can affect the sampling phase of GFAAS equipment. In order to overcome matrix problems which may affect the detection of the water samples, the equipment will automatically performed wash cycle after every injection with ultrapure water. Deuterium background correction was chosen to minimize the background signal in order to reduce the severity of the gas phase interferences resulting from the co-volatilization of the analyte and residual matrix. Three arsenic standard solutions with concentration of 10 ppm, 20 ppm and 30 ppm were prepared for calibration of the instrument. A linear calibration graph was computed with regression line of $r^2 = 1.000$. Each measurement of the environmental samples was performed in triplicates. The results obtained using voltammetry instrument was compared with the results obtained from GFAAS detection.

 Table 3.3:
 Spectrometer parameters for arsenic total determination

Measurement mode Wavelength Lamp Current Bandpass Background correction Absorbance 193.7 nm 75% 0.5 nm D2-Deuterium

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Determination of As(III)

4.1.1 General investigation using Cyclic Voltammetry (CV)

The voltammetric behaviour of As(III) was studied using cyclic voltammetry (CV) in order to obtain qualitative information about the redox reaction at the electrode's surface. Experiment using cyclic voltammetry was conducted in 2 M HCl containing 20 ppm Cu(II) and 5 ppb As(III) in the potential range of -0.5 V to -1.0 V. In order to ensure the reliability of data, each experiment was repeated three times with freshly prepared purged solution in which each scan was also repeated three times. As can be seen in Figure 4.1, only cathodic peak was observed at potential close to -0.8 V. No anodic peak was observed at the reverse potential scans which indicated an irreversible process for re-oxidation. It can also be observed that in Figure 4.1, no cathodic peak was detected in the second and third scan which agrees with Li & Smart (1996) observation. According to the explanation by Li & Smart (1996), the absence of cathodic peak in the successive scan was because of the rapid diffusion of the reduction product (AsH₃) away from the electrode surface as well as slow formation of intermetallic compound (Cu_xAs_y).



Figure 4.1: Cyclic voltammograms of 5 ppb As(III) in 2 M HCl with 20 ppm Cu(II). Scan 1, followed by scan 2 and 3 was preceded by 55 s stirred deposition at deposition potential $E_d = -0.35$ V. The scan rate was 0.1 V/s.

4.1.2 Factors affecting the peak currents

(a) **Deposition potential**

The effect of deposition potentials towards the peak currents of As(III) were studied in the range of -0.25 V to -0.55 V. Figure 4.2 shows the effect of varying deposition potential towards the peak current and peak potential of As(III). As can be observed in Figure 4.2, the peak current increased with deposition potential from -0.25 V to -0.35 V. The absence of arsenic peak at deposition potential more positive than -0.3 V was probably due to incomplete reduction of As(III) to As(0) (Li & Smart, 1996).



Figure 4.2: Effect of varying the deposition potential on SWCS peak current and peak potential of 5 ppb As(III) in 2 M HCl and 20 ppm Cu(II). Deposition potential, Ed, was altered from -0.25 V to -0.55 V. (t_d = 55 s, E_{sw} = 60 mV, f = 149 Hz)

According to the observation by Li & Smart (1996), the decrease of the peak current at deposition potential more negative than – 0.35 V may be related to following reason. Since arsenic(0) can be reduced to arsine at potentials more negative than – 0.35 V, the rate of the reaction was fast and easy to occur at more negative potentials compared to the reaction between arsenic and copper. The rate of reaction between arsenic and copper to form different stoichiometry of Cu_xAs_y intermetallic compound might be slower at negative potentials, thus less Cu_xAs_y are formed at HMDE (Li & Smart, 1996). Besides that, the stoichiometry of the deposited compound on the surface of HMDE could be different at more negative deposition potentials in which the shifting of the peak potential from – 0.78 V ($E_d = -0.35$ V) to – 0.86 V ($E_d = -0.55$ V) provided support for this explanation.

Since $E_d = -0.35$ V gave the optimum peak current and produced the most symmetrical shape curve compared to other deposition potentials, deposition potential at -0.35 V is used in all determinations of As(III).

(b) Copper(II) concentration

Copper is important for the formation of intermetallic compound (Cu_xAs_y) at the electrode surface during the accumulation process in the determination of arsenic. In initial investigation, it was found that no SWCS peak current was detected for 5 ppb As(III) in 2 M HCl in the absence of Cu(II). Cathodic stripping peaks were detected when Cu(II) in the range of 1 – 9 ppm were added into the solution. The same solution was then added with Cu(II) concentration in the range of 10 ppm to 40 ppm with 10 ppm interval. The voltammogram were separated for better observation in which Figure 4.3 shows the voltammograms of 5 ppb As(III) in 2 M HCl in the presence of 0 to 9 ppm Cu(II) and the voltammogram of Cu(II) concentration in the range of 10 ppm to 40 ppm is shown in Figure 4.4. As shown in Figure 4.3, when 1 – 2 ppm Cu(II) were added into the solution, poorly defined curves were detected. Further addition of 5 – 7 ppm Cu(II) yielded poorly defined curves with split peaks. However, the addition of 9 ppm Cu(II) significantly improved the shape of the cathodic stripping peak signal.

Figure 4.5 illustrates the effect of varying Cu(II) concentration on the peak current and peak potential of 5 ppb As(III) in 2 M HCl. As can be seen in Figure 4.5, the peak current of As(III) increased with Cu(II) concentration and reached a maximum value at 10 ppm Cu(II) indicating that a stable Cu_xAs_y intermetallic compound was formed at the surface of HMDE.

When the Cu(II) concentration was increased further from 20 ppm until 40 ppm, the peak current declined with peak potential shifted to more negative potential, which could be due to the formation of other compounds with unknown and different stoichiometry. It is obvious in this study that Cu(II) at 10 ppm is the optimum condition and produced highest sensitivity for As(III) determination. Thus, it is used in all determination of As(III).



Figure 4.3: SWCS voltammograms of 5 ppb As(III) in 2 M HCl. The Cu(II) concentration: 0 ppm, 1 ppm, 2 ppm, 5 ppm, 7 ppm and 9 ppm. ($E_d = -0.35$ V, $t_d = 55$ s, $E_{sw} = 60$ mV, f = 149 Hz)



Figure 4.4: SWCS voltammograms of 5 ppb As(III) in 2 M HCl. The Cu(II) concentration: 10 ppm, 20 ppm, 30 ppm and 40 ppm. The voltammogram showed triplicate scans of each Cu(II) concentration. ($E_d = -0.35$ V, $t_d = 55$ s, $E_{sw} = 60$ mV, f = 149 Hz)



Figure 4.5: Effect of varying Copper(II) concentration on SWCS peak current and peak potential of 5 ppb As(III) in 1 M HCl. Copper(II) concentration ranged from 0 – 40 ppm were investigated. Values shown were the average of three determinations each. ($E_d = -0.35$ V, $t_d = 55$ s, $E_{sw} = 60$ mV, f = 149 Hz)

(c) **Deposition time**

The effects of deposition time on the peak current was studied in the range of 30 s to 180 s at different Cu(II) concentration. Figure 4.6 shows the graph of peak current as a function of deposition time at Cu(II) concentration values in the range of 10 ppm to 40 ppm. As can be seen from Figure 4.6, the peak current increased with the deposition time except for Cu(II) concentration at 40 ppm in which the highest sensitivity was achieved at deposition time of 30 s. According to the findings published by Ferreira and Barros (2002), the higher the Cu(II) concentration, the lower the deposition time required to achieve the maximum current value.

As shown in Figure 4.6 the higher the amount of Cu(II), shorter deposition time was required for the formation of the intermetallic compound at the surface of the electrode which resulted in lower peak current which can be observed for Cu(II) concentration at 30 ppm and 40 ppm, the longer the deposition time, the lower the value of the peak current of As(III) was obtained. At lower Cu(II) concentration of 10 ppm and 20 ppm, longer deposition time was required for the formation of intermetallic compound at the surface of the electrode in which the peak current increased with deposition time but when the maximum value was reached at 90 s and 60 s respectively, the peak current decreased at longer deposition time. At Cu(II) concentration at 30 ppm and 40 ppm does not showed any obvious maximum peak current. Each determination was repeated three times and based on the graph in Figure 4.6, the standard deviation for Cu(II) concentration of 10 ppm, 20 ppm, 30 ppm and 40 ppm was 11.21, 4.54, 1.37 and 0.76 respectively.



Figure 4.6: Effect of varying the deposition time at four copper concentration levels (10 ppm, 20 ppm, 30 ppm, 40 ppm) on the SWCS peak current of 5 ppb As(III) in 2 M HCl. Values shown were the average of three determinations each. ($E_d = -0.35 \text{ V}$; f = 149 Hz; $\Delta E = 5 \text{ mV}$; $E_{sw} = 60 \text{ mV}$)

In most CSV analyses involving the adsorption of a metal complex, the peak current levels off at long deposition times as a result of saturation of the electrode surface (Li & Smart, 1996). Where as in this study, the inconsistency of the peak current at different deposition times may be caused by the change of Cu_xAs_y deposit stoichiometry, which is due to the occurrence of different concentration ratio at the electrode surface. As shown in Figure 4.6, at lower Cu(II) concentration, longer deposition time was required in order to achieve maximum current value in which the maximum current value also increased. In this study, Cu(II) concentration of 10 ppm and deposition time 90 s was needed to achieved the optimum concentration of copper for the formation of intermetallic compound with arsenic at the electrode surface. Eventually, more arsenic was collected at the surface of the electrode due to longer deposition time which resulted in higher peak current. Thus, in this study, the optimum deposition time of 90 s at Cu(II) concentration 10 ppm was used in further determinations.

(d) Pulse amplitude

The influence of the pulse amplitude towards the peak current was observed in the range of 10 mV to 250 mV. Figure 4.7 shows the effect of varying pulse amplitude values on the peak current. Figure 4.7 showed that the peak current increased gradually as the pulse amplitude increased from 10 mV to 200 mV, but, at 250 mV, the peak current suddenly decreased.



Figure 4.7: Effect of varying the pulse amplitude on the SWCS peak current and peak potential of 5 ppb As(III) in 2 M HCl and 10 ppm Cu(II). Pulse amplitude, E_{sw} was altered from 10 – 250 mV. Values shown were average of three determinations each. ($E_d = -0.35$ V; $t_d = 90$ s; f = 149 Hz; $\Delta E = 5$ mV)

Figure 4.8 and Figure 4.9 shows the voltammogram of the peak current with given amplitudes. As shown in Figure 4.8, the changes of the response are still well defined when the pulse amplitudes were increased up to 150 mV, but, the baselines of the response became higher from 150 mV up to 250 mV as illustrated in Figure 4.9. The increased of the baseline current can be contributed by the detection of electroactive species in the solution at higher amplitudes. Besides that, the peak potential also shifted to more positive direction with

increasing pulse amplitude values which could be due to the formation of different stoichiometry of intermetallic compounds at higher amplitudes.



Figure 4.8: SWCS voltammograms of 5ppb As(III) in 2 M HCl and 10 ppm Cu(II). Pulse amplitude in : a = 10 mV, b = 20 mV, c = 30 mV, d = 40 mV, e = 50 mV, f = 60 mV, g = 70 mV, h = 80 mV, i = 90 mV, j = 100 mV, k = 150 mV, l = 200 mV, m = 250 mV. ($E_d = -0.35 \text{ V}$; $t_d = 90 \text{ s}$; f = 149 Hz; $\Delta E = 5 \text{ mV}$)



Figure 4.9: SWCS voltammograms of 5 ppb As(III) in 2 M HCl and 10 ppm Cu(II). Pulse amplitude is in the range of 100 mV to 250 mV. The scans were done in triplicate. ($E_d = -0.35$ V; $t_d = 90$ s; f = 149 Hz; $\Delta E = 5$ mV)

As shown in Figure 4.7 and Figure 4.8, although the peak current sensitivity increased with increasing pulse amplitudes, it can be observed that pulse amplitude at 60 mV showed high sensitivity that was adequate for the measurement of most environmental samples and it was kept constant for further As(III) determinations.

(e) Frequency

Figure 4.10 shows the variation of frequency in the range of 10 Hz to 149 Hz on the peak current. The peak current varies proportionally with increasing frequency. Where as the peak potential shifted to a more negative potential as the frequency increased.



Figure 4.10: Effect of varying the frequency on the SWCS peak current of 5 ppb As(III) in 2 M HCl and 10 ppm Cu(II). Values shown were average of three determinations each. ($E_d = -0.35$ V; $t_d = 90$ s; $\Delta E = 5$ mV)

It is shown in Figure 4.10 that the optimum peak current was achieved at frequency of 149 Hz. Thus, f = 149 Hz was used for further experiments because it showed good sensitivity and an effective scan rate of $\Delta E \ge 0.745$ V/s. This means that the analysis time is drastically reduced; a complete voltammogram can be recorded within a few seconds, as compared with about 2 – 3 min in differential pulse voltammetry (Wang, 2006). Not only that, f = 149 Hz was also the limited maximum frequency allowed in the software programme by the voltammetry instrument.

4.1.3 Quality assurance

(a) Calibration curves and linearity range

In initial investigation, the As(III) calibration curve was studied in the range of 0 to 4 mg/L under the optimized conditions that were determined previously. As can be observed in Figure 4.11, the calibration curve of As(III) exhibited linearity up to 1 mg/L.



Figure 4.11: Calibration curve of As(III) in the range of 0 - 5 mg/L in 2 M HCl and 10 ppmCu(II). (E_d = -0.35 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz)

The linearity in this region was investigated further by constructing another calibration curve in the range of 0 to 1.05 mg/L of As(III). As illustrates in Figure 4.12, the peak current was directly proportional to the concentration of As(III) until 450 μ g/L. The wide linearity

range displayed was a great advantage in quantifying environmental samples in various concentrations without the need of dilution. Dilution could be considered only when the samples contained the total amount of As(III) in standard addition exceeded 450 μ g/L.



Figure 4.12: Calibration curve of As(III) in the range of 0 - 1.05 mg/L in 2 M HCl and 10 ppm Cu(II). (E_d = -0.35 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz)

The linearity of As(III) in concentration ranged within 450 μ g/L was studied further. As shown in Figure 4.13, all the plotted calibration curves showed linearity for low and high concentrations of As(III) in the range of 0.5 – 3.0 μ g/L (Figure 4.13 (a)), 3 – 15 μ g/L (Figure 4.13 (b)) and 15 – 105 μ g/L (Figure 4.13 (c)) with correlation coefficients, R², exceeding 0.99. The quantification of As(III) could be achieved by using a standard curve or standard additions method. However, in order to overcome the matrix effect and the effect of adsorption of surface active organic compounds on HMDE, the standard addition technique was preferred.



(a) As(III) Concentrations : $0.5 - 3.0 \ \mu g/L$



(b) As(III) Concentrations : $3 - 15 \ \mu g/L$



(c) As(III) Concentrations : $15 - 105 \mu g/L$

Figure 4.13: Calibration curves of As(III) in 2 M HCl and 10 ppm Cu(II). ($E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz)

(b) Limit of detection (LOD)

In general terms, limit of detection of an analyte may be described as the concentration which gives an instrumental signal (y), significantly different from the 'blank' or 'background' signal (Miller & Miller, 1993). The calibration curve in the range of 0 to 1 μ g/L was constructed as shown in Figure 4.14. The calculated limit of detection of As(III) based on the calibration curve was summarized in Table 4.1.


Figure 4.14: Calibration curve of As(III) in 2 M HCl and 10 ppm Cu(II). Error bars represent one standard deviation at 95% confidence level. ($E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz)

The calculated limit of detection from the pooled standard deviations of the y-residuals from the calibration curve was estimated to be 0.076 μ g/L. Under the chosen optimum conditions, the calibration plot was linear over the range of 0 to 1 μ g/L with the correlation coefficient of 0.9948.

As(III) Calibration Range (µg/L)	df	$\begin{array}{c} S_{y/x} \\ (n-2) \end{array}$	95% CL $a \pm ts_a$	95% CL b±ts _b	R	LOD (µg/L)
0-1	9	0.2767	0.728 ± 0.427	13.247 ± 0.722	0.9948	0.076

 Table 4.1:
 Summarized statistical determination of limit of detection of As(III).

df is degree of freedom; $S_{y/x}$ is estimated standard deviation of each point on the regression line used as an estimate of the blank (S_B); s_a is standard deviation of intercept; CL is confidence limit; s_b is standard deviation of the slope; R is correlation coefficient; LOD is limit of detection.

(c) Within-run precision

The repeatability of As(III) was obtained by conducting 10 replicates measurements of As(III) in one day. The result of the 10 replicates measurements of As(III) are reported in Table 4.2. The relative standard deviation (RSD) was calculated as 6.67% (n = 10) at 5 ppb As(III) for 90 s deposition time. The calculated RSD of the method was satisfactory with good repeatability.

(d) Between-run Precision

The reproducibility of As(III) was obtained by conducting 10 replicates measurements of As(III) within two weeks. Table 4.3 shows the calculated between-run precision of As(III) over a period of two weeks. The relative standard deviation (RSD) was calculated as 5.47% (n = 10) at 5 ppb As(III) for 90 s deposition time. The data showed good reproducibility and these criteria are required for good analytical technique.

No. of measurement	Mean Peak Current (µA)	Standard Deviation (s)	RSD (%)
1	1.08	0.08	7.41
2	1.09	0.04	3.67
3	1.29	0.09	6.98
4	1.24	0.17	13.71
5	1.19	0.05	4.20
6	1.16	0.08	6.90
7	1.09	0.09	8.26
8	1.21	0.10	8.26
9	1.19	0.05	4.20
10	1.30	0.04	3.08
Mean	1.184	0.079	6.67

Table 4.2:	Within-run	Precision	calculated	from	the	mean	RSD	of	10	replicates
	measuremen	nts of 5 ppb	As(III) pea	k curre	nts.					

No. of measurement	Mean Peak Current (µA)	Standard Deviation (s)	RSD (%)
1	1.13	0.06	5.31
2	1.18	0.13	11.02
3	1.13	0.06	5.31
4	1.05	0.05	4.76
5	0.96	0.01	2.06
6	1.15	0.06	5.22
7	1.04	0.12	11.54
8	1.10	0.03	2.73
9	1.05	0.03	2.86
10	1.03	0.04	3.88
Mean	1.082	0.059	5.47

Table 4.3:Between-run Precision calculated from the mean RSD of 10 replicates
measurements of 5 ppb As(III) peak currents.

(e) Accuracy of the method

The results of the tests conducted on spiked purified water samples in the range 10 to 100 μ g/L were summarized in Table 4.4. As can be seen in Table 4.4, the calculated recoveries were in the range of 94% to 107%. Generally, good recovery indicated good accuracy. The calculated relative standard deviation (RSD) of the three samples were in the range of 6% to 18%.

Sample	А	В	С
As(III) added (µg/L)	10	50	100
As(III) measured (µg/L) (Three replicates)	1. 9.757 2. 11.012 3. 7.558	55.357 56.164 49.957	118.597 94.618 108.042
Mean \pm SD ^a (µg/L) Recovery \pm SD (%) RSD (%)	9.44 ± 1.74 94.4 ± 17.4 18.46	$53.83 \pm 3.37 \\ 107.66 \pm 6.74 \\ 6.26$	$\begin{array}{c} 107.09 \pm 12.02 \\ 107.09 \pm 12.02 \\ 11.2 \end{array}$

Table 4.4:Results obtained for As(III) spiked purified water samples.

^amean value \pm corresponding standard deviation of three determinations

4.2 Determination of As(V)

4.2.1 Chemical reduction of As(V) by thiosulfate

As pentavalent arsenic, As(V), is electroinactive, the determination of total inorganic arsenic generally requires a preliminary step to reduce As(V) to As(III) and then followed by the determination of As(III) species. In this study, the reduction of As(V) using sodium thiosulfate was conducted. In the initial investigation, no peak was found in the absence of sodium thiosulfate in 2 M HCl solution containing 5 ppb As(V) and 10 ppm Cu(II). Figure 4.15 shows the SWCS voltammogram of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II) in the presence of sodium thiosulfate.

As can be seen in Figure 4.15, initial investigation of the voltammograms showed a broad peak was observed in 2 M HCl solution containing 5 ppb As(V) and 10 ppm Cu(II) in the presence of 0.5 mM sodium thiosulfate at $E_d = -0.35$ V. Only when the deposition potential was shifted to -0.45 V, the a quantifiable reduction peak of As(III) was observed at peak potential about -0.72 V. When the sodium thiosulfate concentration was increased further, the peak current increased up to 1.5 mM and then slightly decreased at sodium thiosulfate concentration of 2.0 mM. At sodium thiosulfate concentration of 2.0 mM, another 10 ppm Cu(II) was added into the solution which resulted in a slight increased of the peak current. This could be attributed to the presence of high concentration of Cu(II) which enhances the reduction of As(V).



Figure 4.15: SWCS voltammograms of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II). The Na₂S₂O₄ concentration and deposition potential in : (a) 0 mM, $E_d = -0.35$ V; (b) 0.5 mM, $E_d = -0.35$ V; (c) 0.5 mM, $E_d = -0.45$ V; (d) 1.0 mM, $E_d = -0.45$ V; (e) 1.5 mM, $E_d = -0.45$ V; (f) 2.0 mM, $E_d = -0.45$ V; (g) another 10 ppm Cu(II) was added to (f). (t_d = 90 s, $E_{sw} = 60$ mV, f = 149 Hz).

The effect of Cu(II) towards peak current was investigated further. The procedure above was repeated in the presence of 3.2 mM sodium thiosulfate as optimized by Ferreira & Barros (2002), in the Cu(II) range of 10 – 30 ppm. As can be observed in the voltammograms in Figure 4.16, the highest peak current was at Cu(II) 10 ppm. When the Cu(II) concentration was increased further to 30 ppm, the peak current decreased. This could be due to the precipitation of colloidal sulphur and Cu(I) in the presence of large amount of copper and thiosulfate which makes the accumulation process of Cu-As intermetallic compound at the surface of the HMDE electrode less efficient.



Figure 4.16: SWCS voltammograms of 5 ppb As(V) in 2 M HCl and 3.2 mM Na₂S₂O₄. The Cu(II) concentration in ; (a) 10 ppm; (b) 20 ppm; (c) 30 ppm. ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz).

The above procedure was repeated in the presence of 10 ppm Cu(II) and 5 ppb As(V). Repeated trials at deposition potential of -0.45 V showed consistency of a well defined peak current at peak potential about -0.71 V (Figure 4.17). Thus, deposition potential of -0.45 V was used for further analysis of As(V).



 $\begin{array}{ll} \mbox{Figure 4.17:} & \mbox{Voltammograms of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II) in the} \\ & \mbox{presence of 3.2 mM Na_2S_2O_4. (a) Cyclic linear scan voltammetry at $E_d = -$ 0.45 V, $t_d = 90 s, $scan rate = 0.75 V/s. (b) $SWCSV at $E_d = - 0.45 V, $t_d = 90 s, $E_{sw} = 60 mV, $f = 149 Hz. $ \end{array}$

The sequence of adding in sodium thiosulfate into the solution is important in the reduction process of As(V). Thiosulfate forms sulfur readily in acidic medium especially without the presence of oxidizing agent. In this case, without the presence of As(V) as its oxidizing agent, the added thiosulfate reacted rapidly with the acidic supporting electrolyte forming sulfur which clouded the solution. Thus, when As(V) was added after the addition of thiosulfate, there was no more or small quantity of thiosulfate left for the reduction of As(V). As shown in the voltammograms in Figure 4.18, no peak current was detected when thiosulfate was added into the solution prior to As(V). When As(V) was added prior to thiosulfate, As(V) would be immediately reduce to As(III) producing well defined As(III) peaks.



Figure 4.18: SWCS voltammograms (2 scans) of 5 ppb As(V) in 2 M HCl, 10 ppm Cu(II) and 3.2 mM Na₂S₂O₄. Na₂S₂O₄ was added first into the solution followed by As(V). ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz).

4.2.2 Optimization

(a) **Deposition potential**

Since the determination of As(V) requires the addition of thiosulfate, the deposition potential was being re-examined in the range of -0.25 V to -0.55 V. As can be seen in the voltammograms in Figure 4.19, the deposition potential at -0.45 V produced the highest and optimal peak current. Other peak signals at different deposition potentials are broad, small or not optimal enough to be accepted as the optimized value for arsenic total and As(V) determinations. Thus, in the presence of thiosulfate, deposition potential at -0.45 V was used for arsenic total and As(V) determinations.



Figure 4.19: Effect of deposition potential on the SWCSV determination of 5 ppb As(V) in 2 M HCl, 10 ppm Cu(II) and 3.2 mM Na₂S₂O₄. The deposition potential used in : (a) -0.25 V; (b) -0.30 V; (c) -0.35 V; (d) -0.40 V; (e) -0.45 V; (f) -0.50 V; (g) -0.55 V. (t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz).

(b) Cu(II) concentration

The effect of Cu(II) concentration on the peak current of As(V) was initially studied in the range of 1 ppm to 10 ppm. Figure 4.20 shows the voltammograms of the effect of Cu(II) concentrations towards the peak current of As(V).



Figure 4.20: Effect of Cu(II) concentration on the SWCSV determination of 5 ppb As(V) in 2 M HCl and 3.2 mM Na₂S₂O₄. The Cu(II) concentration used in : (a) 0 ppm; (b) 1 ppm; (c) 3 ppm; (d) 5 ppm; (e) 7 ppm; (f) 10 ppm. From (g) to (h), another 5 and 10 ppb As(V) was added respectively to (f).

As can be observed in Figure 4.20, the peak current increased as the Cu(II) concentration increased from 1 ppm to 10 ppm. When the Cu(II) concentration was increased up to 7 ppm, broad peak signal was observed. As the Cu(II) concentration was increased further to 10 ppm, the broad peak signal diminished and only single peak current was observed at peak potential approximately at -0.73 V. At 10 ppm Cu(II), another 5 ppb and 10 ppb As(V) was added sequentially into the solution. When higher amounts of As(V) were added into the solution, increment of the peak current was observed. This could be due to when higher amount of As(V) was added, more Cu-As intermetallic compound can be formed at the surface of the HMDE electrode.

The appearance of broad peak at 5 ppm - 7 ppm Cu(II) could be due to the precipitation of colloidal sulphur and Copper(I). As the Cu(II) concentration increased, more complex compound was formed and this contributed to the appearance of two peaks at 10 ppm Cu(II). However, when more As(V) was added into the solution, more Cu(II) was required for the formation of Cu-As intermetallic compound rather than for the complex formation. Hence, only single peak was formed at higher As(V) concentration.

The above procedure was repeated with Cu(II) concentration ranged from 10 ppm to 110 ppm. As shown in Figure 4.21, it was obvious that Cu(II) at 10 ppm produced the best sensitivity for the formation of Cu_xAs_y intermetallic compound. Cu(II) at 10 ppm was the optimum concentration and was used for all As(V) and total arsenic determinations.



Figure 4.21: Effect of varying Cu(II) concentration on 5 ppb As(V) in 2 M HCl and 3.2 mM $Na_2S_2O_4$. The Cu(II) concentration was altered from 10 ppm to 110 ppm. (E_d = -0.45 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz).

The optimum Cu(II) concentration at 10 ppm was investigated further by varying the concentration of As(V). Increasing amount of As(V) concentration ranged from 0.1 ppb to 15 ppb was added into the solution. Figure 4.22 shows that Cu(II) concentration at 10 ppm is also produced well defined and repeatable peak signals with consistent increment. Besides that, it shown in the voltammograms in Figure 4.22 that Cu(II) concentration at 10 ppm produced quantifiable peaks which is important for As(V) and total arsenic determinations.



Figure 4.22: SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 3.2 mM $Na_2S_2O_4$. The As(V) concentration used in : (a) 0.1 ppb; (b) 0.5 ppb; (c) 1 ppb; (d) 2 ppb; (e) 4 ppb; (f) 6 ppb; (g) 8 ppb; (h) 10 ppb; (i) 12 ppb; (j) 15 ppb. (E_d = -0.45 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz).

(c) Thiosulfate concentration

In As(V) and total arsenic determinations, thiosulfate is required in excess in order to allow all of As(V) in the solution to be reduced to As(III) prior to determinations. However, large excess of thiosulfate may produce large quantity of sulfur which might interfere with the quantification of As(V) and caused low reproducibility. The reduction of As(V) with thiosulfate prior to the formation of intermetallic compound with Cu(II) occurs according to the equation :

$$AsO_4^{3-} + 2S_2O_3^{2-} + 2H^+ \longrightarrow AsO_3^{2-} + S_4O_6^{2-} + H_2O_6^{2-}$$

Figure 4.23 shows the voltammograms of 5 ppb As(V) in 2 M HCl and 10 ppm Cu(II) with various thiosulfate concentration. As can be observed, the peak current increased with increasing thiosulfate concentration up to 1.5 mM thiosulfate. When the thiosulfate concentration was increased further, the peak current decreased. At thiosulfate concentration of 2.5 mM to 3.5 mM, split peaks were detected and hence could not be quantified. This could be caused by the presence of large excess of thiosulfate might produced colloid of sulfur which makes the accumulation process at the electrode surface less efficient leading to the decrease or split in the peak current and low reproducibility of the result (Junsomboon *et al.,* 2009). Thiosulfate concentration at 1.5 mM produced the optimal peak current and was used for all As(V) and total arsenic determinations.



 $\begin{array}{ll} \mbox{Figure 4.23:} & \mbox{Effect of } Na_2S_2O_4 \mbox{ concentration on the SWCSV determination of 5 ppb} \\ & \mbox{As(V) in 2 M HCl and 10 ppm Cu(II). The } Na_2S_2O_4 \mbox{ used in : (a) 0.5 mM;} \\ & \mbox{(b) 1.0 mM; (c) 1.5 mM; (d) 2.0 mM; (e) 2.5 mM; (f) 3.0 mM; (g) 3.5 mM.} \\ & \mbox{(} E_d = - \mbox{0.45 V}, \mbox{ } t_d = 90 \mbox{ s, } E_{sw} = 60 \mbox{ mV}, \mbox{ } f = 149 \mbox{ Hz}. \end{array}$

(d) **Deposition time**

Figure 4.24 illustrates the variation of peak current as a function of deposition time. It can be observed in Figure 4.24 the peak current increases with increasing deposition time, a behaviour that is different from that observed in the determination of As(III). This could be contributed from the increasing deposition time which resulted in higher amount of Cu-As intermetallic compound being deposited on the surface of the electrode, hence, causing the increment of the peak current. If longer deposition times were used in most CSV analyses, the peak current levels off due to the saturation of the electrode surface.

As can be seen in Figure 4.24, well defined peaks were produced for all of the deposition times except for peak splitting that was observed for the deposition times of 30 s to

40 s. The peak splitting could be caused by the insufficient deposition time for the accumulation or formation of intermetallic compound Cu-As at the surface of the electrode before determination. Even though deposition time of 300 s produced the highest peak current, deposition time 90 s was chosen for all As(V) and arsenic total determinations in order to shorten the time of analysis. But, for the determination of very low concentrations, the sensitivity can be improved using longer deposition times while keeping all other conditions constant.



Figure 4.24: SWCS voltammograms of 2 M HCl containing 5 ppb As(V), 10 ppm Cu(II) and 1.5 mM Na₂S₂O₄. The deposition time used in : (a) 30 s; (b) 40 s; (c) 50 s; (d) 60 s; (e) 90 s; (f) 120 s; (g) 150 s; (h) 180 s; (i) 210 s; (j) 240 s; (k) 270 s; (l) 300 s. ($E_d = -0.45$ V, $E_{sw} = 60$ mV, f = 149 Hz).

4.2.3 Efficiency of reduction

The efficiency of the reduction of As(V) was studied in by comparing the results obtained in the analysis of sample solutions with different ratio of As(III)/As(V), but with the same total arsenic concentrations (8 ppm) (Ferreira & Barros, 2002). The means and standard

deviations of arsenic peak currents obtained are shown in Table 4.5. The two-tailed F-test showed that the variances of 8 ppb As(III) and 2 ppb As(III) + 6 ppb As(V) were significantly different. Even though the variances of 8 ppb As(III) and 2 ppb As(III) + 6 ppb As(V) were different, the respective calculated RSD at 1.27 % and 5.26 % were still acceptable.

As can be seen in Table 4.5, the t-test showed that the mean peak current of 8 ppb As(V) and 2 ppb As(III) + 6 ppb As(V) were not significantly different from 8 ppb As(III). It can also be seen in Figure 4.25, the peak heights and shapes of the reduced As(III) peaks obtained in the solutions with different ratios of As(III)/As(V) were very similar which concludes that the reduction of As(V) and As(III) is quantitative.



Figure 4.25: SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 1.5 mM $Na_2S_2O_4$. The arsenic concentration in : (a) 8 ppb As(III): (b) 8 ppb As(V); (c) 2 ppb As(III) + 6 ppb As(V). (E_d = -0.45 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz).

Concentration of As(III)/As(V) (ppb)	8 ppb As(III)	8 ppb As(V)	2 ppb As(III) +6 ppb As(V)
Mean (µA)	17.39	17.77	18.14
Standard deviation (µA)	0.23	0.47	0.81
RSD (%)	1.27	2.64	5.26
F Calculated Value ($df = 4$)		4.18	12.40
F^{a} Critical Value (P = 0.05) (df = 4)		9.61	9.61
t Calculated Value t Critical Value (P = 0.05)		1.65 (df = 8) 2.31 (df = 8)	2.14 (df = 4) 2.78 (df = 4)

Table 4.5:Statistical comparisons of solutions with the same total arsenic concentration
(8 ppb) but with different ratios of As(III)/As(V).

 F^{a} is the critical value of two-tailed test. df = degree of freedom. The F and |t| values were calculated with respect to As(III).

4.2.4 Quality assurance

(a) Calibration curves and linearity range

The As(V) calibration curve was studied in two different procedures, which is the multiple standard additions of As(V) and the multiple standard additions of As(III). The *'in situ'* calibration curves for As(V) and As(III) are shown in Figure 4.26 and Figure 4.27 respectively. The average correlation coefficients for As(V) and As(III) calibration plots were 0.9977 and 0.9989 respectively. In this investigation, As(V) quantification was simple and sufficiently fast due to the rapid reduction of As(V) to As(III) by thiosulfate. Although the average correlation coefficients for three repeated calibration curves of As(V) to As(III) are almost similar and fitted a straight line, it still remains that As(V) is electroinactive and could not be detected electrochemically. Before it could be quantified, As(V) has to be reduced to As(III).



Figure 4.26: The calibration curve obtained with 9 aliquots of As(V) added into 2 M HCl solution containing 2 ppb As(V). 10 ppm Cu(II) and 1.5 mM Na₂S₂O₄. Each point represents the mean of three determinations. ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz).



Figure 4.27: The calibration curve obtained with 9 aliquots of As(III) added into 2 M HCl solution containing 2 ppb As(V), 10 ppm Cu(II) and 1.5 Mm Na₂S₂O₄. Each point represents the mean of three determinations. ($E_d = -0.45$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz).

According to Miller & Miller (1993), two analytical methods at different analyte concentrations were compared using a regression line in which the more precise method is plotted on the x-axis. In this study, the use of regression line to compare two analytical methods were conducted based on the *'in situ'* calibration by standard additions of As(V) and As(III) respectively in which the most precise method is plotted on the x-axis. However, based on the F-test, there was no significant difference in the variances of both methods. Thus, the results of the peak currents of either these two methods could be plotted on the x-axis. As can be seen in Figure 4.28, the result of the peak current of As(III) was plotted on the x-axis.



Figure 4.28: Comparison of two analytical methods using regression line. The results were obtained from SWCSV peak currents of 9 aliquots of 2 ppb arsenic added into solution containing 2 M HCl, 10 ppm Cu(II) 1.5 mM Na₂S₂O₄ and 2 ppb As(V). The results on x-axis was spiked with 9 x 2 ppb As(III); y-axis was spiked with 9 x 2 ppb As(V). ($E_d = -0.45$ V [As(V)]; $E_d = -0.35$ V [As(III)], $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz).

Based on the method suggested by Miller & Miller (1993), a good linear correlation coefficient, $r^2 = 0.9988$ obeying the equation $y = (1.296 \pm 0.037) \times - (3.152 \pm 0.432)$ was obtained at 95 % confidence levels. Since the calculated intercept and slope at 95 % confidence levels does differ significantly from 0 and 1 respectively, this regression line indicated the existence of systematic errors between the two calibration methods. Not only that, random errors might also occurred in both methods. According to Miller & Miller (1993), in most analyses, the relative standard deviation is roughly constant over a range of concentrations. Hence, the absolute error increases with the concentration of analyte, rather than having the similar value at all concentrations. Thus, the calibration curve of As(V) could not be determined by the standard addition of As(III) but by the standard addition of As(V) solution.

The calibration curve of As(V) by standard addition was investigated in the range of 0 – 200 μ g/L in the presence of thiosulfate. The calibration curve with correlation coefficient, r² = 0.9805 is shown in Figure 4.29. As shown in Figure 4.29, repeated trials showed that the linearity range of As(V) was up to about 125 μ g/L which is narrower than the linearity range of As(III) without the presence of thiosulfate. In this case, only when the total amount of As(V) in the standard additions exceeding 125 μ g/L would dilution be considered.



Figure 4.29: Calibration curve of As(V) in the range of $0 - 200 \ \mu g/L$ in 2 M HCl containing 10 ppm Cu(II) and 1.5 Mm Na₂S₂O₄. (E_d = -0.45 V, t_d = 90 s, E_{sw} = 60 mV, f= 149 Hz).

(b) Limit of Detection

Figure 4.30 shows the calibration curve of As(V) in the range of 0 to 14 μ g/L. The LOD calculated from the pooled standard deviation of the y-residuals from the calibration curve was estimated to be 0.648 μ g/L. The calibration curve was linear over the range of 0 – 14 μ g/L with a correlation coefficient, r² = 0.9983, obeying the equation y = (1.059 ± 0.043) x – (0.183 ± 0.362).



Figure 4.30: Calibration curve of As(V) in the range of $0 - 14 \mu g/L$ in 2 M HCl solution containing 10 ppm Cu(II) and 1.5 Mm Na₂S₂O₄. Error bars represent one standard deviation at 95 % confidence level. (E_d = -0.45 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz).

(c) Within-run precision

The repeatability of As(V) was obtained by conducting 10 replicates measurements of As(V) in a day. The standard deviations of 10 replicates measurements of peak currents taken at 2 ppb As(V) is summarized in Table 4.6. The calculated standard deviation (RSD) obtained at 5.58 % (n = 10) suggested that the within-run precision of the method was satisfactory with good repeatability.

Number of measurement	Mean Peak Current (µA)		
1	3.337		
2	3.560		
3	3.193		
4	3.073		
5	3.293		
6	3.077		
7	3.070		
8	3.563		
9	3.323		
10	3.297		
Mean peak current (µA)	3 279		
Standard deviation (µA)	0.183		
RSD (%)	5.58		

 Table 4.6:
 Within-run Precision calculated from the mean RSD of 10 replicates measurements of 2 ppb As(V) peak currents.

(d) Between-run precision

Table 4.7 shows the calculated between-run precision of 10 replicates measurements of As(V) conducted within 5 days over a period of two weeks. The relative standard deviation (RSD) was calculated at 10.53 % (n = 10) at 5 ppb As(V) for 90 s deposition time. Although the RSD is relatively high, it is still acceptable with satisfactory reproducibility. This could be due to errors that might occur during the preparation of the standard samples, thus, affecting the results obtained within 5 different days in the range of two weeks.

(e) Accuracy and recovery of the reduction of As(V) to As(III)

The accuracy and recovery of the reduction of As(V) to As(III) were conducted in three standard samples with different known As(V) concentrations. Since As(V) is electroinactive, it needs to be reduced to As(III) prior to determination. The results of the recovery tests conducted on spiked purified water samples in the range of 5 µg/L to 50 µg/L As(V) were summarized in Table 4.8. As can be observed in Table 4.8, the calculated recoveries in the form of As(III) (As(V) was reduced to As(III)) were in the range of 102.8 % to 115 %. Although the recovery range is higher than that for As(III), the recoveries of As(V)in the form of As(III) is still satisfactory with an acceptable range in which As(V) was reduced to As(III) in the presence of thiosulfate. The calculated relative standard deviation (RSD) of the three samples was in the range of 1 % to 16 %. In sample A, the presence of As(III) did not interfere with the recovery of As(V) as As(III).

Number of measurement	Mean Peak Current (µA)	Standard Deviation (s)	RSD (%)
1	1.48	0.26	17.57
2	1.84	0.12	6.52
3	1.52	0.16	10.53
4	1.28	0.21	16.41
5	1.48	0.08	5.41
6	1.44	0.06	4.17
7	1.01	0.03	2.97
8	1.20	0.12	10.00
9	1.11	0.25	22.52
10	0.94	0.09	9.57
Mean	1.33	0.14	10.53

Table 4.7:Between-run Precision calculated from the mean RSD of 10 replicates
measurements of 5 ppb As(V) peak currents.

_

Sample	А	В	С
As(V) added (µg/L) As(III) added (µg/L)	5 5	10 0	50 0
Total As(III) measured (µg/L) (Three replicates)	1. 10.114 2. 10.544 3. 9.773	13.251 11.891 9.543	52.833 54.759 54.104
Reduced As(V) (as As(III)) (µg/L) Calculated by difference (Total As(III) measured – As(III) added)	5.114 5.544 4.773	13.251 11.891 9.543	52.833 54.759 54.104
$ \begin{array}{l} Mean \pm SD^{a} \left(\mu g/L \right) \\ Recovery \pm SD \left(\% \right) \\ RSD \left(\% \right) \end{array} $	$5.14 \pm 0.39 \\ 102.8 \pm 7.8 \\ 7.59$	$\begin{array}{c} 11.56 \pm 1.88 \\ 115.6 \pm 18.8 \\ 16.26 \end{array}$	$53.90 \pm 0.98 \\ 107.8 \pm 1.96 \\ 1.82$

Table 4.8:Results obtained for As(V) spiked purified water samples.

^amean value \pm corresponding standard deviation of three independent determinations

4.3 Effect of interferences

Since this work investigates the effect of interferences in the determination of inorganic As(III) and As(V) in natural waters, only compounds that can exist in natural waters were being investigated for interferences. Thus, the interference of surface active compounds was not considered. In this study, the possible influence of the ions Fe^{3+} , Zn^{2+} and Cd^{2+} added at a maximum 1000 µg/L (1 ppm) on the stripping peak of As(III) were tested and the results are summarized in Table 4.9.

These trace elements may deposit on the HMDE or compete with arsenic in its reaction with copper or even in the formation of other compounds with arsenic or arsenic and copper, such as, in the presence of Fe^{3+} , Fe^{3+} might formed compound with either As(III) or Cu(II) or even with both, forming a compound of Fe_x -As_y-Cu_z which may cause interferences in arsenic detection.

Concentration of Ions	Increment or Decrement ^a (%)
500 μg/L Fe ³⁺	-54.0
1000 μg/L Fe ³⁺	-58.0
500 μg/L Cd ²⁺	-79.0
1000 μg/L Cd ²⁺	-57.0
500 μg/L Zn ²⁺ 1000 μg/L Zn ²⁺	+ 1.01 + 1.20

 Table 4.9:
 Percentage increase or decrease of the peak currents of three ions towards As(III)

^a '+' denotes increment and '- ' denotes decrement.

Figure 4.31 shows the voltammograms of the influence of 500 µg/L to 1000 µg/L Fe³⁺ added into 2 M HCl solution containing 10 ppm Cu(II) and 10 ppb As(III). When 500 µg/L of Fe³⁺ was added into the solution, the Fe³⁺ caused 54.0 % depression of the arsenic peak current. However, when the Fe³⁺ concentration was doubled to 1000 µg/L, more than 58.0 % of arsenic peak suppression was observed. In the interference of Cd²⁺, the addition of 500 µg/L and 1000 µg/L of Cd²⁺ caused 79.0 % and 57.0 % decrement of the arsenic peak current respectively. The effect of these metals on the decrement of As(III) peak current may be attributed to the competitive formation of intermetallic compounds either with Cu(II) or As(III), thus, decreasing the amount of copper arsenide formed during the deposition step and hence, suppressed the response (Adeloju *et al.*, 1999).



Figure 4.31: SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 10 ppb As(III). The concentration of Fe³⁺ added : (a) 0 μ g/L; (b) 500 μ g/L; (c) 1000 μ g/L. (E_d = -0.35 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz).

The influence of Zn^{2+} towards the arsenic peak current is shown in Figure 4.32. For Zn^{2+} , the presence of 500 µg/L caused 1.01 % enhancement of the arsenic peak current. When the Zn^{2+} concentration was doubled to 1000 µg/L, 1.20 % of the peak increment was observed.



Figure 4.32: SWCS voltammograms of 2 M HCl containing 10 ppm Cu(II) and 10 ppb As(III). The concentration of Zn^{2+} added : (a) 0 µg/L; (b) 500 µg/L; (c) 1000 µg/L. (E_d = -0.35 V, t_d = 90 s, E_{sw} = 60 mV, f = 149 Hz)

4.4 Application on environmental samples

4.4.1 Water pretreatment

The water samples collected at different depth (surface, 6 metre and 11 metre) from Tasik Biru, Bau were filtered through 0.45 μ m membrane filter in order to remove suspended particulates available in the water samples which might interfere with quantification. In

addition, the water samples were acidified to $pH \sim 2$ with hydrochloric acid in order to prevent the deposition of arsenic during storage.

4.4.2 Determination of As(III) and total inorganic arsenic

The water samples collected from different depth (surface to 11m) at Tasik Biru were analyzed. The calibration graphs and concentrations obtained were automatically generated by the voltammetry software and the results derived from the software were summarized in Table 4.10. The result showed that the water samples collected contained both oxidation states of arsenic at different concentrations.

In the determination of As(III) and total inorganic arsenic, Student's t-test at 95 % confidence level was used to compare the concentration measured at certain depth with the depth nearest to it. In both measurement, it was found that there was no significant difference in the concentrations of As(III) and total inorganic arsenic at different depths profile, although each depth varies in concentration. Although these variations are not clearly understood, it could be due to the different oxic (oxygen level) conditions existed in the lake.

In the determination of total inorganic arsenic in the environmental samples, poorly defined or broad curves were detected in the first few scans which made it unsuitable for quantification. This could be due to the presence of matrix interference in the environmental samples which might interfered with the reduction of As(V) to As(III) and also the accumulation of As(III) at the surface of the electrode. However, with repeated scanning, the matrix interference have been minimized and As(V) became dominant. Thus, As(V) could be

successfully reduced to As(III) and the reduced As(III) could be accumulated onto the surface of the electrode resulting in a well defined quantifiable curves. Figure 4.33 and 4.34 shows the voltammograms obtained from the detection of As(III) and total inorganic arsenic in the environmental samples at 6 m depth. As can be seen in the voltammograms, well defined peak currents were obtained with repeated scanning.

As(V) concentrations were obtained from the difference of total inorganic arsenic and As(III) concentrations. As can be seen in Table 4.10, As(V) concentrations ranged from 0.10 mg/L to 0.19 mg/L were obtained from the calculation of the difference between total inorganic arsenic and As(III) concentrations. As(III) concentrations were the highest measured in all three depths indicating As(III) is the dominant form of inorganic arsenic species in Tasik Biru, Bau.

The highest As(III) concentration measured was at 11 m, where as the lowest As(III) concentration measured was at the surface of the lake. For As(V) concentrations, the highest and lowest concentrations measured were at the surface and 11 m depth respectively. Figure 4.35 shows one of the calibration graphs used to derive the concentration of total inorganic arsenic (11 m depth) in the water samples. The variations in the concentrations of As(III) and As(V) in lake water could probably be due to several factors such as redox conditions and biological activity, an increasing ratio of As(III) to As(V) with depth and an influx of mining-contaminated sediment pore waters at the sediment water interface (Smedley & Kinniburgh, 2002). It could also be caused by the aerobic and anaerobic system that might exist in the natural water.
The depletion of oxygen levels in the bottom lake water as a result of biological activity and abundant of nutrients are likely cause of higher As(III) concentration in the deeper depth (Smedley & Kinniburgh, 2002). While at the surface of the water, the concentration of As(V) is dominant which could be contributed by the conversion of As(III) to As(V) under a well aerated surface water.

As presented in Table 4.10, it can be seen that Tasik Biru contained high concentrations of total inorganic arsenic. The concentrations of arsenic in Tasik Biru is 10 to 48 more times higher than the maximum permissible value of arsenic in drinking water recommended by WHO (10 μ g/L). Hence, it can be concluded that Tasik Biru is not safe for consumption or any recreational activities.



Figure 4.33: SWCS voltammograms of determination of As(III) in water samples from Tasik Biru (6 m depth) in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz. (Each scan was repeated three times)



Figure 4.34: SWCS voltammograms of determination of total inorganic arsenic in water samples from Tasik Biru (6 m depth) in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz. (Each scan was repeated three times)



Figure 4.35: Calibration graph obtained from the determination of total inorganic arsenic (11 m depth) of water sample from Tasik Biru in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz. (Each determination of the water samples was repeated three times and the mean concentration is the average of three determinations. Voltammetry software automatically generated the calibration graph and concentration)

Sample	Surface	6 m	11 m
As(III) measured (mg/L)	0.136 0.328 0.202	0.272 0.365 0.288	0.223 0.413 0.499
Mean As(III) \pm SD (mg/L)	0.22 ± 0.10	0.31 ± 0.05	0.38 ± 0.14
Total inorganic arsenic measured (mg/L) Mean ± SD (mg/L)	$\begin{array}{c} 0.434 \\ 0.431 \\ 0.364 \\ 0.41 \pm 0.04 \end{array}$	$\begin{array}{c} 0.471 \\ 0.433 \\ 0.464 \\ 0.46 \pm 0.02 \end{array}$	$\begin{array}{c} 0.431 \\ 0.494 \\ 0.505 \\ 0.48 \pm 0.04 \end{array}$
As(V) Calculated by difference (mg/L)	0.298 0.103 0.162	0.199 0.068 0.176	0.208 0.081 0.006
Mean As(V) \pm SD (mg/L)	0.19 ± 0.10	0.15 ± 0.07	0.10 ± 0.10

Table 4.10:Determination of inorganic As(III) and total inorganic arsenic in
environmental samples collected from Tasik Biru, Bau.

4.4.3 Determination of Arsenic total

The determination of arsenic total was conducted on the digested samples collected from Tasik Biru, Bau at different depth profile. The calibration graphs and concentrations obtained were automatically generated by the voltammetry software as shown in Figure 4.36, the calibration graph obtained from the software at 6 m depth. The results of the concentrations of arsenic total at different depth level derived from voltammetry software were summarized in Table 4.11. The total concentration of arsenic was the highest at 6 m depth and lowest at 11 m depth.

Based on Table 4.11, student's t-test at 95 % confidence level was used to compare the difference in the concentrations obtained at a certain depth with the depth nearest to it. According to the student's t-test, there was no significance difference of arsenic total concentration detected in the water samples collected from surface to the 11 m depth although each depths varies in concentrations with 6 m depth recorded the highest arsenic total concentration.

The difference in the concentrations of arsenic total in digested water samples and total inorganic arsenic in the water samples at the same level were compared by applying student's t-test at 95 % confidence level. Based on the results obtained from the student's t-test, the concentrations of arsenic total from the surface to 11 m depth are significantly difference than the concentrations of total inorganic arsenic at the same depth level. This could be contributed by the presence of organoarsenic compounds in the water. The concentration of organic arsenic compounds that can be present in the lake water largely

depends on biological processes in which organic arsenicals compounds are produced by the transformation of inorganic arsenic salts and also affected by human activities such as agriculture. Thus, the chemical species of arsenic that exist in the water are in the form of organoarsenic compounds and inorganic arsenic.

As a verification of the results obtained using SWCSV, the determination of arsenic total in the digested sample was also conducted using Atomic Absorption Spectrometry (AAS) instrument. The results obtained from both SWCSV and AAS method are summarized in Table 4.12. Figure 4.37 shows the calibration graph with $r^2 = 1.000$ obtained from the detection of arsenic standard solution from blank to 10 ppm, 20 ppm and 30 ppm by AAS instrument. The calibration graph is used to derive the concentration of arsenic in the water samples. The arsenic total concentration increases up to 6 m depth in which it decreases at a depth of 11 m. Based on Table 4.12, student's t-test at 95% confidence level was used to compare the difference in the arsenic total concentrations detected by AAS at a certain depth with the depth nearest to it. Although the concentration of arsenic total from surface to 11 m varies with 11 m recorded the highest arsenic total concentration, based on the student's t-test, there was no significance differences in the arsenic total concentration between the depth in the water samples. Based on Table 4.12, there are significant differences in the concentration range between these two different detection methods and F-test at 95 % confidence level was employed to compare the significant difference in the concentration obtained. According to the two-tailed F-test conducted, there was no significant difference in the standard deviation of the concentration of arsenic total detected with SWCSV and AAS. Thus, SWCSV method is suitable and sensitive for the routine analysis of arsenic in the environmental samples.



Figure 4.36: Calibration graph obtained from the determination of arsenic total (6 m depth) of digested water sample collected from Tasik Biru in 2 M HCl, 10 ppm Cu(II) and 1.5 mM thiosulfate run at $E_d = -0.35$ V, $t_d = 90$ s, $E_{sw} = 60$ mV, f = 149 Hz. (Each determination of the water samples was repeated three times and the mean concentration is the average of three determinations)



Figure 4.37: Calibration graph obtained from the detection of arsenic standard solution (blank, 10 mg/L, 20 mg/L and 30 mg/L) by AAS instrument. The calibration graph exhibited a linearity with $r^2 = 1.000$.

Sample	Surface	6 m	11 m
As Total measured (mg/L)	1.660 1.348 1.602	1.534 1.630 1.722	1.626 1.690 1.132
Mean Total Arsenic measured ± SD (mg/L)	1.54 ± 0.17	1.63 ± 0.09	1.48 ± 0.31

Table 4.11:	Determination of arsenic total in digested environmental samples collec	ted
	from Tasik Biru, Bau.	

Depth	SWCSV*	AAS*	
Surface 6 m 11 m	$\begin{array}{l} 1.54 \pm 0.17 \ \text{mg/L} \\ 1.63 \pm 0.09 \ \text{mg/L} \\ 1.48 \pm 0.31 \ \text{mg/L} \end{array}$	$\begin{array}{l} 1.82 \pm 0.12 \ mg/L \\ 1.88 \pm 0.06 \ mg/L \\ 1.38 \pm 0.14 \ mg/L \end{array}$	

Table 4.12:Comparison between the results obtained by SWCSV and by AAS for the
determination of arsenic total (digested samples)

*The results obtained was the Mean As Total ± SD (mg/L)

CHAPTER 5

CONCLUSION

In the initial investigation, cyclic voltammetry (CV) was used for the determination of As(III) in order to obtain the qualitative information about the redox reaction of As(III) at the surface of the mercury electrode. It was observed that in 2 M HCl containing 10 mg/L Cu(II) and 5 μ As(III), only cathodic peak was detected at about – 0.8 V and no anodic peak was detected at the reverse potential indicating the irreversible process for re-oxidation of As(III).

For the analysis of inorganic As(III), the optimum deposition potential, Cu(II) concentration and deposition time was at – 0.35 V, 10 ppm and 90 s respectively. A longer deposition time means that the analyte are deposited at the surface of HMDE with a longer period. Pulse amplitude that gave the most sensitivity was at 60 mV, where as, frequency at 149 Hz was employed for all the determinations because it is the limit maximum frequency allowed in the software programme. The newly optimized method exhibited linearity range up to 1 mg/L with calculated limit of detection was estimated to be 0.076 μ g/L. This newly modified also exhibited a good range of precision and accuracy. No significant interference was observed with Zn²⁺, where as, the addition of Cd²⁺ and Fe³⁺ caused significant peak suppression.

In the analysis of total inorganic arsenic, a preliminary step was required for the reduction of As(V) to As(III) and then followed by the reduction of As(III) species. The optimized deposition potential and Cu(II) concentration in the presence of 3.2 mM thiosulfate

was at -0.45 V and 10 mg/L respectively. Even though the peak current increases with the increasing deposition time up to 300 s, deposition time at 90 s was selected in order to shorten the time of analysis. The optimized thiosulfate concentration that was required for the reduction of As(V) to As(III) was obtained at 1.5 mM and was used for all total inorganic arsenic and arsenic total determinations. Linearity range up to 125 µg/L was obtained with calculated limit of detection was estimated to be at 0.648 µg/L.

The newly optimized method was found to be suitable for the determination of toxic inorganic As(III) in low $\mu g/L$ concentration even in the presence of inorganic As(V) and was applied for the determination of inorganic As(III) in the environmental samples collected from Tasik Biru, Bau. Since As(V) is electroinactive and a reduction step is required prior to itd determination, the optimized method for total inorganic arsenic [As(III) + As(V)] was applied in the environmental samples collected from Tasik Biru, Bau, in which the concentrations of As(V) in the environmental samples was obtained by calculating the differences between the concentration of [As(III) + As(V)] and As(III). According to Student's t-test, no significant differences were observed in the concentrations of inorganic As(III) and total inorganic arsenic [As(III) + As(V)] at different depth profile. The results showed that the environmental samples contained high concentrations of both As(III) and As(V) species which were more than 10-48 times higher than the maximum permissible value of $10\mu g/L$ arsenic in the drinking water recommended by WHO. Analysis of the digested samples showed the presence of organoarsenic compounds in the water samples collected from Tasik Biru. It can be concluded that Tasik Biru is not safe for human consumption and any recreational activities.

This work shows that besides using other analytical instrumentation which is more expensive and complicated such as AAS, IC and HPLC, voltammetry is a reliable, sensitive and inexpensive instrument technique which can be employed for the measurement and speciation of arsenic in the environmental samples. Unlike any other previous method for the reduction of As(V) to As(III) which requires heating of the samples prior to determination, in this study, a less aggressive reducing agent, sodium thiosulfate, was employed in the reduction of As(V) to As(III). The optimum condition for the reduction process was also studied in the determination of total inorganic arsenic [As(III) + As(V)]. The overall procedure was simple as there was no need for sample heating or removal of the excess reducing agent before the voltammetric measurement. The reduction process was also fast as it could be completed during the preparation of the solution for analysis. This work also shows that SWCSV is suitable for quantitative measurement of arsenic.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE WORKS

Although the analysis of arsenic using SWCSV produced a satisfying result, there is still room for improvement in this technique in order to ensure a better analysis in the future. A better analysis to determine SWCSV suitability for the determination of heterogeneous samples should be further studied and validated. In order to ensure its validity and applicability for arsenic speciation analysis, SWCSV analysis of arsenic should be evaluated and tested further on environmental, biological and soil samples and also drinking water. In method validation, since environmental samples have different matrices compared with purified water, recovery test could be conducted by spiking environmental water samples instead of purified water with either As(III) or As(V) standard solution or even both elements and analysed by SWCSV.

The analysis of arsenic using the newly modified method could be conducted using different working electrodes material such as platinum or gold. Besides using AAS as a verification purposes, the results obtained using SWCSV could also be further verified with other more established methods such as ICP-MS or Ion Chromatography (IC). Since the concentrations and relative proportions of As(III) and As(V) vary according to redox conditions and biological activity, in future study, the redox potential and dissolved oxygen of the environmental samples at different depth could also be measured in order to understand the variation of the inorganic and organic samples in natural water. Not only that, the

environmental samples should also be sampled more than once in order to study the concentrations and variations of arsenic in the environmental samples.

REFERENCES

- Adeloju, S.B., Young, T.M., Jagner, D., and Batley, G.E. (1999). Constant current cathodic stripping potentiometric determination of arsenic on a mercury film electrode in the presence of copper ions. *Analytica Chimica Acta*, **381**, 207-213.
- Ahuja, S. (2008). Contamination of groundwater : Mechanism, analysis and remediation.John Wiley & Sons, Hoboken.
- Andrea, M.O. (1978). Distribution and speciation of arsenic in natural waters and some marine algae. *Deep-Sea Research*, **25**, 391-402.
- Andrea, M.O. (1979). Arsenic speciation in seawater and interstitial waters: The influence of biological-chemical interactions in the chemistry of a trace element. *Limnology Oceanography*, 24, 440-45.
- ATSDR (2007). Public health statement: Arsenic. Agency for Toxic Substances and Disease Registry, United States of America.
 Available from: http://www.atsdr.cdc.gov/toxprofiles/phs2.html
 Accessed on: 3/5/2010
- Bard, A.J., and Faulkner, L.R. (1980). *Electrochemical methods: Fundamentals and applications*, John Wiley & Sons, Inc. pp. 1-248.

- Bard, A.J., and Faulkner, L.R. (2001). *Electrochemical methods: Fundamentals and applications*, 2nd edition. John Wiley & Sons, Inc. pp. 1-466.
- Barra, C.M., and Correia dos Santos, M.M. (2001). Speciation of inorganic arsenic in natural waters by square-wave cathodic stripping voltammetry. *Electroanalytical Chemistry*, 13(13), 1098-1104.
- Batley, G.E. (1989). *Trace element speciation: Analytical methods and problems*. CRC Press, Inc.
- Batley, G.E. (1989). Collection, preparation and storage of samples for speciation analysis. In:
 Trace element speciation analytical methods and problems (Batley, G.E. ed.), CRC
 Press, Inc. pp 1-9
- Berg, M., Tran, H.C., Nguyen, T.C., Pham, H.V., Schertenleib, R., Giger, W. (2001). Arsenic contamination of groundwater and drinking water in Vietnam: A human health threat. *Environmental Science and Technology*, 35, 2621-2626.
- Bhumbla, D.K. and Keefer, R.F. (1994). Arsenic mobilization and bioavailability in soils. In: Arsenic in the environment. Part I: Cycling and characterization (Nriagu, J.O. ed.), John Wiley & Sons, Inc. pp. 51-74.
- Bond, A.M. (1980). *Modern polarographic methods in analytical chemistry*. Marcel Dekker, Inc. pp. 169-235; 435-468.

- Borgono, J. M. and Greiber, R. (1972). Epidemiological study of arsenism in the city of
 Antofagasta. In: *Trace substances in environmental health*. (Hemphill, D.D. ed.),
 University of Missouri. pp. 13-24.
- Bott, A.W. (1995). Voltammetric determination of trace concentrations of metals in the environment. *Current Separations*, **14**(1), 24-30.
- Britton, W.E., Brooks, M.A., Curran, D.J., Dryhurst, G., Enke, C.G., Faulkner, L.R., Frank, S.N., Fry, A.J., Galus, Z. and Geiger, W.E. (1984). *Laboratory Techniques in electroanalytical Chemistry* (Kissinger, P.T. & Heineman, W.R. eds.), Marcel Dekker, Inc.
- Brook, F.J., and Moore, J.N. (1988). Particle-size and chemical control of As, Cd, Cu, Fe, Mn, Ni, Pd and Zn in bed-sediment from the Clark Fork River, Montana (U.S.A). *Science* of the Total Environment, **76**, 247-266.
- Cebrian, M.E., Albrose, A., Aguilar, M. and Blakely, E. (1983). Chronic arsenic poisoning in the North of Mexico. *Human Toxicology*, **2**, 121-133.
- Chakraborti, D., Nichols, D.L. and Irgolic, K.J. (1984). Determination of arsenite and arsenate by differential pulse polarography. *Fresenius Z Analytical Chemistry*, **319**, 248-251

- Chakraborti, D., Das, B., Rahman, M.M. (2008 in press). Status of groundwater arsenic contamination in the state of West Bengal, India: A 20 years study report. *Molecular Nutrtion and Food Research*.
- Chatterjee, A. (1994). Ground water arsenic contamination in residential area and surroundings of P. N. Mitra Lane, Calcutta, due to industrial effluent discharge. Ph.D. thesis, Jadavpur University, Calcutta, India.
- Chatterjee, A., Das, D., Mandal, B.K., Chowdhury, T.R., Samantha, G. and Chakraborty, D. (1995). Arsenic in groundwater in six districts of West Bengal, India: The biggest arsenic calamity in the world. In: Part 1. Arsenic species in drinking water and urine of the affected people. *Analyst*, **120**, 643-656.
- Chen, C.J., Chen, C.W., Wu, M.M., and Kuo, T.L. (1992). Cancer potential in liver, lung, bladder, and kidney due to ingested inorganic arsenic in drinking water. *British Journal of Cancer*, **66**, 888-892.
- Chen, C.J., and Lin, L.J. (1994). Human carcinogenicity and atherogenicity induced by chronic exposure to inorganic arsenic. In: *Arsenic in the Environment, Part II, Human Health and Ecosystems Effects* (Nriagu, J.O. ed.), Wiley, New York.
- Chu, H.Y. (1994). Arsenic distribution in soils. In: Arsenic in the environment. Part I: Cycling and characterization (Nriagu, J.O. ed.), John Wiley & Sons, Inc. pp. 17-41.

- Chunguo, C., and Zihui, L. (1988). Chemical speciation and distribution of arsenic in water, suspended solids and sediment of Xiangjiang River, China. *Science of the Total Environment*, **77**, 69-82.
- Clement, W.H., and Faust, S.D. (1981). The release of arsenic from contaminated sediments and muds. *Journal Environmental Science Health*, A16, 87-122.
- Crecelius, E.A., Johnson, C.J., and Hofer, G.C. (1974). Contamination of soils near a copper smelter by arsenic, antimony, and lead. *Water, Air, Soil Pollution,* **3**, 337-342.
- Cullen, W.R., and Reimer, K.J. (1989). Arsenic speciation in the environment. *Chemical Reviews*, **89**, 713-764
- Dang Q. Hung., Olga Nekrassova, and Richard G. Compton. (2004). Analytical methods for inorganic arsenic in water: a review. *Talanta*. **64**, 269-277.
- Dasgupta, P.K., and Huang, H. (1999). A field-deployable instrument for the measurement and speciation of arsenic in potable water. *Analytica Chimica Acta*, **380**, 27-37.
- Demesmay C., Olle M. and Porthault M. (1994) Arsenic speciation by coupling high performance liquid chromatography with induced coupled plasma mass spectrometry. *Fresenius J Analytical Chemistry*, **348**, 205-210.

- de Oliveira, E., McLaren, J.W., and Berman, S.S. (1983). Simultaneous determination of arsenic, antimony, and selenium in marine samples by inductively coupled plasma atomic emission spectrometry. *Analytical Chemistry*, **55**, 2047-2050.
- Done, A.K. and Peart, A.J. (1971). Arsenic: Occurrence, toxicity and speciation techniques. *Water Research*, **34**(**17**), 4304-4312.
- Eaton, D., Wang, H.C. and Northington, J. (1998). *Analytical chemistry of arsenic in drinking water*. American Water Works Association Research Foundation and American Water Works Association, USA.
- Evans, L.J. (1989). Chemistry of metal retention by soils. *Environmental Science Technology*, **23**, 1046-1056.
- Farrell, J.R., Iles, P.J. and Yuan, Y.J. (1996). Determination of arsenic by hydride generation gas diffusion flow injection analysis with electrochemical detection. *Analytica Chimica Acta*, 334, 193-197.
- Feng, Y.L., Chen, H.Y., Tian, L.C., and Narasaki, H. (1998). Off-line separation and determination of inorganic species in natural water by high resolution inductively coupled plasma mass spectrometry with hydride generation combined with reaction of As(V) and L-cysteine. *Analytica Chimica Acta*, **375**, 167-175.

- Ferreira, M.A., and Barros, A.A. (2002). Determination of As(III) and As(V) in natural waters by cathodic stripping voltammetry at a hanging mercury drop electrode. *Analytica Chimica Acta*, **459**, 151-159.
- Ferguson, J.F., and Gavis, J. (1972). A review of the arsenic cycle in natural water. *Water Research*, **6**, 1259-1274.
- Finkelman, R.B., Belkin, H.E., and Zheng, B. (1999). Health impacts of domestic coal use in China. *Proceedings of the Natural Academy of Sciences (U.S.A.).* **96**, 3427-3431.

Florence, T.M. (1982). The speciation of trace elements in waters. *Talanta*, 29, 345-369.

- Florence, T.M. (1989). Electrochemical techniques for trace elements speciation in waters. In: *Trace element speciation analytical methods and problems* (Batley, G.E. ed.), CRC Press, Inc. pp 77-107
- Frankenberg Jr., W.T. (2002). *Environmental chemistry of arsenic*. Marcel Dekker, Inc. pp 1-183.
- Feldmann, J., Grumpling, R. and Hirner, A.V. (1994). Determination of volatile metals and metalloid compounds in gases from domestic waste deposits with GC-ICP-MS. *Fresenius J Analytical Chemistry*, **350**, 228-235.

- Forsberg, G., O'Laughin, J.W., Megargle, R.G., and Koirtyohann, S.R. (1975). Determination of arsenic by anodic stripping voltammetry and differential pulse anodic stripping voltammetry. *Analytical Chemistry*, **47**, 1586-1592.
- Francesconi, K.A., Edmonds, J.S. and Morita, M. (1994). Determination of arsenic and arsenic species in marine environmental samples. In: Arsenic in the environment. Part I: Cycling and Characterization (Nriagu, J.O ed.), John Wiley & Sons, Inc. pp. 189-202.
- Francesconi, K.A. and Edmonds, J.S. (1997). Arsenic and marine organisms. Advances in Inorganic Chemistry, 44, 147-189.
- Francesconi, K.A. and Kuehnelt, D. (2002). Arsenic compounds in the environment. In: *Environmental chemistry of arsenic* (Frankenberger Jr., W.T. ed.), Marcel Dekker, Inc.
- Fry, A.J., and Britton, W.E. (1984). Solvents and supporting electrolytes. In: Laboratory techniques in electroanalytical chemistry (Kissinger, P.T. and Heineman, W.R. eds.), Marcel Dekker, Inc. pp. 367-381.
- Galus, Z. (1984). Mercury electrodes. In: Laboratory techniques in electroanalytical chemistry (Kissinger, P.T. and Heineman, W.R. eds.), Marcel Dekker, Inc. pp. 267-284.
- Gibbon-Walsh, K., Salaün, P. and van den Berg, C.M.G. (2010). Arsenic speciation in natural waters by cathodic stripping voltammetry. *Analytical Chimica Acta*, **662**, 1-8.

- Goessler, W. and Kuehnelt, D. (2002). Analytical methods for the determination of arsenic and arsenic compounds in the environment. In: *Environmental chemistry of arsenic* (Frankenberger, Jr. 2002 ed.), Marcel Dekker, Inc. pp. 27-44.
- Gorby, M.S. (1994). Arsenic in human medicine. In: Arsenic in the environment. Part I: Cycling and characterization (Nriagu, J.O. ed.), John Wiley & Sons, Inc. pp. 1-16.
- Grabinski, A.A. (1981). Determination of arsenic(III), arsenic(V), monomethylarsonate, and dimethylarsinate by ion-exchange chromatography with flameless atomic absorption spectrometric detection. *Analytical Chemistry*, **53**, 966-968.
- Greschonig, H., and Irgolic, K.J. (1992). Electrochemical methods for the determination of total arsenic and arsenic compounds. *Applied Organometallic Chemistry*, **6**, 565-577.
- Greulach, U., and Henze, G. (1995). Analysis of Arsenic(V) by cathodic stripping voltammetry. *Analytica Chimica Acta*, **306**, 217-223.
- Hata, N., Yamada, H., Kasahara, S., and Taguchi, S. (1999). Membrane solubilization with tetramethylammonium hydroxide for the preconcentration and electrothermal atomic absorption spectrometric determination of trace amount of arsenic in water. *Analyst*, 124, 23-26.

- Hawkridge, F.M. (1984). Electrochemical cells. In: Laboratory techniques in electroanalytical chemistry (Kissinger, P.T. & Heineman, W.R., eds.), Marcel Dekker, Inc. pp. 337.
- He, Y., Zheng, Y., Ramnaraine, M. and Locke, D.C. (2004). Differential pulse cathodic stripping voltammetric speciation of trace level inorganic arsenic compounds in natural water samples. *Analytica Chimica Acta*, **511**, 55-61.
- Heineman, W.R., Mark, Jr. H.B., Wise, J.A. and Roston, D.A. (1984). Electrochemical Preconcentration. In: *Laboratory techniques in electroanalytical chemistry* (Kissinger, P.T. and Heineman, W.R. eds.), Marcel Dekker, Inc. pp. 499-535.
- Heineman, W.R. and Kissinger, P.T. (1984). Larger-amplitude controlled-potential techniques.
 In: Laboratory techniques in electroanalytical chemistry (Kissinger, P.T. and Heineman, W.R. eds.), Marcel Dekker, Inc. pp. 51.
- Henke, K.R. (2009). Arsenic in natural environment. In: Arsenic-environmental chemistry, health, throats and waste treatment (Henke, K.R. ed.), John Wiley & Sons, Inc. pp. 69-235.
- Henze, G., Wagner, W. and Sanders, S. (1997). Speciation of arsenic(V) and arsenic(III) by cathodic stripping voltammetry in fresh water samples. *Fresenius J Analytical Chemistry*, 358, 741-744.

- Horowitz, A.J., Elrick, K.A., and Callender, E. (1988). The effect of mining on the sedimenttrace element geochemistry of cores from the Cheyenne River Arm of Lake Oahe, South Dakota, U.S.A. *Chemical Geology* **67**, 17-33.
- Horowitz, A.J., Elrick, K.A., and Cook, R.B. (1990). Arsenopyrite in the bank deposits of the Whitewood Creek-Belle Fourche-Cheyenne River-Lake Oahe systems, South Dakota, U.S.A. Science of the Total Environment. 97/98, 219-233.
- Howard, A.G., Apte, S.C., Comber, S.D.W., and Morris, R.J. (1988). Biogeochemical control of the summer distribution and speciation of arsenic in the Tamar Estuary. *Estuarine and Coastal Marine Science* **27(4)**, 427-443.
- Hughes, M.F., Thomas, P.J. and Kenyon, E.M. (2009). Toxicology and epidemiology of arsenic and its compounds. In: *Arsenic-environmental chemistry, health threats and waste treatment* (Henke, K.P. ed.), John Wiley & Sons, Ltd. pp. 237-264.
- Hung, D.Q., Wekrassora, O. and Compton, R.G. (2004). Analytical methods for inorganic arsenic in water: a review. *Talanta*, **64**, 269-277.
- Jain, C.K., and Ali, I. (2000). Arsenic : Occurrence, toxicity and speciation techniques. *Water Research*, **34(17)**, 4304-4312.

- Jekel, M.R. (1994). Removal of arsenic in drinking water treatment. In: Arsenic in the environment. Part I: Cycling and characterization (Nriagu, J.O. ed.), John Wiley & Sons, Inc. pp. 119-132.
- Johnston, R.P., Sarker, M.H., (2007). Arsenic mitigation in Bangladesh: National screening data and case studies in three upazilas. *Journal of Environmental Science and Health, Part A.* **42**, 1889-1896.
- Jones, H., Visoottiviseth, P., Md. Khoda Bux, Földenyl. R., Kováts, N., Borbély, G., Calbács,
 Z. (2008). Case Reports: Arsenic pollution in Thailand, Bangladesh, and Hungary.
 Reviews of Environmental Contamination, **197**, 163-186.
- Junsomboon, J., Sooksamiti, P., Grudpan, K., Lapanantnoppakhun, S., Thavornyuthikarn, P., and Jakmunee, J. (2009). Cathodic stripping voltammetric procedure for the determination of some inorganic arsenic species in water, soil and ores samples. *Chiang Mai Journal of Science* **363(3)**, 369-383
- Kamenev, A.I., Orlov, S.E., and Lyakhov, A.B. (2001). Electrochemical preconcentration of Arsenic(III) in its determination by stripping voltammetry at graphite electrodes modified with gold and copper. *Journal Analytical Chemistry*, **56(9)**, pp. 850-854.
- Kanazawa, A., Tohyama, T., and Baba, Y. et al. (2000). 40 years follow-up study on mental sequelae to an accidental mass arsenic poisoning in Japan. In: *Metal ions in biology*

and medicine (Centeno, J.A., Collery, P., Vernet, G., Finkelman, R.B., Gibb, H., Etienn J.C., eds.), pp. 74-76. Vol. 6. Rome: John Libbey Eurotext,

- Kissinger, P.T., and Heineman, W.R. (1984). *Laboratory techniques in electroanalytical chemistry*. Marcel Dekker, Inc.
- Kissinger, P.T. (1984). Small-Amplitude and related controlled-potential techniques. In: *Laboratory techniques in electroanalytical chemistry* (Kissinger, P.T. & Heineman, W.R. eds.), Marcel Dekker, Inc. pp. 157-158.
- Koch, I., Feldman, J., Wang, L., Andrewes, P., Reimer, K.J., and Cullen, W.R. (1999). Arsenic in the Meager Creek hot springs environment, British Columbia, Canada. Science of the Total Environment, 236, 101-107.
- Kopanica, M., and Norotoný. (1998). Determination of traces of arsenic(III) by anodic stripping voltammetry in solutions, natural waters and biological material. *Analytica Chimica Acta*, 368, 211-218.
- Kounaves, S.P. (1990). *Chapter 37: Voltammetric Techniques*. Department of Chemistry, Tufts University. pp. 709-722.
- Kumaresan, M., and Riyazuddin, P. (2001). Overview of speciation chemistry of arsenic. *Current Science*, **80(7)**, 837-846.

- Le, C.X. (2002). Arsenic speciation in the environment and humans. In: *Environmental chemistry of arsenic* (Frankenberger Jr., W.T. ed.), Marcel Dekker, Inc.
- Léonard, A. (1991). Arsenic. In: *Metals and their compounds in the environment* (Merian, E. ed.), Weinheim:VCH. pp. 751-774.
- Li, H. and Smart, R.B. (1996). Determination of sub-nanomolar concentration of arsenic(III) in natural waters by square wave cathodic stripping voltammetry. *Analyitca Chimica Acta*, **325**, 25-32.
- Londesborough, S., Mattusch, J., and Wennrich, R. (1999). Separation of arsenic species by HPLC-ICP-MS. *Fresenius Journal of Analytical Chemistry*, **363**, 577-581.
- Lu, F.J. (1990a). Review of fluorescent humic substances and Blackfoot disease in Taiwan. *Applied Organometallic Chemistry*, **4**, 191-195.
- Lu, F J. (1990b). Blackfoot Disease: Arsenic in humic acid. The Lancet. 336, 115-116.
- Luong, J.H.T., Majid, E. and Malc, K.B (2007). Analytical tools for monitoring of arsenic in the environment. *The Open Analytical Chemistry Journal*, **1**, 7-14.
- Mandal, B.K., Chowdhury, T.R., Samanta, G., Basu, G.K., Chowdhury, P.P., Chandra, C.R., Lodh, D., Karan, N.K., Dhar, R.K., Tamili, D.K., Das, D., Saha, K.C. and Chakraborti,

D. (1996). Arsenic in groundwater in seven districts of West Bengal, India: The biggest calamity in the world. *Current Science*. **70**, 976-986.

- Meier, P.C., Zünd, R.E. (1993). Statistical methods in analytical chemistry. John Wiley & Sons, Inc. pp. 1-76; 81-129
- Michalke, K., Wickenheiser, E.B., Mehring, M., Hirner, A.V. and Hensel, R. (2000).
 Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. *Applied and Environmental Microbiology*, 66, 2791-2796.
- Miller, J.C. and Miller, J.N. (1993). *Statistical for analytical chemistry, 3rd edition*. Ellis Harwood Limited. pp. 15-137.
- Mok, W.M., Shah, N.K. and Wai, C.M. (1986). Extraction of arsenic(III) and arsenic(V) in natural waters for neutron activation analysis. *Analytical Chemistry*, **58.** 110.
- Mok, W.M., Riley, J.A., and Wai, C.M. (1988). Arsenic speciation and quality of groundwater in a lead-zinc mine, Idaho. *Water Research*, **22**, 769-774.
- Mok, W.M., and Wai, C.M. (1990). Distribution and mobilization of arsenic and antimony species in the Coeur D'Alene River System, Idaho. *Environmental Science Technology*, **24**, 102-108.

- Morrison, G.M.P. (1989). Trace element speciation and its relationship to bioavailability and toxicity in natural waters. In: *Trace element speciation analytical methods and problems* (Batley, G.E. ed.) CRC Press, Inc. pp. 26-38.
- Myers, D.J., and Osteryoung, J. (1973). Determination of arsenic(III) at the parts-per-billion level by differential pulse polarography. *Analytical Chimica Acta*, **45**(2), 267-271.
- Naqvi, S.M., Vaishnavi, C. and Singh, H. (1994). Toxicity and metabolism of arsenic in vertebrates. In: Arsenic in the environment. Part II: Human health and ecosystem effects (Nriagu, J.O. ed.), John Wiley & Sons. pp. 55-77.
- Neeb, R. (1989). Stripping Voltammetry. Metrohm AG, 9101, Herisau, Switzerland.
- Niedzielski, P., Siepak, M., Przybylek, J. and Siepak, J. (2002). Atomic absorption spectrometry in determination of arsenic, antimony and selenium in environmental samples. *Journal of Environmental Studies*, **11(5)**, 457-466.
- Nriagu, J.O., and Azcue, J.M. (1990). Environmental sources of arsenic in food. *Advances in Environmental Science and Technology*, **23**, 103-127.
- Nriagu, O. J. (1994). Arsenic in the environment. Part I: Cycling and characterization. John Wiley and Sons, Inc. pp. 1-216.

- Nürnberg, H.W., and Mart, L. (1998). Water Chemistry. In: *The determination of trace metals in natural waters* (West, T.S. & Nürnberg, H.W. eds.), Blackwell Scientific Publications.
- Onishi, H., and Sandell, E.B. (1955). Geochemistry of arsenic. *Geochimica et Cosmochimica* Acta, 7, 1.
- Palus, A.F. (1990a). Voltammetry. In: Analytical Instrumentation handbook (Ewing, G.W. ed.), Marcel Dekker, Inc. pp. 603-619.
- Palus, A.F. (1990b). Coulometry. In: Analytical instrumentation handbook (Ewing, G.W. ed.), Marcel Dekker, Inc. pp. 661-673.
- Pinto, S.S. and Mcgill, C.M. (1953). Arsenic trioxide exposure in industry. *Industrial Medicine and Surgery*, **22**, 281-287.
- Peters, G.R., McCurdy, R.F., and Hindmarsh, J.T. (1996). Environmental aspects of arsenic toxicity. *Critical Reviews in Clinical Laboratory and Sciences*, **33**, 457-493.
- Peterson, P.J., Benson, L.M., and Zieve, R. (1981). Metalloids, Sec.3- Arsenic. In: *Effect of heavy metal pollution on plants*. (Lepp, N.W., ed.), Vol. 1, Applied Science Publ., London. pp. 299-322.

- Profumo, A., Merli, D. and Pesaranto, M. (2005). Voltammetric determination of inorganic As(III) and total inorganic As in natural waters. *Analytica Chimica Acta*, **539**, 245-250.
- Quastel, J.H. and Scholefield, P.G. (1953). Arsenic oxidation in soil. Soil Science, 75, 279.
- Ragaini, R.C., Ralston, H.R., and Roberts, N. (1977). Environmental trace metal contamination in Kellogg, Idaho, near a lead smelting complex. *Environmental Science Technology*, **11**, 773-781.
- Richardson, D.H.S. (1985). Application of voltammetry in environmental science. *Environmental Pollution*, **10**, 261-276.
- Xie, R., Johnson, W., Spayd, S., Hall H.S., and Buckley B. (2006). Arsenic speciation analysis on human urine using ion exchange chromatography coupled to plasma mass spectrometry. *Analyitca Chimica Acta*, **578**, 186-194.
- Sadana, R. (1983). Determination of arsenic in the presence of copper by differential pulse cathodic stripping voltammetry at a hanging mercury drop electrode. *Analytical Chemistry* **55**, 304-307.
- Salaün, P., Planer-Friedrich, B. and van der Berg, C.M.G. (2007). Inorganic arsenic speciation in water and seawater by anodic stripping voltammetry with a gold microelectrode. *Analyitca Chimica Acta*, 585, 312-322.

- Sandberg, G.R. and Allen, I.K. (1975). A proposed arsenic cycle in an agronomic ecosystem. *ACS Symposium Series*, **7**, 124-147.
- Sanders, J.G., Riedel, G.F. and Osman, R.W. (1994). Arsenic cycling and its impact in estuarine and coastal marine ecosystems. In: Arsenic in the environment. Part I: Cycling and characterization (Nriagu, J.O. ed.), John Wiley & Sons, Inc. pp. 289-308.
- Sawyer, D.T. and Robert, Jr. J.L. (1974). *Experimental electrochemistry for chemists*. John Wiley & Sons, Inc.
- Skoog, D.A. and Leary, J.J. (1992). *Principles of instrumental analysis*, 4th edition. Havcourt Brace College Publishers. pp. 462-485; 535-563.
- Smedley, P.L. and Kinniburgh, D.G. (2001). Arsenic in groundwater across the world. In: Arsenic contamination in Bangladesh. Vol 2: Final report, British Geological Survey Technical Report WC/00/19 (Kinniburgh, D.G., & Smedley, P.L. eds.), pp. 1-257 Available from: <u>http://www.bgs.ac.uk/arsenic/bphase2/reports.htm</u>. Accessed on: 6/5/2010.
- Smedley. P.L. and Kinniburgh, D.G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry*, **17**, 517-568.

- Stojanovic, R.S., Bond, A.M., and Butler, E.C.V. (1990). Liquid chromatographyelectrochemical detection of inorganic Arsenic using a wall jet cell with conventional and microsized platinum disk electrodes. *Analytical Chemistry*, **62(24)**, 2692-2697.
- Takamatsu, T., Aoki, H. and Yoshida, T. (1982). Determination of arsenate, arsenite, monomethylarsonate and dimethylarsinate in soil polluted with arsenic. *Soil Science* 133(4), 239-246.
- Taylor, P.R., Qiao, Y.L., Schatzkin, A., Yao, S.X., Lubin, J., Mao, B.L., Rao, J.Y., McAdams, M. and Xuan, X.Z. (1989). Relation of arsenic exposure to lung cancer among tin miners in Yunnan Province, China. *British Journal of Industrial Medicine*, 46, 881-886.

The New Straits Times (28 October 1999). Tasik Biru unsafe due to arsenic in water.

- Tseng, W.P. (1977). Effects of dose-response relationships of skin cancer and Blackfoot disease with arsenic. *Environmental Health Perspectives*, **19**, 109-119.
- U.S. EPA (1998). Research plan for Arsenic in drinking water. Environmental Protection Agency, United States of America, EPA/600/R-98/042, February 1998.
 Available from: http://www.epa.gov/ord/htm/documents.arsenic.pdf
 Accessed on: 6/5/2010.

- U.S.EPA (1999). Analytical methods support document for Arsenic in drinking water.
 Environmental Protection A gency, United States of America, December 1999.
 Available on: http://www.epa.gov/safewater/arsenic/pdfs/methods.pdf
 Accessed on: 3/5/2010.
- U.S.EPA (2000). Arsenic in drinking water rule: Economic analysis. Environmental Protection Agency, United States of America.
 Available from: http://www.epa.gov/safewater/arsenic/pdfs/econ_analysis.pdf
 Accessed on: 3/5/2010
- U.S. EPA (2002). Arsenic in drinking water: Analytical methods. Environmental Protection Agency, United States of America.
 Available from: <u>http://www.epa.gov/safewater/ars/ars7.html</u> Accessed on: 3/5/2010
- U.S. EPA (2006). Arsenic in drinking water. Environmental Protection Agency, United States of America. Available from: <u>http://www.epa.gov/safewater/arsenic/index.html</u> Accessed on: 3/5/2010
- Vandenhecke, J., Waeles, M., Riso, R.D., and Le Corre, P. (2007). A stripping chronopotentiometric (SCP) method with a gold film electrode for determining inorganic arsenic species in seawater. *Analytica Chimica Acta*, **388**, 929-937.

- Van Holderbeke, M., Zhao, Y., Vanhaecke, F., Moens, L., Damns, R., and Sandra, P. (1999). Speciation of six arsenic compounds using capillary electrophoresis-inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, 14, 229-234.
- Villaescusa, I. and Bollinger, J-G. (2008). Arsenic in drinking water: sources, occurrence and health effects (a review). *Environmental Science and Biotechnology*, **7**, 307-323.
- Walsh, L.M., Summer, M.E. and Keeney, D.R. (1977). Occurrence and distribution of arsenic in soils and plants. *Environmental Health Perspectives* **19**, 67-71.
- Wang, J. (1985). Stripping analysis: Principles, instrumentation and applications. VCH Publishers.
- Wang, J. (1990). Instrumentation for stripping analysis. In: Analytical instrumentation handbook (Ewing, G.W. ed.), Marcel Dekker, Inc. pp. 619-641.
- Wang, J. (2006). Analytical electrochemstry, 3rd ed. John Wiley & Sons, Inc. pp. 80-82.
- Wang, C.H., Hsiao, C.K. and Chen, C.L. (2007). A review of the epidemiologic literature on the role of environmental arsenic exposure and cardiovascular diseases. *Toxicology* and Applied Pharmacology, 223(3), 315-326.

- WHO (1981). International programme in chemical safety. Environmental Health Criteria 18.
 Arsenic. World Health Organisation, Geneva. Available from: <u>http://www.inchem.org/document/ehc/ehc/ehc018.html</u> Accessed on: 6/5/2010
- WHO (2008). Guidelines for drinking water quality. Volume I: Recommendations, 3rd ed.World Health Organization, Geneva.
- Xie, R., Johnson, W., Spayd, S., Hall, G.S. and Buckley, B. (2006). Arsenic speciation analysis of human urine using ion exchange chromatography coupled to inductively coupled plasma mass spectrometry. *Analytica Chimica Acta*, 578, 186-194.
- Yamauchi, H. and Fowler, B.A. (1994). Toxicity and metabolism of inorganic and methylated arsenicals. In: Arsenic in the environment. Part II: Human health and ecosystem effects (Nriagu, J.O. ed.), John Wiley & Sons, Inc. pp. 35-55.
- Yan-Chu, H. (1994). Arsenic distribution in soils. In: Arsenic in the environment. Part I: Cycling and characterization (Nriagu, J. O. ed.), John Wiley & Sons, Inc. pp. 17-51.
- Zhang, Q., Minami, H., Inoue, S. and Atsuya, I. (2004). Differential determination of trace amounts of arsenic(III) and arsenic(V) in seawater by solid sampling atomic absorption spectrometry after preconcentration by coprecipitation with nickelpyrrolidine dithiocarbamate complex. *Analytica Chimica Acta*, **508**, 99-105.
Zima, J. and van den Berg, C.M.G. (1994). Determination of arsenic in seawater by cathodic stripping voltammetry in the presence of pyrrolidine dithiocarbamate. *Analytica Chimica Acta*, **289**, 291.